Mirza Hasanuzzaman *Editor*

Approaches to the Remediation of Inorganic Pollutants

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This book is dedicated to
My Wife
Dr. Kamrun Nahar

Preface

Rapid urbanization, industrialization, and various natural hazards lead to environmental pollutions by different organic and inorganic pollutants. Inorganic pollutant in nature is mainly a result of anthropogenic activity. They are mainly toxic metals and metalloids, different salts, toxic ions, etc. Inorganic contaminants come from natural processes such as volcanic eruptions and continental dusts. They are mineral based and have a wide range of anthropogenic sources within the environment including extraction of ores, industrial processing of many kinds, gas generation, landfill, and transport. Among the inorganic pollutants, metals/metalloids have drawn considerable attention due to their vast damages to plants and animals. These pollutants adversely affect plant growth and productivity when present in high concentrations in the agricultural soil. Various transporters are accountable for the entry and distribution of different elements inside the plant. Therefore, there is a high priority to cope with the toxicity of heavy metals.

Inorganic pollutants can be managed by monitoring natural attenuation strategy. In recent decades, many approaches have been developed by the researchers which can clean up these pollutants from the environment or make plants tolerate these elements. Phytoremediation is one such approach which is used since many decades to clean the toxicity of different contaminants present in soil, groundwater, and other contaminated areas. It is an ecologically friendly and cost-effective technology, which can be applied both for in situ and ex situ treatments. The method can serve as an alternative tool against physical and chemical treatment methods which demand high capital inputs and are labor and energy-intensive. Phytoremediation in combination with other remediation processes, viz. microbial remediation can be effectively employed in the treatment of wetlands, establishment of crops in stressed soils, and promoting sustainable agriculture. Recently, the synergistic (plant–microbe) interaction is found as an important role in the management of polluted soils. For instance, bacterial-assisted phytoremediation is found to have great potential for the restoration of metal-contaminated environments which is considered as an economical and environmentally sustainable solution. However, the information on the physiological and molecular bases as well as appropriate approaches for improvement of plants having remediation capacities need to be organized in a book.

This book "*Approaches in the Remediation of Inorganic Pollutants*" contains 19 chapters on the most recent information with up-to-date citations, which will provide comprehensive literature of recent advances on the different approaches in the remediation of inorganic pollutants. Emphasis also has been given to elaborate the recent advancement on the techniques of different types of remediation techniques. The aim is to bring together a group of scientists in the field of plant physiology, ecology, crop science, and environmental sciences to present the latest developments in this area.

We believe that this book will be of greater use for the students and researchers, particularly Environmental Scientists, Biotechnologists, Plant Physiologists, Soil Chemists, and Agronomists, to understand the injury and tolerance mechanisms and subsequently improvement of plant-based remediation.

I like to give special thanks to the authors for their outstanding and timely work in producing such fine chapters. Our profound thanks also to Dr. Md. Mahabub Alam, Department of Agronomy, Sher-e-Bangla Agricultural University, for his valuable support in formatting and incorporating all editorial changes in the manuscripts. Special thanks to Prof. Dr. M.N.V. Prasad, Department of Plant Sciences, University of Hyderabad, Hyderabad, India for helping in organizing the contents and critical reviews of some chapters. I am highly thankful to Ms. Lee, Mei Hann, Editor (Editor, Environmental Sciences and Agriculture), Springer, Japan and Aakanksha Tyagi, Associate Editor, Springer India for their prompt responses during the acquisition. I am also thankful to Mr. Beracah John Martyn, Production Editor (Books), Springer Nature, and all other editorial staff for precious help in formatting and incorporating editorial changes in the manuscripts.

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Mirza Hasanuzzaman is a Professor of Agronomy at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. He received his PhD on "Plant Stress Physiology and Antioxidant Metabolism" from the United Graduate School of Agricultural Sciences, Ehime University, Japan with Japanese Government (MEXT) Scholarship. Later, he completed his postdoctoral research in Center of Molecular Biosciences (COMB), University of the Ryukyus, Okinawa, Japan with "Japan Society for the Promotion of Science (JSPS)" postdoctoral fellowship. Subsequently, he joined as Adjunct Senior Researcher at the University of Tasmania with Australian Government's Endeavour Research Fellowship. He has been devoting himself to the research in the field of Crop Science, especially focused on Environmental Stress Physiology since 2004. He has published over 100 articles in peer-reviewed journals and books. He has edited 13 books and written 35 book chapters on important aspects of plant physiology, plant stress responses, and environmental problems in relation to plant species. These books were published by internationally renowned publishers. He is a research supervisor of undergraduate and graduate students and supervised 20 M.S. students so far. He is Editor and Reviewer of more than 50 peer-reviewed international journals and recipient of "Publons Global Peer Review Award 2017, 2018, and 2019." He is an active member of about 40 professional societies and acting as Publication Secretary of Bangladesh Society of Agronomy. He has been honored by different authorities for his outstanding performance in different fields like research and education. He has received the World Academy of Science (TWAS) Young Scientist Award 2014. He has attended and presented 25 papers and posters in national and international conferences in different countries (USA, UK, Germany, Australia, Japan, Austria, Sweden, Russia, etc.).

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Ion Homeostasis and Its Role in Salt Remediation by Halophytes

Pedro García-Caparrós, Mirza Hasanuzzaman, and María Teresa Lao

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Abstract

Salinity is an abiotic stress which reduces the growth in plants mainly due to the osmotic and ionic effects at cellular level, therefore under high level of saline conditions, the maintenance of an adequate ion homeostasis in plants is essential. For instance, one common feature triggered by halophytes is to maintain a low Na^+ concentration in the cytosol and a higher K^+/Na^+ ratio. Considering this fact, the introduction of halophytic species in reclamation of salt-affected soils involves many advantages because these species are able to uptake toxic ions such as Na^+ and Cl^-, which is an economic solution mainly for developing

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countries or areas without any possibilities of growing another commercial species.

Keywords

Desalination · Heavy metals · Nutrient fluxes · Salinity

1 Introduction

Salinity is an abiotic stress that reduces the growth and consequently the yield of crops. It is estimated that approximately 830 to 950 million of ha in the world are affected (Teakle and Tyerman 2010; García-Caparrós and Lao 2018).

Salt-affected soils can be classified into three main groups: saline soils, sodic soils, and saline–sodic soils (Nouri et al. 2017). The saline soils contain predominantly Na^+ and Cl^- ions whereas in sodic soils CO_3^{2-} and HCO_3^- are more common (Sastre-Conde et al. 2015). It is necessary to mention that the tolerance of one species to sodic conditions does not involve a tolerance to NaCl; e.g., rice is much more tolerant to sodicity than salinity (Singh and Flowers 2011).

Under salt stress, plants suffer an osmotic and ionic stress. The osmotic stress involves a disturbance of water uptake capacity and the ionic stress is associated with the uptake of toxic ions (Na^+ and Cl^-). Both stresses result in a suppression of many physiological and biochemical processes in plants (Munns and Tester 2008).

The cellular homeostasis is essential for an adequate growth of plants and is based on the maintenance of its internal steady state, especially under disturbed conditions such as salinity. In this sense, the balance of ion uptake in plants is controlled through the ion fluxes (Nieves-Cordones et al. 2012).

The most important ions at cellular level are K^+ and Na^+ mainly due to their implications in different physiological processes such as enzyme activation and conservation of membrane potential and osmotic potential, therefore the maintenance of appropriate K^+ and Na^+ concentrations at intracellular level is crucial in plant homeostasis (Hajiboland 2012).

The maintenance of ion homeostasis at cellular level through the performance of different transporters is crucial in plants grown under saline conditions and especially is more accentuated in halophytic species (García-Caparrós et al. 2018). In this chapter, we will encompass on nutrient homeostasis under saline conditions in halophytic species. We will also shed light on the role of halophytes in phytoremediation of saline soils and soils with a high concentration of heavy metals.

2 Ion Homeostasis Under Saline Conditions

Under adequate growth conditions, ion homeostasis plays an important role in many biological processes in higher plants, therefore under stressed conditions like salinity, the maintenance of the ion homeostasis is even more crucial, since there is a deregulation of ion fluxes in plants due to the high concentration of saline ions (Nieves-Cordones et al. 2012).

2.1 Chloride Homeostasis

In saline environments, plants require to control the concentration of chloride at cellular level in order to avoid toxic damages, therefore the maintenance of an adequate efflux and influx of chloride is essential (Li et al. 2017). The entrance of Cl^- ions in a cell is adjusted by high- and low-affinity Cl^-/H^+ symport activities while the efflux of these ions is carried on passively with the help of a gradient between the cell and the external medium (Teakle and Tyerman 2010). The movement of chloride in plants is performed through different transporters such as chloride channels (CLCs) proteins involved in vacuolar sequestration of Cl^- ions (Zifarelli and Pusch 2010) and electroneutral cation-chloride-cotransporters (CCCs) settling the movement of Cl^- ions across the plasma membrane (Colmenero-Flores et al. 2007).

2.2 Calcium Homeostasis

Calcium is involved in the conservation of membrane integrity but also participates in crucial phases of plant growth and development acting as a messenger, hence the disturbance of calcium uptake under saline conditions in higher plants has to be compensated through the use of different transporters in order to maintain the cell homeostasis (Manishankar et al. 2018). The approach of Ca^{2+} influx is regulated by depolarization and hyperpolarization-activated Ca^{2+}-permeable channels (DACCs and HACCs, respectively) (Miedema et al. 2008). With regard to calcium efflux, two types of transporters are involved: Ca^{2+}-ATPases and Ca^{2+}-exchangers (CAXs) which act jointly since CAXs reduce the cellular Ca^{2+} concentration until low limits (μM) and then Ca^{2+}-ATPases are responsible for the control of this low Ca^{2+} concentration (Miedema et al. 2008; Bose et al. 2011).

2.3 Sodium Homeostasis

Under saline conditions, sodium concentration is high in the environment and its homeostasis at cellular level in plants is controlled through different transporters. The approach of Na^+ influx is carried on through the following transport systems: non-selective cation channels (NSCC), high affinity K^+ transporters (HAK/KUP/KT/HKT), and cation-Cl^- cotransporters (Kronzucker and Britto 2011). The first group of transporters (NSCC) is ubicated in plasmatic membrane and in the tonoplast of plant cells. Although these transporters are crucial in the movement of cations between cells mainly due to their high selectivity over anions, they have a main drawback since they are not able to distinguish between K^+ and Na^+ ions

(Pottosin and Dobrovinskaya 2014). Transporters such as HAK/KUP/KT are involved in K^+ homeostasis and also play a crucial role in Na^+ control at cellular level (Adams and Shin 2014). With regard to HKT, it is necessary to mention that they are classified into two groups according to the constitutional amino acid sequence of the first pore domain (PD) (S-G-G-G motif (class I) and G-G-G-G (class II)) (Platten et al. 2006). These structural differences led to a distinctive cation specificity being Na^+ uniport in the first group whereas the second group are Na^+/K^+ symport (Kronzucker and Britto 2011).

As far as sodium efflux is concerned, it is necessary to highlight that it is an active process carried on with the help of Salt Overly Sensitive (SOS) transporters (Na^+/H^+ antiporter) (Maathuis et al. 2014). These antiporters are responsible for the movement of Na^+ toward the apoplast using a H^+ gradient across the plasma membrane (Núñez-Ramírez et al. 2012).

2.4 Potassium Homeostasis

Potassium is crucial in an adequate plant's growth since it participates in different biochemical processes such as enzyme activation, membrane transport, anion neutralization, and osmoregulation (Wang and Wu 2013). Due to the high importance of this nutrient at cellular level, the maintenance of an appropriate flux is essential and is depending on the equilibrium potential (Ek) and membrane potential (Em). If the value of Em is more negative than Ek, there is a process of K^+ influx into the cells while on the contrary, if Em is less negative than Ek, K^+ is shifted to the outside of the cell (Sun et al. 2009).

The homeostasis of potassium in a cell is maintained through the performance of different transporters. These transporters can be categorized into two differentiated groups: (1) potassium permeable channels (shaker-type located in the plasma membrane and "two-pore" and non-selective cation channels (NSCCs) ubicated in the tonoplast) and (2) potassium transporters (KUP/HAK/KT/HKT and K^+/H^+ antiporters) (Benito et al. 2014).

Due to the chemical similarity between K^+ and Na^+ ions and the corresponding competence by their uptake sites, it is necessary to determine the respective K^+/Na^+ ratio in plants grown under saline conditions (Adams and Shin 2014). Halophyte plants usually have a low K^+/Na^+ ratio since they are able to use Na^+ as a cheap way for the maintenance of the osmotic potential (Flowers and Colmer 2008). It is necessary to highlight that the discriminating capacity of halophytes for Na over K is variable between species (Flowers et al. 1986). On the same vein, Flowers and Colmer (2008) reported that halophytes range between mean values of 9 and 60 in terms of net K/Na selectivity (a determination more accurate since takes into account K and Na concentration in the plant with respect to the concentration recorded in the root medium).

3 Halophytes Classification

The definition of halophyte would be the following “species able to grow and complete the life cycle under saline concentrations of at least 200 mM NaCl (~20 dSm^{-1})” according to the recommendations given by Flowers et al. (1986). On the same hand, Flowers et al. (1986) reported that halophyte plants can overcome saline concentrations which can cause the death of at least 99% of other species.

The most frequently ions in the seawater are Na^+ and Cl^- which play an essential role in the osmotic adjustment of halophytes (Flowers et al. 2010). Among halophytic species, approximately 500 species can be grown under seawater salinity. Nevertheless, most halophytes cannot tolerate these high levels of saline concentrations (Flowers et al. 2010).

One important feature in halophytes is the high adaptability to survive in different ecological ranges such as coastal regions, salt marshes, inland deserts, and salt steppes (Flowers and Colmer 2008).

There are approximately 20 orders of halophytes being Caryophyllales the order with the highest number of halophytes followed by Alismatales. With regard to Caryophyllales, it is necessary to point out that this order includes genus belonging to family Chenopodiaceae such as Atriplex, Salicornia, and Tecticornia, whereas the order Alismatales includes marine flowering plants such as Zostera and Thalassia (Flowers and Colmer 2008).

Literature reports on halophytes classification have been established following different criterion like ecological aspects or salt tolerance mechanisms. For instance, considering the habitat traits of each species, they can be categorized into three classes: (a) obligate with an adequate growth in salt natural environments with high saline concentrations; (b) facultative which can able to grow in salty conditions but the optimal growth occurs under low or without salt conditions and (c) habitat-indifferent halophytes without any crucial preference for the natural environment in which they are growing (Hasanuzzaman et al. 2014).

As far as salt tolerance mechanisms are considered, the halophytes categorization is based on the prevailing salt tolerance mechanism evolved in each species. For instance, salt excluding species are distinguished by the presence of a root system with an ultrafiltration system which avoids the uptake of saline ions. Mangrove species such as *Rhizophora mucronata* and *Kandelia candel* are typical salt excluder species. Another genera belonging to halophytes are salt excreting overcoming this abiotic stress through the use of specialized foliar glands. Some examples of these kinds of halophytic species are *Avicennia officinalis*, *Aegiceras corniculatum*, and *Acanthus ilicifolius*. Lastly, other halophytes are salt accumulators since they are able to manage increasing salt concentrations at cellular and tissue level through an increase of succulence. *Sonneratia acida*, *Salvadora persica*, and *Sesuvium portulacastrum* are representative species of this group of halophytes (Hasanuzzaman et al. 2014).

4 Halophytes-Assisted Salt Phytoremediation

The huge increase of salinization at worldwide level has involved the non-use of saline soils to grow many salt sensitive crops mainly due to the yield restriction. This constraint is more accentuated especially in arid and semiarid regions with scarce rainfall and inadequate irrigation systems to decrease the salt concentration in the rhizosphere of plants (Hasanuzzaman et al. 2014, 2019).

The lack of suitable physiochemical methods and the high cost of them to overcome the damage caused by saline soils in the crops have led to the search of new sustainable alternatives in order to combat this abiotic stress. Among these alternatives, the use of halophytes plants in saline soils has been considered as an effective and environmentally acceptable solution (Panta et al. 2016). This solution is based on the potential of halophytes to accumulate very large concentrations of saline ions (Na^+ and Cl^-) in the aerial parts (Shabala 2013). The viability of saline soil phytoremediation using halophytes was firstly reported by Boyko (1966). Nowadays, there are numerous researches corroborating the high capacity of soil desalination of different halophytic species. For instance, Qadir et al. (2006) and Abdelly et al. (2007) reported the potentiality of halophytes for the remediation of saline soils. On the same hand, Archana and Kumar (2014) carried out an study in India in order to decrease the degree of salinization in the soil using different halophytic species (*Suaeda fruticosa*, *Suaeda maritima*, and *Tamarix aphylla*) and the results obtained reported that these species were able to reduce the salt concentration in the root medium through the accumulation of salts mainly in roots.

Following with the same line, there are several updated references in the last 10 years in the Table 1, where the halophytic species are classified considering if they are phytoextractors of Na^+ and Cl^- jointly or only Na^+ or Cl^-.

Table 1 Summary of recent reports about phytoextraction capacity of different halophytes

Halophyte species	Target ions	References
Borago officinalis	Na^+ and Cl^-	Badi and Sorooshzadeh (2011)
Atriplex nummularia	Na^+ and Cl^-	Belkheiri and Mulas (2013) and Silva et al. (2016)
Atriplex halimus and *A. hortensis*	Na^+ and Cl^-	Suaire et al. (2016)
Salsola soda, Portulaca oleracea	Na^+ and Cl^-	Karakas et al. (2017)
Aster tripolium	Na^+ and Cl^-	Wiszniewska et al. (2019)
Atriplex lentiformis	Na^+	Glenn et al. (2009)
Bassia indica	Na^+	Shelef et al. (2012)
Chenopodium quinoa	Na^+	
Sulla carnosa	Na^+	Jlassi et al. (2013)
Sesuvium portulacostrum	Na^+	Muchate et al. (2016) and Ramasamy et al. (2017)
Arthrocnemum macrostachyum	Na^+	Barcia-Piedras et al. (2019)
Phragmites australis, Puccinellia nuttalliana, Spartina pectinata	Cl^-	McSorley et al. (2016)
Spartina pectinata, Distichlis spicata	Cl^-	Yun et al. (2019)

5 Conclusion and Future Perspectives

Under saline conditions, plants suffer a growth and yield reduction mainly due to the ionic and toxic stresses caused by this abiotic factor. The homeostasis of several ions such as Cl^-, Na^+, K^+, and Ca^{2+} is disrupted by high NaCl concentrations in the root medium. The capacity of saline ions uptake and the survivance of halophytes in these environments show the feasibility of these species in remediation of saline soils. On the same hand, the potential of extracting heavy metal in polluted areas strengthen the use of these species as a cheap and environmentally solution. Learning from the capacity of halophytes to uptake saline and heavy metal ions and using these features as a possible alternative for soils phytoremediation are the future tasks for plant biologists and agronomists. These knowledge about the use of halophytes as phytoremediators can be useful for the determination of specific genes involved in these chemical pathways in order to be transferred to salt sensitive crops.

References

Abdelly C, Barhoumi Z, Ghnaya T, Debez A, Hamed KB, Ksouri R, Talbi O, Zribi F, Ouerghi Z, Smaoui A, Huchzermeyer B, Grignon CD (2007) Potential utilisation of halophytes for the rehabilitation and valorisation of salt-affected areas in Tunisia. In: Öztürk M, Waisel Y, Khan MA, Görk G (eds) Biosaline agriculture and salinity tolerance in plants. Birkhäuser, Basel, pp 161–170

Adams E, Shin R (2014) Transport, signaling, and homeostasis of potassium and sodium in plants. J Integr Plant Biol 56:231–249

Archana P, Kumar A (2014) Bioremediation of saline soils using aquatic plants. J Pharm Biol Sci 2:63–67

Badi HN, Sorooshzadeh A (2011) Evaluating potential of borage (*Borago officinalis* L.) in bioremediation of saline soil. Afr J Biotech 10:146–153

Barcia-Piedras JM, Pérez-Romero JA, Mateos-Naranjo E, Camacho M, Redondo-Gómez S (2019) Effect of prior salt experience on desalination capacity of the halophyte *Arthrocnemum macrostachyum*. Desalination 463:50–54

Belkheiri O, Mulas M (2013) The effects of salt stress on growth, water relations and ion accumulation in two halophyte *Atriplex* species. Environ Exp Bot 86:17–28

Benito B, Haro R, Amtmann A, Cuin TA, Dreyer I (2014) The twins K^+ and Na^+ in plants. J Plant Physiol 171:723–731

Bose J, Pottosin I, Shabala SSS, Palmgren MG, Shabala S (2011) Calcium efflux systems in stress signaling and adaptation in plants. Front Plant Sci 85:1–17

Boyko H (1966) Basic ecological principles of plant growing by irrigation with highly saline or seawater. In: Boyko H (ed) Salinity and aridity. Springer, Dordrecht, pp 131–200

Colmenero-Flores JM, Martinez G, Gamba G, Vazquez N, Iglesias DJ, Brumos J, Talon M (2007) Identification and functional characterization of cation-chloride cotransporters in plants. Plant J 50:278–292

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963

Flowers TJ, Hajibagheri MA, Clipson NJW (1986) Halophytes. Q Rev Biol 61:313–337

Flowers TJ, Galal HK, Bromham L (2010) Evolution of halophytes: multiple origins of salt tolerance in land plants. Funct Plant Biol 37:604–612

García-Caparrós P, Lao MT (2018) The effects of salt stress on ornamental plants and integrative cultivation practices. Sci Hortic 240:430–439

García-Caparrós P, Hasanuzzaman M, Lao MT (2018) Ion homeostasis and antioxidant defense toward salt tolerance in plants. In: Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B (eds) Plant nutrients and abiotic stress tolerance. Springer, Singapore, pp 415–436

Glenn EP, Mckeon C, Gerhart V, Nagler PL, Jordan F, Artiola J (2009) Deficit irrigation of a landscape halophyte for reuse of saline wastewater in a desert city. Landsc Urban Plan 89:57–64

Hajiboland R (2012) Effect of micronutrient deficiencies on plants stress responses. In: Ahmad P, Prasad MNV (eds) Abiotic stress responses in plants: metabolism, productivity and sustainability. Springer, New York, pp 283–329

Hasanuzzaman M, Nahar K, Alam M, Bhowmik PC, Hossain M, Rahman MM, Vara Prasad MN, Ozturk M, Fujita M (2014) Potential use of halophytes to remediate saline soils. Biomed Res Int 2014:8. https://doi.org/10.1155/2014/589341

Hasanuzzaman M, Shabala S, Fujita M (2019) Halophytes and climate change: adaptive mechanisms and potential uses. CAB International, Oxfordshire, p 400

Jlassi A, Zorrig W, Khouni AE, Lakhdar A, Smaoui A, Abdelly C, Rabhi M (2013) Phytodesalination of a moderately-salt-affected soil by *Sulla carnosa*. Int J Phytoremediation 15:398–404

Karakas S, Cullu MA, Dikilitaş M (2017) Comparison of two halophyte species (*Salsola soda* and *Portulaca oleracea*) for salt removal potential under different soil salinity conditions. Turk J Agric For 41:183–190

Kronzucker HJ, Britto DT (2011) Sodium transport in plants: a critical review. New Phytol 189:54–81

Li B, Tester M, Gilliham M (2017) Chloride on the move. Trends Plant Sci 22:236–248

Maathuis FJ, Ahmad I, Patishtan J (2014) Regulation of Na^+ fluxes in plants. Front Plant Sci 5:467. https://doi.org/10.3389/fpls.2014.00467

Manishankar P, Wang N, Köster P, Alatar AA, Kudla J (2018) Calcium signaling during salt stress and in the regulation of ion homeostasis. J Exp Bot 69:4215–4226

McSorley KA, Rutter A, Cumming R, Zeeb BA (2016) Chloride accumulation vs chloride excretion: phytoextraction potential of three halophytic grass species growing in a salinized landfill. Sci Total Environ 572:1132–1137

Miedema H, Demidchik V, Very AA, Bothwell JH, Brownlee C, Davies JM (2008) Two voltage-dependent calcium channels co-exist in the apical plasma membrane of *Arabidopsis thaliana* root hairs. New Phytol 179:378–385

Muchate NS, Nikalje GC, Rajurkar NS, Suprasanna P, Nikam TD (2016) Physiological responses of the halophyte *Sesuvium portulacastrum* to salt stress and their relevance for saline soil bio-reclamation. Flora 224:96–105

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Ann Rev Plant Biol 59:651–681

Nieves-Cordones M, Aleman F, Fon M, Martínez V, Rubio F (2012) K^+ nutrition, uptake, and its role in environmental stress in plants. In: Ahmad P, Prasad MNV (eds) Environmental adaptations and stress tolerance of plants in the era of climate change. Springer, New York, pp 85–112

Nouri H, Borujeni SC, Nirola R, Hassanli A, Beecham S, Alaghmand S, Mulcahy D (2017) Application of green remediation on soil salinity treatment: a review on halophytoremediation. Process Saf Environ Protec 107:94–107

Núñez-Ramírez R, Sánchez-Barrena MJ, Villalta I, Vega JF, Pardo JM, Quintero FJ, Martínez-Salazar J, Albert A (2012) Structural insights on the plant salt-overly-sensitive 1 (SOS1) Na^{+}/H^{+} antiporter. J Mol Biol 424:283–294

Panta S, Lane P, Doyle R, Hardie M, Haros G, Shabala S (2016) Halophytes as a possible alternative to desalination plants: prospects of recycling saline wastewater during coal seam gas operations. In: Khan MA, Ozturk M, Gul B, Ahmed MZ (eds) Halophytes for food security in dry lands. Academic Press, Amsterdam, pp 317–329

Platten JD, Cotsaftis O, Berthomieu P, Bohnert H, Davenport RJ, Fairbairn DJ, Horie T, Leigh RA, Lin HX, Luan S, Maser P, Pantoja O, Rodriguez-Navarro A, Schachtman DP, Schroeder JI, Sentenac H, Uozumi N, Very AA, Zhu JK, Dennis ES, Tester M (2006) Nomenclature for HKT transporters, key determinants of plant salinity tolerance. Trends Plant Sci 11:372–374

Pottosin I, Dobrovinskaya O (2014) Non-selective cation channels in plasma and vacuolar membranes and their contribution to K^+ transport. J Plant Physiol 171:732–742

Qadir M, Schubert S, Noble AD, Saqib M, Saifullah T (2006) Amelioration strategies for salinity-induced land degradation CAB reviews: perspectives in agriculture, veterinary science. Nutr Nat Res 1:69

Ramasamy J, Periasamy K, Venugopal B (2017) Phytoremediation potential of *Sesuvium portulacastrum* on remediating salt affected soil. Curr World Environ 12:687

Sastre-Conde I, Carmen Lobo M, Beltrán-Hernández RI, Poggi-Varaldo HM (2015) Remediation of saline soils by a two-step process: washing and amendment with sludge. Geoderma 247:140–150

Shabala S (2013) Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. Ann Bot 112:1209–1221

Shelef O, Gross A, Rachmilevitch S (2012) The use of *Bassia indica* for salt phytoremediation in constructed wetlands. Water Res 46:3967–3976

Silva YJ, Silva YJ, Freire MB, Lopes EA, Santos MA (2016) *Atriplex nummularia* Lindl. as alternative for improving salt-affected soils conditions in semiarid environments: a field experiment. Chil J Agr Res 76:343–348

Singh RK, Flowers TJ (2011) Physiology and molecular biology of the effects of salinity on rice. In: Pessarakli M (ed) Handbook of plant and crop stress. CRC, Boca Raton, pp 899–939

Suaire R, Durickovic I, Framont-Terrasse L, Leblain JY, De Rouck AC, Simonnot MO (2016) Phytoextraction of Na^+ and cl^- by *Atriplex halimus* L. and *Atriplex hortensis* L.: a promising solution for remediation of road runoff contaminated with deicing salts. Ecol Eng 94:182–189

Sun J, Chen SL, Dai SX, Wang RG, Li NY, Shen X, Zhou XY, Lu CF, Zheng XJ, Hu ZM, Zhang ZK, Song J, Xu Y (2009) Ion flux profiles and plant ion homeostasis control under salt stress. Plant Signal Behav 4:261–264

Teakle NL, Tyerman SD (2010) Mechanisms of cl^- transport contributing to salt tolerance. Plant Cell Environ 33:566–589

Wang Y, Wu WH (2013) Potassium transport and signaling in higher plants. Ann Rev Plant Biol 64:451–476

Wiszniewska A, Koźmińska A, Hanus-Fajerska E, Dziurka M, Dziurka K (2019) Insight into mechanisms of multiple stresses tolerance in a halophyte *Aster tripolium* subjected to salinity and heavy metal stress. Ecotox Environ Saf 180:12–22

Yun KB, Koster S, Rutter A, Zeeb BA (2019) Haloconduction as a remediation strategy: capture and quantification of salts excreted by recretohalophytes. Sci Total Environ 685:827–835

Zifarelli G, Pusch M (2010) CLC transport proteins in plants. FEBS Lett 584:2122–2127

Role of Transporters in Accumulating Salt Ions by Halophytes

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Abstract

Ionic stress is the major component of salinity and is brought about by excess ions accumulation, especially in the aerial parts of plants. Salinization is the major problem affecting crop productivity. The effective way to understand, to cope the salt effectiveness and increase crop productivity is to study the tolerance mechanism of halophytes. Halophytes always have a remarkable ability to tolerate salinity that is dangerous for most other crops. This chapter summarizes the present knowledge about ions absorption, accumulations and transportation patterns in halophytes under salinity. Moreover, the role of sodium (Na^+), potassium (K^+), chloride (Cl^-) and calcium (Ca^+) transporters and channels in uptake and translocation of these ions has been shown and discussed. It is concluded that further studies are needed to better understand the ions transportation in halophytes and to explore the novel regulatory pathways, which augment the complexity of salt ions and could increase tolerance under salinity.

Keywords

Avoidance · Ion compartmentalization · Osmotic adjustment · Salinity tolerance mechanism

1 Introduction

Salinization of soil is a serious issue in the world (Feng et al. 2015; Khalid et al. 2019). Among 230 million hectares of land currently in use for agriculture, 20% are affected by salt, and this number increases every year as a result of inconsistent crop irrigation practices, excessive fertilization and excessive ploughing as well as natural causes such as salt intrusion into coastal zones resulting from the sea-level rise (Smajgl et al. 2015; Lin et al. 2018). At present, due to the poor salt tolerance of the crop's plants, it is impossible to plant crops on the saline-alkali land (Hussain et al. 2018). The challenge of feeding about 10 billion people by the next century is forcing agricultural production into salinized wasteland so that it appears likely to be achieved only through a major breakthrough in breeding crops for salinity tolerance. Therefore, understanding the salt-tolerant mechanism of plants especially halophytes, which can survive and complete their life cycles in harsh, saline environments, will be a key step in improving crop salt tolerance for adaptation to saline habitats (Shabala et al. 2014).

Halophytes are generally recognized as plants that can survive high concentrations of electrolytes in their environments. Ecologically, halophytes have been defined as "the native flora of saline soils" (Jennings 1976). In this context, saline soils are those containing solutions with an osmotic pressure of at least 3.3 bar, which is equivalent to 70 mM monovalent salts (Feng et al. 2015). Recently, halophytes refer to the plants that are adapted to saline soil environments and are able to survive and reproduce at salt concentrations of 200 mM or greater (Guo et al. 2018). German botanist Breckle classified halophytes into three major categories on the physiological basis of salt tolerance as well as the accumulation and transport of ions. (A) Recretohalophytes can secrete salt from the plant body to the outside through salt glands (as in *Limonium bicolor*) or into salt bladders for temporary storage and then the salt will scatter from salt bladders when it encounters strong winds or other external stimuli (as in *Chenopodium quinoa*). Salt entering the plant body is secreted by salt glands or salt bladders, which reduces the salt content of plant life tissue and guarantees its normal growth and development in saline soil environments (Yuan et al. 2016). (B) Euhalophytes such as *Suaeda salsa* and *Mesembryanthemum crystallinum*, which are divided into two categories known as leaf succulent euhalophytes and stem succulent euhalophytes accumulate salt in the vacuoles of succulent green tissues of leaves or stems, respectively (Xu et al. 2016). Euhalophytes compartmentalize excessive salt ions, which enter the plant cells into the vacuole (Maathuis et al. 1992) . On the one hand, this reduces the water potential of the plant and helps it to absorb water from the saline soil and, on the other hand, this reduces the ions content in cytoplasm and avoids damage to enzymes and biological substances in the cytoplasm (Song and Wang 2015). (C) Salt-exclusion halophytes, which are also called salt excluders such as reed plant (*Phragmites communis* L.), accumulate much more salt in the vacuoles of parenchyma tissues and parenchyma of roots and xylem than in the shoot.

Salt-exclusion halophytes mostly transport 2% of the salt absorbed by the roots to the shoots, and the remaining salt is excluded into the soil solution (Husain et al. 2004). At present, the key locations for salt exclusion in salt-exclusion halophytes are reported to be the pericycle and xylem parenchyma cells, the root cortex (especially the casparian strip) and the phloem cells (Munns et al. 2006).

2 Ions and Plant Relation

2.1 Tissue-Specific Compartmentation

Salt concentration for the normal growth of monocotyledonous species is much lower (Glenn et al. 1999) as compared to dicotyledonous halophytes, which show optimal growth in 100–250 mM NaCl. Dicotyledonous halophytes generally accumulate more NaCl in shoot tissues than their monocotyledonous counterparts (Table 1), with total 'ash' content in the former often exceeding 50% of the shoot dry mass (Flowers and Colmer 2008). It is not clear whether this difference is attributed to specific anatomical features, higher transpiration rates or higher activity of Na^+ transporters in some plant tissues. Much less (three- to fourfold) Na^+ is accumulated in halophyte roots compared with shoots (Table 2), while K^+ content in both organs is comparable (Tables 1 and 2).

The most obvious 'physiological hallmark' distinguishing halophytes from glycophytes is their ability to select K^+ from a mixture dominated by Na^+ and yet accumulate sufficient Na^+ for the purposes of osmotic adjustment. At the

Table 1 Sodium and potassium content in leaves of selected halophyte species grown at high external salinities (200–500 mM NaCl range)

Species	$[Na^+]$	$[K^+]$	Reference
Aster tripolium	125–275	65	Ueda et al. (2003)
Atriplex nummularia	330–870	65–100	Ramos et al. (2004)
Atriplex prostrata	660–1205		Flowers (1985)
Atriplex spongiosa	400–650	60–200	Storey et al. (1983)
Atriplex portulacoides	220–320	140–160	Redondo-Gómez et al. (2007)
Atriplex centralasiatica	325–440		Qiu and Lu (2003)
Bolboschoenus maritimus	60–400		Albert (1975)
Chenopodium glaucum	150–380		Albert (1975)
Crypsis aculeata	100–125		Albert (1975)
Jaumea carnosa	165–500		Ramos et al. (2004)
Juncus gerardii	40–150		Albert (1975)
Lepidium crassifolium	90–380		Albert (1975)
Mesembryanthemum crystallinum	240–690	50–110	Agarie et al. (2007)
Puccinellia distans	50–130		Albert (1975)
Salicornia prostrata	380–620		Albert (1975)
Scorzonera parviflora	75–125		Albert (1975)
Spergularia media	240–450		Albert (1975)
Suaeda salsa	340–1150	35–80	Wang et al. (2001)
Suaeda monoica	750–880	25–40	Storey and Jones (1979)
Suaeda maritima	380–660	35–160	Wang et al. (2007)
Thellungiella halophila	200–395		Vera-Estrella et al. (2005)
Triglochin maritimum	170–260		Albert (1975)
Plantago maritima	100–300		Albert (1975)

Data were collated from published sources and expressed as millimoles of leaf sap $[Na^+]$ or $[K^+]$ for the purposes of consistency

whole-plant level, the selectivity between K^+ and Na^+ ($S_{K/Na}$) in halophytes is within the range of 100–200, even at external salinities exceeding sea water levels (Storey et al. 1983). Specific details beyond this selectivity remain obscure.

Tissue-specific compartmentalization appears to play an important role in controlling the above selectivity. Pronounced Na^+ gradients were detected between EBCs, leaf epidermis and mesophyll cells in *M. crystallinum*. Gradients in $S_{K/Na}$ selectivity were also reported between the bundle sheath cells and the bladder cells in *A. spongiosa* (Storey et al. 1983). However, over 50% of halophytes do not have glands or external bladders to modulate their tissue ion concentration (Flowers and Colmer 2008), so the above explanation is not applicable to all situations. It appears that vacuolar sequestration fulfils this role in bladder-less species. Indeed, even within species possessing bladders, Na^+ may be sequestered in salt bladders primarily in young leaves, while in mature leaves, the major Na^+ storage site are vacuoles (Storey et al. 1983).

The above high $S_{K/Na}$ selectivity in halophytes appears to be intrinsically related to tissues' (and, specifically, roots') ability to retain K^+. Contrary to glycophytes

Table 2 Sodium and potassium content in roots of selected halophyte species grown at high external salinities (200–500 mM NaCl range)

Species	[Na^+]	[K^+]	Reference
Atriplex prostrata	130–315	50–65	Anderson et al. (1977)
Atriplex spongiosa	80	120	Storey et al. (1983)
Atriplex nummularia	105–160	85–95	Ramos et al. (2004)
Atriplex halimus	70–95		Hassine et al. (2008)
Atriplex portulacoides	60–100	50–60	Redondo-Gómez et al. (2007)
Disphyma crassifolium	220–475		Neales and Sharkey (1981)
Mesembryanthemum crystallinum	110–150		Barkla et al. (2002)
Puccinellia maritima	110–140		Flowers (1985)
Porteresia coarctata	80–100	45–60	Flowers et al. (1990)
Salicornia bigelovii	220–270	75–100	Ayala and O'leary (1995)
Sarcocornia natalensis	20–150	65–220	Naidoo and Rughunanan (1990)
Suaeda maritima	105–200	105–215	Hajibagheri et al. (1984)
Sporobolus virginicus	39–99	30–45	Marcum and Murdoch (1990)
Thellungiella halophila	70–210		Vera-Estrella et al. (2005)
Triglochin maritimum	148	70	Jefferies (1973)

Data were collated from published sources and expressed as millimoles of root sap [Na^+] or [K^+], for the purposes of consistency

(Tester and Davenport 2003), increasing salinity stimulated K^+ accumulation in halophyte roots (e.g. *Suaeda monoica* or *Triglochin maritima*) and resulted in plants' ability to maintain fairly constant K^+ concentrations in the shoot. Volkov et al. (2003) have concluded that the main difference between cell-type-specific ion distribution in *Arabidopsis thaliana* and its halophyte relative *Thellungiella halophila* during salt stress was related to K^+ rather than Na^+. These authors have reported that *Thellungiella* plants accumulated very high K^+ concentrations in the epidermis under control conditions, although during salt stress, epidermal K^+ concentrations decreased dramatically, whereas bulk K^+ concentrations increased. The authors suggested that the epidermis played the role of storage site for K^+ in this species (Volkov et al. 2003).

2.2 Inorganic Ion Accumulation and Osmotic Adjustment

Halophytes are adapted to low water potential by their capacity for osmotic adjustment. It is widely accepted that cell turgor is maintained by storage of Na^+ and Cl^- in vacuoles, with the solute potential of the cytosol adjusted by accumulation of K^+ and organic solutes (Glenn et al. 1999; Storey 1995). According to Glenn et al. (1999), the three major inorganic ions, Na^+, K^+ and Cl^-, account for 80–95% of the cell sap osmotic pressure in both halophyte grasses and dicots. As a result, halophytes accumulate substantial amounts (>10% of dry weight each) of Na^+ and Cl^- in their shoots, predominantly in vacuoles (Flowers and Colmer 2008). At the same time, cytoplasmic K^+ concentrations in halophytes are similar to those of glycophytes (Flowers and Colmer 2008). As a result, halophytes have a rather high (~5) vacuole/cytosol Na^+ ratio and, at the same time, a high (~4) cytosol/vacuole K^+ ratio (Ye and Zhao 2003).

2.3 Organic Osmolytes: Osmotic Adjustment or Osmoprotection?

It has long been thought that halotolerance depends on the osmotic adjustment of the cytoplasm, achieved by the accumulation of compatible solutes. The two major osmolytes are glycine betaine (GB) and proline, although others such as inositol, pinitol, sorbitol, mannitol and ononitol are also reported (Agarie et al. 2007; Hassine et al. 2008).

The expression patterns of compatible solutes vary dramatically in time, as well as between species and tissues. As such, proline accumulated as early as 24 h after stress imposition while GB concentration culminated after 10 days of stress and did not decrease after the stress was relieved, in *Atriplex* (Hassine et al. 2008). While root betaine concentrations in *Armeria maritima* increased under saline conditions, no significant increase in betaine in leaves was detected, where concentrations remained between 2 and 4 mM. At the same time, much higher (120 mM) GB levels were reported in the leaves of *Sporobolus virginicus* (Marcum and Murdoch 1992).

Even assuming that compatible solutes (such as GB or proline) are located exclusively within the cytosol, the reported range of concentrations (typically 20–150 mM) is hardly sufficient for full osmotic adjustment in the cytosol, given the reported Na^+ concentrations in the vacuole (e.g. 500–600 mM) or external media (200–500 mM) found under typical conditions. Hence, it is more likely that the major role of compatible solutes in halophytes may not be in conventional water retention but in osmoprotection and/or ROS scavenging (Bohnert and Shen 1998; Hassine et al. 2008).

We have previously reported that compatible solutes are potent regulators of K^+-permeable ion channels in the plasma membranes of root epidermal cells (Cuin and Shabala 2007). In this study, root pretreatment with physiologically relevant concentrations of amino acids resulted in a significant reduction of detrimental salinity effects on cytosolic K^+ homeostasis. Similar results were obtained for physiologically relevant concentrations of GB, a representative of another class of compatible solutes (Cuin and Shabala 2007). Moreover, low (5 mM) concentration of compatible solutes such as GB (a quaternary amine), proline (amino acid), and mannitol or *myo*-inositol (polyoles) was also efficient in reducing disturbance to intracellular K^+ homeostasis caused by oxidative stress (OH• treatment; Cuin and Shabala 2007). Taken together, these results suggest that physiologically relevant concentrations of compatible solutes might contribute to plants' adaptive responses to salinity by regulating K^+ transport across the plasma membrane, thus enabling maintenance of an optimal K^+/Na^+ ratio, arguably the most important feature of salinity tolerance in plants (Tester and Davenport 2003). Surprisingly, no such work has been conducted on halophytes. Thus, the questions remain to be answered: Can plasma membrane transporters in halophyte cells be gated by the stress-induced changes in the osmolyte levels under saline conditions? and whether there is a difference in the gating properties between halophyte and glycophyte species?

3 Ion Channels and Ion Transporters

One controversial question in ion transport is the relative role of ion channels and transporters. It is commonly accepted that ion channels in an open state/conformation allow passage of over 10^6–10^8 ions per second via a selective pore formed within a protein molecule. The diameter of the pore is determined by the molecular structure of the ion channel, from 1.2 nm for the potassium channel KcsA with a narrow part of 0.4 nm in diameter (MacKinnon 2004) to 1.5 nm and even 2.8 nm diameter of the general bacterial porins with low selectivity that allow the passage of small hydrophilic molecules (about 0.6 nm pores for the highly selective porins; Galdiero et al. 2012). The diameter of the pore and the nature of the amino acids lining it essentially determine the ion selectivity of the ion channel and the potential number of ions passing per unit of time. To put these dimensions in context, the diameter of a non-hydrated Na^+ ion is about 0.2 nm and of K^+ is 0.3 nm, while effective diameters of the hydrated ions are estimated as 0.72 nm for Na^+ and 0.62 nm for K^+ (Mähler and Persson 2012). In spite of the similarities of size, the selectivity could, for example, be over 1000 for K^+ over Na^+ in potassium-selective ion channels or over 10 for Na^+ over K^+ in sodium-selective channels, all due to selectivity filters defined by conserved amino acids for specific channel types. Often the amino acid sequence glycine-tyrosine-glycine (GYG) indicates selectivity for K^+. Introducing mutations into the pore to change the amino acids can convert a potassium-selective ion channel to a nonselective one (Heginbotham et al. 1992). The interactions of ions with the protein molecule of ion channels are not yet well understood but probably involve non-electrostatic ion–ion interactions, van der Waals forces, interaction with water molecules and numerous other interactions. Several methods of modelling and of simulations of molecular dynamics have been applied within at least the last 30 years; a sharp increase in computing power has allowed the lipid environment of membranes, pH and known biochemical factors and regulators to be included in the models (Maffeo et al. 2012).

Direct measurements are the basis for investigating ion fluxes via ion channels; these methods provide information about permeating ions, number of ions crossing the membrane per second, selectivity and gating during the transport processes. For example, a small current of 1 pA can be measured by patch clamp. This corresponds to an ion (monovalent) current of 10^{-12} A/(1.6×10^{-19} coulombs) $\approx 6 \times 10^6$ ions/second ($+1.6 \times 10^{-19}$ coulombs is the elementary charge, a charge of a monovalent cation or opposite to the negative charge of an electron).

Transporters can be considered as enzymes where conformational changes of a protein molecule are required for a complete transport cycle of ions (Gadsby 2009). The turnover rate of the transporter is the number of complete transport cycles performed per second (Longpré and Lapointe 2011). The plant plasma membrane H^+-ATPase pumps about 100 ions per second, which is at the lower end of estimates for turnover rate of transporters. The value is, however, comparable to 20–100 H^+ per second by yeast plasma membrane ATPase Pma1 (Serrano 1988) and a turnover rate of 160 s^{-1} of animal Na^+/K^+-ATPase (Skou 1998). Similar or even lower turnover rates, from 3 to 60 s^{-1}, were shown for a human sodium/glucose cotransporter (Longpré and Lapointe 2011), while a turnover rate of about 500 s^{-1} was estimated for sucrose/H^+ cotransporter from maize ZmSUT1 (Carpaneto et al. 2010). The highest possible turnover rate for activity of ion transporters has been assessed from studies of protein structure and frequency of conformational changes with an estimated upper limit of 10^6 s^{-1} (Chakrapani and Auerbach 2005), although this seems, from actual measurements, to be an overestimated value.

4 Ions Transport Mechanism in Halophytes

4.1 Radial Ion Transport in Halophytes

It is generally accepted that weakly voltage-dependent nonselective cation channels (NSCCs) represent a major pathway for Na^+ uptake by glycophyte roots (Tyerman and Skerrett 1998). Among other candidates, dual-affinity HKT transporters and low-affinity LCT transporters have been named as potential mediators of Na^+ uptake by plant roots under saline conditions. However, some other authors deny HKT involvement in root Na^+ uptake and instead suggest its involvement in Na^+ retrieval from the xylem (Berthomieu et al. 2003; Tester and Davenport 2003).

Reports on sodium transport in halophytes are much more limited and usually confined to only a few model species such as *Thellungiella halophila* or *M. crystallinum* (Table 3). A significant part of these reports employed various molecular tools to study the effect of salinity on the tissue-specific expression of a particular transporter or reported its activity after expression in some heterologous system. However, heterologous expression systems have often failed to produce functional channels from transcripts encoding both cyclic nucleotide-gated channels (CNGCs) and glutamate receptor-like channels, which are prime candidates for Na^+ uptake channels in *A. thaliana* (Davenport 2002; Maathuis and Sanders 2001). Also, despite the presence of two Na^+/H^+ exchanger isoforms in the plasma membranes of *Thellungiella* root tissue, no Na^+/H^+ exchange was detected (Vera-Estrella et al. 2005), suggesting that some of these transporters may require activation by regulatory molecules that are not present in vitro in transport assays with isolated membrane vesicles.

Direct patch-clamp experiments on root plasma membrane transporters mediating ionic homeostasis in halophyte roots are very rare and confined to only one species, *T. halophila* (Volkov and Amtmann 2006). These studies have identified three distinct types of ion currents in *T. halophila* root cells: (1) time-dependent inward currents, (2) time-dependent outward currents and (3) instantaneous currents.

All three current types in *T. halophila* root cells appear to provide a very limited pathway for Na^+ uptake (Volkov and Amtmann 2006), and at physiological membrane potentials, the instantaneous current constitutes the only pathway for Na^+

Table 3 Root plasma membrane transport systems reported in halophytes

Transporter	Species	Method	Reference
Na^+/H^+ exchanger	*Thellungiella*	Expression studies	Oh et al. (2007)
Na^+/H^+ exchanger	*Thellungiella*	Expression studies	Vera-Estrella et al. (2005)
Voltage-independent K^+-selective channel	*Thellungiella*	Patch-clamp	Volkov and Amtmann (2006)
Time-dependent inward rectifying channel	*Thellungiella*	Patch-clamp	Volkov and Amtmann (2006)
Time-dependent outward rectifying channel	*Thellungiella*	Patch-clamp	Volkov and Amtmann (2006)
HAK high-affinity K^+ uptake transporter	*Thellungiella*	Patch-clamp	Alemán et al. (2009)
HAK family transporters	*Mesembryanthemum*	Expression studies	Su et al. (2002)
HKT transporter	*Mesembryanthemum*	TEVC in heterologous expression system	Su et al. (2002)
H^+-ATPase	*Atriplex nummularia*	Expression studies	Niu et al. (1993)

uptake. These instantaneous currents were fivefold more selective for potassium in *T. halophila* than in *A. thaliana* ($P_{N}a/P_{K} = 0.12$ vs. 0.67, respectively; Volkov et al. 2003). This is consistent with high $S_{K/Na}$ selectivity ratio observed in halophytes at the whole-plant level (Storey et al. 1983).

Given the fact that halophytes use Na^+ as a major osmolyte for osmotic adjustment, it is logical to expect that they must possess rather efficient systems for Na^+ vacuolar sequestration. Indeed, a tonoplast Na^+/H^+ exchanger gene was isolated and characterized in the roots of *Atriplex gmelinii* (Hamada et al. 2001). Also, tonoplast Na^+/H^+ antiport activity was induced by NaCl treatment in roots of salt-tolerant *Plantago maritima* species, but not in salt-sensitive *Plantago media* (Staal et al. 1991). However, while NHX1 activity was detected in the plasma membrane fractions of *Mesembryanthemum* roots (Vera-Estrella et al. 2005), tonoplast Na^+/H^+ exchanger activity was not observed in either control or NaCl-treated roots (Barkla et al. 2002). Hence, the relation between salinity and a tonoplast Na^+/H^+ exchanger in halophyte root activity is not as straightforward as one would expect.

Na^+ vacuolar sequestration in halophyte roots has to be energized; both tonoplast H^+-ATPases and PP-ases may be involved. However, while some authors have reported NaCl-induced increases in both H^+ transport and H^+- ATPase hydrolytic activity in the tonoplast of *Thellungiella* root cells (Vera-Estrella et al. 2005), specific V-ATPase activities in roots were similar in roots of the halophyte *P. maritima* and the glycophyte *P. media* and did not change after exposure to 50 mM NaCl (Staal et al. 1991). Moreover, down-regulation of V-ATPase expression was reported in *Mesembryanthemum* roots (Golldack and Dietz 2001), suggesting that roots are apparently unable to accumulate Na^+ and presumably pass it to the xylem for translocation to the leaves. As for the V-PPase, only a moderate (10%) increase in the hydrolytic activity was detected in salt-treated *Thellungiella* roots (Vera-Estrella et al. 2005), leading to the conclusion that V-PPase plays a minor role in energizing the tonoplast. Taken together, it appears that vacuolar sequestration of Na^+ in roots is not a key determinant of salinity tolerance in halophytes.

4.2 Xylem Ion Loading

Control of Na^+ loading into the xylem has long been considered one of the key determinants of salinity tolerance in glycophytes (Tester and Davenport 2003). Surprisingly, specific details of this process are lacking. Also controversial are estimates of the amount of sodium taken up by roots and loaded into the xylem to be delivered to the shoot. While Munns et al. (2000) estimated that at 200 mM external Na^+, about 97% of all Na^+ presented to the root surface must be excluded; regardless of whether the species is a glycophyte or halophyte, Volkov and Amtmann (2006) were more conservative in their estimates and believed that both *T. halophila* and *A. thaliana* (a halophyte and a glycophyte, respectively) export 70% of the Na^+ taken, back into the external media. Even more controversial are reports by Balnokin and coauthors who have found that in *Suaeda altissima* concentration of Na^+ in the xylem was 1.3–1.6 times higher than in the bath solution and reached as high as 350 mM (Balnokin et al. 2005). Such conflicting estimates call for a more thorough comparison between glycophyte and halophyte species, under some sort of standardized conditions. Also highly controversial are reports on the effect of growth conditions on xylem Na^+ content. While Clipson and Flowers (1987) have reported about a twofold night time increase in the xylem Na^+ content, other authors believed that xylem sap salt concentration is highest during the day. The reason for such discrepancies is yet to be understood.

Although the above report of 350 mM Na^+ in the xylem sap of *S. altissima* is clearly outside the typical range found in other halophytes (Table 4), concentrations of around 50 mM may be taken as a reasonable estimate. Halophytes are using Na^+ as a cheap osmoticum to maintain cell turgor and thus, some significant amounts of Na^+ are expected to be loaded into the xylem. Also, it was argued that high xylem

Table 4 Xylem Na^+ concentration in some selected halophyte species grown at high external salinities (200–500 mM NaCl range)

Species	Xylem [Na^+] (mM)	Reference
Suaeda maritima	46	Flowers (1985)
Atriplex littoralis	30–48	Rozema et al. (1981)
Salicornia virginica	19–38	Ownbey and Mahall (1983)
Suaeda maritima	50–60	Clipson and Flowers (1987)
Avicennia species	5–122	Scholander et al. (1962)
Suaeda altissima	50–350	Balnokin et al. (2005)

Na^+ concentrations are important for formation of water potential gradients to drive water transport to the shoot (Balnokin et al. 2005).

The underlying transport mechanisms involved in both Na^+ loading into the xylem and its retrieval from the transpiration stream remain highly controversial. To the best of our knowledge, not a single electrophysiological study has been undertaken to address this issue in halophytes. In glycophytes, both passive (Wegner and De Boer 1997) and active (Munns and Tester 2008) models have been vigorously advocated. This results mainly from the great uncertainty regarding the range of Na^+ concentrations in the cytosol of xylem parenchyma cells (originating from the methodological difficulties of such measurements). However, even assuming it being as high as 100 mM (Hajibagheri et al. 1985), a twofold Na^+ gradient between the cytosol and the xylem will make passive Na^+ loading thermodynamically plausible only if the membrane potential values of xylem parenchyma cells are less negative than −17 mV. This seems not to be the case, with some authors reporting highly negative values for halophyte parenchyma cells (e.g. −130 to −140 mV in *Atriplex hastata*; Anderson et al. 1977). This leaves active Na^+ loading as the only option. The most likely candidate is an SOS1 putative Na^+/H^+ antiporter. In glycophytes, such an exchanger is preferentially expressed at the xylem/symplast boundary of roots, as indicated by promoter–GUS fusions (Shi et al. 2002). Although no direct evidence for halophytes was presented, the observed acidification of the root exudating from 5.5 to 4.5 in response to NaCl treatment in *S. altissima* (Balnokin et al. 2005) is consistent with this model.

4.3 Unloading and Ion Transport in Leaves

Maintenance of low cytosolic Na^+ levels in photosynthetically active mesophyll cells is considered to be absolutely essential for glycophytes grown under saline conditions. Given the fact that the sensitivity of key cytosolic enzymes to Na^+ appears to be similar for both glycophytes and halophytes (Flowers and Colmer 2008), halophyte species must also possess some effective mechanisms to keep cytosolic Na^+ levels under control while accumulating Na^+ in leaf tissues for the purposes of osmotic adjustment. This process involves Na^+ transport control at both the plasma and tonoplast membranes.

The activity of an Na^+/H^+ exchanger is energized by the plasma membrane H^+-ATPase pump. Consistent with this, salt treatment increased both the H^+ transport and hydrolytic activity of plasma membrane H^+-ATPases in salt cress leaves (Vera-Estrella et al. 2005). However, in *Aster tripolium* NaCl-induced stimulation of P-ATPase activity was observed only after 1 day of salt treatment and was followed by the pronounced decline of the P-ATPase activity (Ramani et al. 2006). In *S. bigelovii*, highest plasma membrane H^+-ATPase activity was observed in plants grown at optimal (200 mM NaCl) salt concentration (Ayala et al. 1996), while in another halophyte, *P. maritima*, such treatment caused a decrease in the plasma membrane H^+-ATPase activity in leaves. Also, accumulation of NaCl-induced H^+-ATPase mRNA was observed only in fully expanded leaves but not in expanding leaves, or in stems (Niu et al. 1993), and NaCl effects on H^+-ATPase activity showed a clearly pronounced concentration dependence, even within the same species (Ramani et al. 2006). Taken together, these observations may suggest that up-regulation of the plasma membrane H^+-ATPase in leaf mesophyll cells is not a facultative feature of halophytes.

Vacuolar Na^+ sequestration is a physiological hallmark of halophytes. This sequestration is achieved via tonoplast Na^+/H^+ antiporters (Barkla et al. 1995; Flowers and Colmer 2008). Tonoplast antiporters are constitutive in halophytes (Barkla et al. 1995; Glenn et al. 1999), whereas they must be activated by NaCl in salt-tolerant glycophytes (e.g. Garbarino and DuPont 1988), while in salt-sensitive plants, their expression levels are extremely low and not salt inducible (Zhang and Blumwald 2001). Evidence for both constitutive expression of tonoplast Na^+/H^+ antiporters and stimulation of their activity under saline conditions was reported for at least several halophyte species including *Mesembryanthemum*, *Salicornia* and *Atriplex*. Overexpression of an Na^+/H^+ antiporter from *Suaeda salsa* in *Arabidopsis* conferred salt tolerance and correlated with Na^+ accumulation in the vacuoles of the latter species (Li et al. 2007). The *Km* values for vacuolar Na^+/H^+ transporter range from 2.4 mM in *P. maritima* (Staal et al. 1991) to 14 mM in *A. gmelinii* (Matoh et al. 1989).

Na^+/H^+ antiporter activity could be energized by both vacuolar H^+-ATPases and H^+-PPases. Indeed, numerous studies have reported a rather strong increase in both the expression levels and hydrolytic activity of V-ATPase in a range of halophyte species. In general, salinity leads to an increase in V-ATPase activity in halophytes, while in glycophytes it remains constant or declines (Wang et al. 2001). It also appears that the effects of salinity on V-ATPase activity are NaCl specific (Wang et al. 2001) and not related to the osmotic component of salt stress.

The role of the V-PPase in halophyte salinity tolerance is more controversial. While overexpression of V-PPase from *Suaeda* in *Arabidopsis* conferred salt tolerance (Guo et al. 2006), V-PPase hydrolytic activity and amount of the protein increased in *Salicornia* plants grown at 200 NaCl but were inhibited by 50 mM NaCl (Parks et al. 2002). This led to the conclusion that V-PPase plays a relatively minor role in energizing the tonoplast under the increasing demands imposed by high levels of Na^+/H^+ exchange (Vera-Estrella et al. 2005). This may be partially explained by the fact that the activity of the H^+-PPase is K^+ dependent and, hence, may be down-regulated by K^+ leak from the cytosol under saline conditions (Shabala and Cuin 2008).

Another possible pathway for loading of potentially toxic Na^+ and Cl^- ions into vacuoles is via pinocytosis. Pinocytic invaginations have frequently been found in the shoot cells of several salt-accumulating halophytes such as *Seidlitzia rosmarinus*, *Salicornia europaea*, *Climacoptera lanata*, *Suaeda arcuata* and *S. altissima* (Balnokin et al. 2007). Several types of pinocytotic structures were observed. The most typical, defined as ‘type 1’ structures, consisted of pinocytotic invaginations of the PM and the tonoplast. They were surrounded by a double membrane (plasmalemma and tonoplast) and protruded deeply into the vacuole (Balnokin et al. 2007). The ‘floating’ multivesicular bodies were also observed in the vacuoles, which apparently originated from pinocytic invaginations. The pinocytotic structures contained high concentrations of Na^+ and Cl^-, and it was suggested that pinocytosis is instrumental in both transport of toxic ion from the apoplast and their subsequent sequestration in the vacuoles of the shoots cells in salt-accumulating halophytes (Kurkova and Balnokin 1994). Salt-induced pinocytosis was also observed in glycophyte species such as barley and beans (Nassery and Jones 1976). Efficient ion compartmentalization relies not only on transport across the tonoplast but also on retention of ions within vacuoles. Indeed, given the four- to fivefold concentration gradient between the vacuole and the cytosol, Na^+ may easily leak back, unless some efficient mechanisms are in place to prevent this process. Modified lipid composition of the tonoplast has been mentioned (Glenn et al. 1999), but the specific details remain obscure. In addition, tonoplast cation channels, through which Na^+ might leak back to the cytoplasm, were found to be closed at physiological concentrations of Na^+ in patch-clamp experiments on *S. maritima* vacuoles. However, given the several fold difference in vacuolar Na^+ accumulation between different halophyte families (Flowers and Colmer 2008), this finding cannot be extrapolated to all species. Hence, the need to explicitly characterize the functional properties and gating modes of vacuolar channels mediating Na^+ and K^+ transport across the tonoplast in different halophyte families remains high on the agenda.

4.4 Ion Transport in Guard Cells

In glycophytes, increasing salinity causes a marked decline in stomatal conductance (g_s), reducing both net CO_2 assimilation (P_n) and transpiration rates. It is believed that halophytes exhibit reduced g_s values compared with glycophytes (Lovelock and Ball 2002) but reports on transpirational studies in halophytes are confusing. While most authors agree that halophytes generally show a decline in transpiration rate at salinities above optimal (Debez et al. 2006), some exceptions are reported. For example, no difference in g_s values was reported for leaves of *S. bigelovii* grown at 200 mM (optimal) and 600 mM NaCl levels (Ayala and O'Leary 1995), and no significant effect of salinity on either P_n or g_s was found in *Spartina densiflora* after 90-day treatment with NaCl levels up to 500 mM (Mateos-Naranjo et al. 2010). At suboptimal salinities, some species show a greater rate of transpiration while others show a reduced rate. A perennial halophyte *Salvadora persica* exposed to 200 mM NaCl had slightly higher gas exchange properties relative to non-NaCl controls (Maggio et al. 2000). Similarly, the maximum P_n value in *A. macrostachyum* was observed at 510 mM NaCl. In mangrove species, *Avicennia germinans*, all gas exchange parameters reached their maximum at a salinity level of about 170 mM and P_n rates in *Bruguiera parviflora* were higher in plants grown at 100 mM NaCl compared with non-saline controls (Parida et al. 2004). However, some studies report much higher *gs* and transpirational values for suboptimal salinities compared with NaCl levels considered to be optimal for plant growth (e.g. *S. bigelovii*, *Atriplex centralasiatica*, *Urochondra setulosa*, *Phragmites australis*).

It should be noted that the observed decline in stomatal conductance is not always accompanied by a loss of leaf water content (Redondo-Gómez et al. 2007), and effects of NaCl and water stress on leaf gas exchange in some halophyte species were strikingly different. Moreover, the presence of NaCl alleviated detrimental effects of osmotic stress caused by the presence of PEG in the growth media of *Atriplex halimus*. These and similar results led to the conclusion that the observed NaCl-induced reduction in g_s is likely to be a result of signalling processes rather than a general loss of turgor (Redondo-Gómez et al. 2007).

The observed reduction in stomatal conductance in halophyte leaves is assumed to be important for improvements in water use efficiency (Glenn et al. 1999) and may originate from both morphological and physiological adaptive responses to salinity. A comparison between glycophyte species *A. thaliana* and its halophytic relative *T. halophila* (salt cress) revealed about twofold higher stomata density in *Thellungiella* leaves. At the same time, the overall transpiration rate was higher in *Arabidopsis*, suggesting more efficient CO_2 distribution to mesophyll cells at low stomatal apertures in salt crest. Given the fact that the above paper by Inan et al. (2004) is arguably the only work making such a direct comparison, it remains open as to whether these findings can be generalized to other halophyte species. Also, an increase in leaf thickness (succulency) may affect the number of stomata per unit of leaf volume, and a decline in stomatal densities was reported for *S. maritima*, *Distichlis spicata* and *Kochia prostrata* (Karimi et al. 2005) species under saline conditions.

Potassium efflux in both *Aster* species was mediated by depolarization-activated outward-rectifying KOR channels. These channels were also highly selective for K^+ over Na^+ ($P_{Na}/P_K = 0.05$), and I_{Kout} were twice as large in *A. tripolium*. Remarkably, outward K^+ currents in this species were inhibited by cytosolic Na^+; this inhibition was absent in *A. amelus*. Na^+ entry into the guard cells occurred via weakly rectifying NSCC. The whole-cell inward current through NSCC was reported to be around 17 pA, which could lead to an accumulation of ~500 mM Na in just under 1.5 h. These numbers are consistent with the ones previously reported in the literature (Raschke 1975).

The above results suggest that the fundamental difference in the response of halophyte and non-halophyte *Aster* species arises from the regulation of cation permeability by cytosolic Na^+ but not by the basic properties of ion channels such as their conductances, voltage dependence, selectivity and sensitivity to Ca^{2+}. The specific details of this regulation remain obscure. Indirect effects are most likely, given the fact that the above deactivation of the inward-rectifying K^+ conductance in *A. tripolium* was absent when cytosolic pH and Ca^{2+} were buffered. Also, responses were delayed by 20–30 min pointing to the existence of some signal transduction pathway responding to cytosolic Na concentration, most likely mediated by an increase in cytosolic Ca^{2+}. It should be added that NaCl-induced cytosolic Ca^{2+} concentrations have been previously reported in guard cells of non-halophyte species (Ranf et al. 2008) but still have to be confirmed in halophytes.

4.5 Salt Glands and Bladders

As previously mentioned, some halophytes have evolved specialized epidermal cells, such as glands or bladders, for the elimination or sequestration of excess salt from metabolically active tissues (Flowers and Colmer 2008). Being very large and containing a large central fluid-filled vacuole, such cells serve as a peripheral salinity and water storage organ. Lefevre (2007) demonstrated that over 50% of the accumulated Na^+ may be excreted at the leaf surface via such specialized structures, and mutants lacking EBCs showed much poorer growth and reduced leaf succulence and water content (Agarie et al. 2007).

Very high (typically between 500 and 1000 mM) Na^+ and Cl^- concentrations are reported for EBCs in salt-stressed plants. EBCs may also store organic osmolytes such as ononitol, pinitol and proline; together with inorganic ions, this generates a turgor gradient that can accelerate the growth of new cells. EBCs also contain a substantial (although much lower) amount of K^+. In particular, numerous raphide crystals were found inside EBCs in some species like *M. crystallinum*; these crystals contain no Na^+ and were suggested to serve as a potassium reservoir to maintain the Na^+/K^+ homeostasis in this halophyte. The latter suggestion was made on the basis that K^+ content in raphide crystals of high salinity grown *Mesembryanthemum* plants rapidly disappeared, presumably as a result of K^+ remobilisation to increase cytosolic K^+ concentration of nearby mesophyll cells.

The functional role of EBCs may also differ significantly between species. For example, it appears that, in *Atriplex hortensis*, bladder hairs removed almost all the Na^+ from the young leaf lamina, while they contributed little to the control of Na^+ content in a fully expanded leaf. At the same time, juvenile leaves in *Mesembryanthemum* had fewer and smaller EBCs compared with mature leaves (Agarie et al. 2007). Given the limited number of reports on this topic, this controversy is yet to be resolved.

Another important issue is the actual mechanism of control of Na^+ uptake into gland cells and their ability to discriminate between Na^+ and K^+. Na^+ and Cl^- are the predominantly secreted salts, with plants usually excreting three to four times the amount Na^+ present in leaves on daily basis. At the same time, K^+ excretion is at least an order of magnitude lower (e.g. 12-fold in *D. spicata*). As a result, the Na/K ratio in the hairs of salt-grown halophytes is much higher than that in the mesophyll cells (Flowers et al. 1990). With no electrophysiological work available in the literature, the mechanisms for such selectivity remain a complete mystery.

Only a very limited number of papers have attempted to reveal the identity of specific ion transporters in EBCs; nearly all of them are related to the tonoplast Na^+/H^+ exchanger. It is assumed that an increase in the accumulation of Na^+ in the EBCs is achieved by the high tonoplast Na^+/H^+ antiport and V-type ATPase activity (Barkla et al. 2002). This may explain the mechanisms by which Na^+ is removed from the EBC cytosol but does not address the issue of how K^+ is retained in the EBC cytosol, given the expected dramatic plasma membrane depolarisation following the massive Na^+ intake into the EBCs. Also unclear are the identity and gating modes of specific transporters mediating Na^+ movement across the EBC plasma membrane.

5 Role of Transporters in Salt Stress Adaptation

5.1 Sodium Uptake from Soil

The excess of salts in the soil solution poses a challenge to the plant. Na^+ and other ions taken up by roots are transported to shoots in the transpiration stream, where they accumulate over time. Elevated concentrations of salts are built up in the apoplast and eventually inside the cell, as water evaporates. The accumulation of ions in plant tissues results in progressive damage. These ionic-specific stress effects are superimposed on those caused by hyperosmolarity (Munns and Tester 2008). Whether plants have specific transport systems for low affinity Na^+ uptake from soil remains an open question, and the exact mechanisms responsible for root Na^+ and Cl^- uptake are only partially clear and likely include transporters from several gene families and transport classes.

5.1.1 The Role of Nonselective Cation Channels in Na^+ Uptake

In the last few years, evidence has been presented supporting the existence of weakly voltage-dependent nonselective cation channels (*NSCCs*) that are the main pathway for Na^+ entry into the roots, at high soil NaCl concentrations. Although there are many candidate genes in the databases that could encode these *NSCC* channels, their identity remains elusive. Two families of nonselective cation channels, *CNGCs* (cyclic nucleotide-gated channels) and *GLRs* (glutamate-activated channels), have been suggested to be candidate *NSCC* channels (Fig. 1) (Tester and Davenport 2003). The inhibition of Na^+ influx and *NSCC* currents upon addition of membrane permeable cyclic nucleotide analogues provided correlative evidence for the operation of *CNGCs* in plants; a family of plant channels that in *Arabidopsis* comprise 20 members (Talke et al. 2003). To date, five *AtCNGCs* have been characterized (*AtCNGC 1, 2, 3, 4* and *10*). Electrophysiological studies have suggested that *AtCNGC1* and *AtCNGC4* are equally permeable to K^+ and Na^+ and when expressed in *Xenopus oocytes*, they displayed activation by cyclic nucleotides. *AtCNGC2* appears to be selective for K^+ and to discriminate against Na^+. *AtCNGC10* rescued K^+ transport defective mutants of *E. coli*, yeast and *Arabidopsis akt1-1*, suggesting that *AtCNGC10* mediates the transport of K^+ into the roots. *AtCNGC3* was recently characterized by functional complementation of yeast and by characterization of *Arabidopsis* T-DNA knockout mutants. *AtCNGC3* was primarily expressed in the cortical and epidermal root cells. Growth of the mutant seedlings in toxic NaCl (and KCl) concentrations was improved, suggesting a restricted ion influx in the mutant plants. Ionotropic glutamate receptors (*GLRs*) are proteins that interact with glutamate and form cation channels with a wide range of permeabilities. In *Arabidopsis*, the family of putative *GLRs* comprises 20 members (Demidchik et al. 2004). Glutamate-activated Na^+ and Ca^{2+} voltage-independent currents were characterized

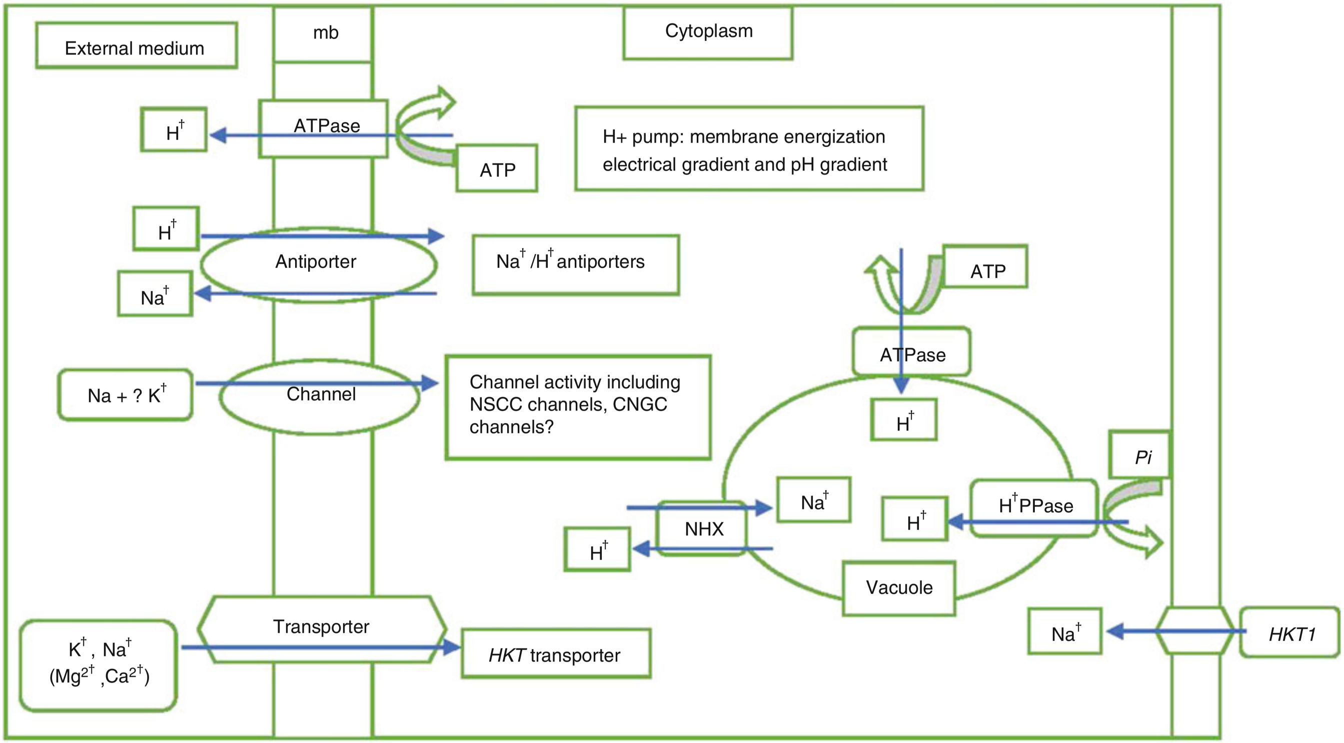

Fig. 1 The main transport systems identified so far at the plasma membrane

in *Arabidopsis* roots. Demidchik et al. (2004) noted that although the effects of environmental factors on apoplastic glutamate remain unclear, the concentrations of glutamate required for half-activation of these channels correlated well with the range of apoplastic glutamate concentrations reported (0.2–0.5 mM), suggesting a role of these channels in Na^+ uptake.

Sodium has a strong inhibitory effect on K^+ uptake by cells, presumably by interfering with transporters in the root plasma membrane such as the Shaker-type K^+ channels (*KAT1* and *AKT1* form the predominant inward K^+ conductance observed in plant plasma membranes). Such channels generally have a high K^+/Na^+ selectivity and were generally regarded not to play a significant role in Na^+. However, a more recent work suggests that the picture is more complex and there may be ecophysiological variations in this respect. Wang et al. (2007) used a pharmacological approach to characterize Na^+ uptake in the halophyte *Suaeda maritima* and concluded that the low-affinity Na^+ uptake pathway in this species resembles an *AKT1* channel. Similarly, Kader and Lindberg (2005) provided evidence that K^+ channels mediate substantial Na^+ influx in a salt-sensitive rice cultivar but not in a tolerant one. In both cases the conclusions were derived from applying channel blockers and inhibitors, which can be notoriously nonspecific, but these findings do suggest that K^+ channels are potential pathways for root Na^+ influx. In addition, the study by Wang et al. (2007) suggests that basic processes such as Na^+ uptake may be considerably different in halophytes and such diversity could be an important contributor to salt tolerance. However, the scarcity in data from halophytes in this respect forms a large hindrance in testing this hypothesis.

5.1.2 Carrier-Type Transporters That Mediate Na^+ Uptake

HKTs (high-affinity potassium transporters) are carrier-type proteins that mediate Na^+ and K^+ transport (Fig. 1). Members of the *HKT* gene family are Na^+-specific transporters (although they were initially described as high-affinity K^+ transporters and hence their name) that mediate either preferential Na^+ transport or Na^+-K^+ symport, partly depending on whether the specific transporter has a highly conserved serine (subfamily 1) or glycine (subfamily 2) residue in the first pore loop of the protein and on the extracellular Na^+-K^+ ratio (Horie et al. 2009). Generally, *HKT* members of subfamily 1 have a relatively higher Na^+-to-K^+ selectivity than subfamily 2 *HKT* transporters. In *Arabidopsis*, loss of function of the only *HKT1;1* gene encoding an Na^+-selective transporter caused the accumulation of Na^+ in leaves but reduced Na^+ concentrations in roots, with little effect on the net uptake of Na^+ by the plant. *AtHKT1;1* is preferentially expressed in the vasculature, where it is thought to regulate the Na^+ distribution between roots and shoots. Two complementary functions for *AtHKT1;1* have been proposed (Pardo 2010). The phloem recirculation model posits that Na^+ is loaded into shoot phloem cells by *AtHKT1;1* and then transferred to roots via the downward stream of phloem, preventing Na^+ overaccumulation in shoots. However, there seems to be little (10% or less) re-translocation of Na^+ from leaves via the phloem relative to the amount imported in the transpiration stream via the xylem. On the other hand, *AtHKT1;1* is generally accepted to mediate the retrieval of Na^+ from the xylem sap, thereby restricting the amount of Na^+ reaching the photosynthetic tissues. These two Na^+ transport processes could be functionally linked to achieve basi-petal translocation of Na^+ because ions that were unloaded by xylem parenchyma cells might be transported into the phloem via symplastic diffusion (Pardo 2010).

5.1.3 Transporters Involved in Cl^- Uptake

Chloride (Cl^-) is a major osmotically active solute in the vacuole involved in both turgor and osmoregulation processes (Marschner 1995), with implications for the proper development of plants (Colmenero-Flores et al. 2007). Despite its importance in plant biology, Cl^- is one of the less studied essential nutrients at the physiological and molecular levels. In contrast to Na^+, Cl^- uptake in most conditions must be energized, but although there is a substantial amount of information regarding K^+ and Na^+ transport in plants, very little is clear about the molecular mechanisms behind the substantial Cl^- influx that results from salinization (Flowers and Colmer 2008). Genes and proteins involved in Cl^- transport have been very poorly studied in plants. The attention has been mainly focused on the voltage-dependent Cl^- channel *CLC* family. Phylogenetic and functional analyses have shown that plant *CLC* genes encode anion channels and active Cl^-/H^+ antiporters localized in endosomal compartments, which are involved in NO^{3-} compartmentalization and pH regulation in the *trans*-Golgi system (Fecht-Bartenbach et al. 2007). Although the transcript abundance of several *CLCs* is affected by salinity, they are unlikely to contribute to root Cl^- uptake: First, plant *CLCs* have only been detected at endomembranes that appear to exclude a role in Cl^- uptake, and second the thermodynamics of Cl^- uptake role out passive-channel-type mechanisms. A second class of potential Cl^- transporters is formed by the cation chloride cotransporters (CCCs) encoding one gene in *Arabidopsis* and two genes in rice. The *A. thaliana* cation-Cl^- cotransporters (*AtCCCs*), expressed in root and shoot tissues, mediate the coordinated symport of K^+, Na^+ and Cl^- and have been postulated to participate in the long-distance transport of Cl^-. Loss of function of *AtCCC* in *Arabidopsis* led to a changed root:shoot Cl^- ratio but also to a large increase in net Cl^- uptake arguing against a role of *AtCCC* in the uptake of this ion. More recently, the *Arabidopsis* slow anion channel associated 1 (At*SLAC1*) gene was shown to encode for the guard cell plasma membrane S-type anion channel involved in stomatal closure. Another member of this family, At*SLAH1*, is expressed in the root vasculature suggesting a potential involvement in the long-distant transport of anions (Negi et al. 2008).

5.2 Transporters Involved in Salt Efflux

It is essential that plants possess adequate efflux systems to remove potentially dangerous ions such as Na^+ from the cytosol. Inevitably, the mechanisms to extrude Na^+ into the apoplast or vacuole have to be energized, which typically occurs via H^+-coupled antiport (Blumwald et al. 2000), whereas those for Cl^- may be (partially) passive. Early studies on tonoplast antiporters showed significant up-regulation of their pumping capacity after plant exposure to salt. In the plasma membrane too, evidence for H^+:Na^+ antiporters was obtained underlining the relevance of such systems to plant salt tolerance. Data dealing with Cl^- efflux are scarce: using compartmental flux analysis, Britto and Kronzucker (2006) showed large Cl^- efflux when plants were exposed to 100 mM NaCl. Just as is the case for Na^+, the majority (up to 90%) of Cl^- that entered the symplast was quickly removed. Although some of the Cl^- efflux could theoretically be mediated by anion channels, no data are available regarding the mechanistic details or regarding the identity of the proteins involved.

5.2.1 Na^+ Efflux Mechanisms at the Plasma Membrane

Comparisons of unidirectional Na^+ fluxes and rates of net accumulation of Na^+ in roots indicate that 70–95% of the Na^+ fluxed into the root symplast is extruded back to the apoplast and that small differences in Na^+ exclusion capacity lead to major changes in the net accumulation of Na^+ (Tester and Davenport 2003). In *Arabidopsis*, the plasma membrane Na^+/H^+ exchanger *SOS1* (Salt Overlay Sensitive) facilitates Na^+ homeostasis by extruding the ion from root epidermal cells at the root–soil interface (Fig. 1). *SOS1* is preferentially expressed in xylem parenchyma cells, and analyses of the Na^+ root/shoot partitioning in roots of *sos1* plants under different salt regimes indicate that *SOS1* participates in the redistribution of Na^+ between the root and shoot, likely working in concert with *AtHKT1;1* at the plasma membrane of xylem parenchyma cells (Pardo 2010). Additional evidence of the involvement of *SOS1* in long-distance Na^+ transport has been produced recently in the halophytic *Arabidopsis* relative *Thellungiella salsuginea* (a.k.a. *T. halophila*) and in tomato (Oh et al. 2009). Lower net Na^+ flux was observed in the xylem sap of tomato plants with suppressed *SOS1* activity (Olías et al. 2009). Down-regulation of *ThSOS1* in *Thellungiella* increased Na^+ accumulation in the root tip and in the stele. Maximal Na^+ accumulation, concomitant with a decrease in the K^+ content, was found in the root xylem parenchyma. These cells presented an Na^+-K^+ ratio more than 12 times higher than equivalent cells in wild-type plants. Reduced or abolished activity of *SOS1* interferes with K^+ nutrition and long-distance transport (Olías et al. 2009). Mutations in rice and *Arabidopsis HKT* Na^+ transporters also reduce K^+ accumulation in shoots during salt exposure. The activity of the *SOS1* exchanger is regulated through protein phosphorylation by the *SOS2–SOS3* kinase complex in *Arabidopsis*. *SOS2/CIPK24* is a serine/threonine protein kinase of the *SnRK3/CIPK* family. *SOS3/CBL4* is a myristoylated membrane-bound Ca^{2+} sensor belonging to the recovering-like family of *SCaBPs/CBLs*. Upon Ca^{2+} binding, *SOS3* binds to and enhances the protein kinase activity of *SOS2*. Besides activating *SOS2*, *SOS3* was shown to recruit *SOS2* to the plasma membrane to facilitate interaction with *SOS1*. *SOS2* also interacts with *SCaBP8/CBL10* to form an alternative protein kinase complex that regulates *SOS1* at the plasma membrane. *SOS2* has recently been shown to phosphorylate *SCaBP8/CBL10* at its C-terminus, thus adding a new layer of regulation to CBL proteins besides Ca^{2+} binding and fatty acyl modifications. This phosphorylation was induced by salt stress, occurred at the membrane, stabilized the *SCaBP8-SOS2* interaction and enhanced plasma membrane Na^+/H^+ exchange activity. Surprisingly, interaction of *SOS2/CIPK24* with

SCaBP8/CBL10 may also result in localization of the kinase complex at the vacuolar membrane where it mediates salt tolerance by regulating the accumulation of Na^+ in shoot tissues by an as yet undefined mechanism that may involve regulation of the Na^+/H^+ exchange at the tonoplast. Regulation of the tonoplast *V-ATPase* by *SOS2* in the absence of CBL proteins has also been reported (Batelli et al. 2007). Presumably, the post-translational modifications of *SCaBP8/CBL10* or the interaction of combinatorial protein kinase complexes with specific targets in different cellular membranes may ultimately define the localization of the protein kinase in vivo.

5.2.2 The Vacuolar Na^+/H^+ Antiporter and the H^+ Pump

The compartmentation of Na^+ ions into vacuoles provides an efficient mechanism to avert the toxic effects of Na^+ in the cytosol. The transport of Na^+ into the vacuoles is mediated by cation/H^+ antiporters that are driven by the electrochemical gradient of protons generated by the vacuolar H^+-translocating enzymes, the H^+ ATPases and the H^+ pyrophosphatase (H^+-PPase) (Fig. 1). Although the activity of these cation/H^+ antiporters was demonstrated more than 20 years ago, their molecular characterization was only possible after the *Arabidopsis* genome-sequencing project. Na^+ compartmentation in the vacuole occurs in all tissues and is an important mechanism for osmotic adjustment and Na^+ detoxification in the cytosol. There are eight *NHX* gene family members in *Arabidopsis*, and the functions of *AtNHX1, 4, 7* and *8* have been studied. *AtNHX7* is also known as *AtSOS1*, and *AtNHX8* has been shown to be an Li^+/H^+ antiporter, although the biological relevance of Li^+ transport remains obscure. *AtNHX4* is localized to the vacuole and might function in concert with *AtNHX1*. Several reports indicate that constitutive overexpression of the vacuolar transporters increases the salt tolerance of a variety or species. Constitutive overexpression of the *Arabidopsis* vacuolar Na^+/H^+ antiporter, *AtNHX1*, appears to increase salinity tolerance significantly in yeast, *Arabidopsis*, tomato, *Brassica napus* and cotton. Similarly, constitutive overexpression of various cereal homologues has been reported to improve the salinity tolerance of *Arabidopsis*, rice and barley. The overexpression of *NHX1* appears to increase the capacity of the plant to regulate cytoplasmic and vacuolar pH (Viehweger et al. 2002). The cation selectivity is regulated by a luminal C-terminus. The overexpression of *NHX1* in *Arabidopsis* led to a small increase in shoot Na^+ accumulation, possibly allowing the cells to maintain a favourable osmotic balance, yet maintaining low cytoplasmic Na^+ levels due to sequestration of the Na^+ within the vacuole. The *nhx1* mutant had significantly lower Na^+/H^+ and K^+/H^+ exchange capabilities in isolated vacuoles, fewer large epidermal cells and less overall leaf area, indicating that *NHX1* also plays a developmental role. Overexpression and knockout of the *NHX1* gene in *Arabidopsis* have been shown to significantly and differentially alter the expression of a large number of genes involved in the response to salt stress, indicating that *Arabidopsis* can respond to a change in one Na^+ transporter by regulating other genes (Sottosanto et al. 2007). Other members of the *NHX* family are also capable of moving Na^+. Yokoi et al. (2002) reported that *AtNHX2* and *AtNHX5* could be important salt-tolerant determinants and observed that *AtNHX2* has a major function in vacuolar Na^+ sequestration. H^+ pumps in the plasma membrane and tonoplast energize solute transport necessary to compartmentalize cytotoxic ions away from the cytoplasm and to facilitate the function of ions as signal determinants. That is these pumps provide the driving force (H^+ electrochemical potential) for secondary active transport and function to establish membrane potential gradients that facilitate electrophoretic ion flux. The plasma membrane-localized H^+ pump is a P-type ATPase and is primarily responsible for the large pH and membrane potential gradient across this membrane. A vacuolar-type H^+-ATPase and H^+-PPase generate the ΔpH and membrane potential across the tonoplast. The activity of these H^+ pumps is increased by salt treatment, and induced gene expression may account for some of the up-regulation. The H^+-PPases are considered to form a multigene family. Two cDNA clones (*OVP1* and *OVP2*) encoding vacuolar H^+-PPases isolated from rice were reported. Indeed, there are two genes in *Arabidopsis* annotated as inorganic pyrophosphatase H^+-PPase (*AVP1, AVP3*) and a third loci encoding a pyrophosphatase like (*AVP2* = *AVPL1*), more than five isoforms in rice and at least three isoforms in barley.

5.2.3 Role of Cl^- Channels in Vacuolar Cl^- Compartmentation

In addition to Na^+, Cl^- compartmentation is also important for salt tolerance, as elevated levels of Cl^- in the cytosol may be harmful, particularly in the case of citrus crops. Since the vacuole is moderately positive with reference to the cytoplasm, part of the vacuolar Cl^- sequestration could proceed through ion channels, and several voltage-gated anion channels of the *CLC* family have been detected in the tonoplast of various species. In *Arabidopsis*, *CLCa* was recently shown to function primarily as a H^+-coupled antiporter to drive vacuolar nitrate accumulation, whereas *CLCc* may also be involved in NO^{3-} homeostasis rather than vacuolar Cl^- sequestration. However, *CLC* transcription has been found to respond to salinity: In rice, *OsCLCa* was significantly up-regulated in salt-sensitive cultivars in response to salinity stress and *OsCLCc*, which is expressed in both leaves and roots, showed transcript reduction in the chloride accumulating salt-sensitive *IR29* while transient induction occurred in the chloride excluding variety Pokkali. Diédhiou and Golldack (2006) showed a coordinated regulation of anion and cation homeostasis in salt-treated rice and suggested a function for *OsCLCc* in osmotic adjustment at high salinity. Nakamura et al. (2006) showed that the same *CLC* channels partially complimented the yeast *gef1* mutant, which lacks the yeast *CLC* channel. In conjunction, these findings suggest that *CLC*-type anion channels are important in mediating Cl^- sequestration in vacuole.

6 Oxidative Signalling and Damage Repair in Halophytes

When plants are exposed to salt stress condition, they induce oxidative damage (Khalid et al. 2020). It has been repeatedly stated that the capacity to limit oxidative damage is important for halophytes' salt tolerance (Parida and Jha 2010). It is also believed that halophytes possess higher oxidative stress tolerance than glycophytes. However, given the fact that these conclusions are derived from comparisons of only a rather limited number of species (e.g. *T. halophila* and *A. thaliana*), these reports need to be treated with some caution. Similar to glycophytes, halophytes are capable of up-regulating ROS scavenging systems under high-salinity conditions. As a rule of thumb, activity of all major antioxidant systems increases noticeably under salt stress.

A rather substantial difference in the kinetics of antioxidant activity appears to exist between roots and leaves, and salinity effects on the activity of major antioxidant enzymes display a clearly pronounced dose and time dependence (Amor et al. 2007). This complexity most likely reflects the above dual role of ROS in plant–environment interaction.

Of specific importance is an increase in the antioxidant activity in chloroplasts. Chloroplast SOD activity is markedly enhanced with the increase of NaCl or with time, especially in its thylakoid-bound fraction. This is believed to be essential in protecting leaf photosynthetic machinery and enabling its optimal functioning under saline conditions. Interestingly, NaCl salinity and osmotic stress lead to a differential regulation of distinct SOD isoenzymes. Such differential regulation may play a major role in plant stress tolerance.

There also appear to be a few rather specific features characteristic to halophytes only. The facultative halophyte *M. crystallinum* (ice plant) shifts its mode of carbon assimilation from the C-3 pathway to crassulacean acid metabolism (CAM) in response to many factors that lead to the generation of ROS at the cellular level.

This is believed to be important for effectively protecting *M. crystallinum* against oxidative damage (Hurst et al. 2004) and is attributed to the reduced H_2O_2 levels in CAM plants under saline conditions compared with C3 photosynthesis. In this context, much lower H_2O_2 levels were detected in *M. crystallinum* WT compared with a mutant unable to switch to CAM metabolism. Extensive studies on another model halophyte species, *T. halophila* (salt crest), has led to the suggestion that increased proline accumulation may be one of the key features conferring its salinity tolerance (Kant et al. 2006). However, proline-accumulating species were found among both halophytes (e.g. *Artemisia lerchiana* and *T. halophila*) and glycophytes (e.g. *P. major* and *Mycelis muralis*), suggesting that proline accumulation cannot be named as a specific hallmark of halophytes. Interestingly, a negative correlation was found between proline content and SOD activity in these species. Thus, it appears that proline's major role is not in conventional water retention (as assumed for most osmolytes) but in antioxidant defence. These studies also led to the conclusion that high-SOD activity is not an obligatory trait of salt tolerance. Moreover, plants with high-peroxidase activity and active proline accumulation could acclimate to salt stress independently of SOD activity. Another possible class of ROS scavengers in halophytes are polyamines (Kuznetsov et al. 2007; Shevyakova et al. 2006; Stetsenko et al. 2009).

7 Conclusion

Halophytes are highly valuable model species in physiological studies on plant salinity tolerance, and a potential source of salinity tolerance genes in crops. It appears that halophytism is an integration of a large number of physiological adaptive mechanisms rather than a distinct morphological or anatomical feature. Remarkably, very little is known about molecular and cellular aspects of halophytes' remarkable tolerance to salinity. It appears that halophyte roots operate mainly in the 'passage mode' and that xylem Na^+ loading is crucial for shoot osmotic adjustment and maintenance of sustained growth under high-salinity conditions. These findings question the existing strategies to improve salinity tolerance in cereals and other crops by maximizing plants' ability to retrieve Na^+ from the xylem. Vacuolar sequestration of Na^+ in roots appears not to be a key determinant of salinity tolerance in halophytes (Vera-Estrella et al. 2005), while such sequestration in leaf vacuoles is absolutely essential to confer the latter trait. Both the process of xylem Na^+ loading and its sequestration in leaf cell vacuoles are mediated by active transport systems; the expression of these transporters appears to show high tissue specificity.

While only a few direct electrophysiological studies were undertaken to investigate the identity and functional role of key transport systems mediating Na^+ and K^+ homeostasis in halophyte cells, all of them point to the importance of regulation of cation permeability, while basic properties of ion channels such as their conductance, voltage dependence and selectivity appear not to be very different between halophytes and glycophytes. Remarkably, this seems to be the case for such diverse systems as root tonoplast channels and plasma membrane channels in stomata guard cells. The specific details of this regulation remain an enigma and call for more active research in this direction. In this context, the regulatory role of compatible solutes and antioxidant molecules should be reexamined, and a causal relationship between oxidative and salinity stress tolerance in halophytes should be studied in a greater detail. Also essentially unexplored remains an issue of mechanisms of Na^+ loading and regulation of Na^+/K^+ selectivity in epidermal bladder and gland cells. Understanding this and the above issues can be instrumental in developing a better understanding of what appears to be the complex and highly orchestrated regulation of ion transport and sequestration in halophytes, opening up the prospect of improving salinity tolerance in crops.

References

Agarie S, Shimoda T, Shimizu Y, Baumann K, Sunagawa H, Kondo A, Ueno O, Nakahara T, Nose A, Cushman JC (2007) Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. J Exp Bot 58(8):1957–1967

Albert R (1975) Salt regulation in halophytes. Oecologia 21(1):57–71

Alemán F, Nieves-Cordones M, Martínez V, Rubio F (2009) Differential regulation of the HAK5 genes encoding the high-affinity K+ transporters of Thellungiella halophila and Arabidopsis thaliana. Environ Exp Bot 65(2):263–269

Amor NB, Jiménez A, Megdiche W, Lundqvist M, Sevilla F, Abdelly C (2007) Kinetics of the antioxidant response to salinity in the halophyte *Cakile maritima*. J Integr Plant Biol 49(7):982–992

Anderson WP, Willcocks DA, Wright BJ (1977) Electrophysiological measurements on the root of *Atriplex hastata*. J Exp Bot 28(4):894–901

Ayala F, O'Leary JW (1995) Growth and physiology of *Salicornia bigelovii* Torr. at suboptimal salinity. Int J Plant Sci 156(2):197–205

Ayala F, O'Leary JW, Schumaker KS (1996) Increased vacuolar and plasma membrane H+-ATPase activities in *Salicornia bigelovii* Torr. in response to NaCl. J Exp Bot 47(1):25–32

Balnokin YV, Myasoedov NA, Shamsutdinov ZS, Shamsutdinov NZ (2005) Significance of Na+ and K+ for sustained hydration of organ tissues in ecologically distinct halophytes of the family Chenopodiaceae. Russ J Plant Physiol 52(6):779–787

Balnokin YV, Kurkova EB, Khalilova LA, Myasoedov NA, Yusufov AG (2007) Pinocytosis in the root cells of a salt-accumulating halophyte Suaeda altissima and its possible involvement in chloride transport. Russ J Plant Physiol 54(6):797–805

Barkla BJ, Zingarelli L, Blumwald E, Smith JAC (1995) Tonoplast Na+/H+ antiport activity and its energization by the vacuolar H+-ATPase in the halophytic plant *Mesembryanthemum crystallinum* L. Plant Physiol 109(2):549–556

Barkla BJ, Vera-Estrella R, Camacho-Emiterio J, Pantoja O (2002) Na+/H+ exchange in the halophyte Mesembryanthemum crystallinum is associated with cellular sites of Na+ storage. Funct Plant Biol 29(9):1017–1024

Batelli G, Verslues PE, Agius F, Qiu Q, Fujii H, Pan S, Schumaker KS, Grillo S, Zhu JK (2007) SOS2 promotes salt tolerance in part by interacting with the vacuolar H+-ATPase and upregulating its transport activity. Mol Cell Biol 27(22):7781–7790

Berthomieu P, Conéjéro G, Nublat A, Brackenbury WJ, Lambert C, Savio C, Uozumi N, Oiki S, Yamada K, Cellier F, Gosti F (2003) Functional analysis of AtHKT1 in Arabidopsis shows that Na+ recirculation by the phloem is crucial for salt tolerance. EMBO J 22(9):2004–2014

Blumwald E, Aharon GS, Apse MP (2000) Sodium transport in plant cells. BBA-Biomembranes 1465(2):140–151

Bohnert HJ, Shen BO (1998) Transformation and compatible solutes. Sci Hortic 78(1–4):237–260

Britto DT, Kronzucker HJ (2006) Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends Plant Sci 11(11):529–534

Carpaneto A, Koepsell H, Bamberg E, Hedrich R, Geiger D (2010) Sucrose-and H+-dependent charge movements associated with the gating of sucrose transporter ZmSUT1. PLoS One 5(9)

Chakrapani S, Auerbach A (2005) A speed limit for conformational change of an allosteric membrane protein. Proc Natl Acad Sci USA 102(1):87–92

Clipson NJ, Flowers TJ (1987) Salt tolerance in the halophyte *Suaeda maritima* (L.) Dum. The effect of salinity on the concentration of sodium in the xylem. New Phytol 105(3):359–366

Colmenero-Flores JM, Mart'ınez G, Gamba G (2007) Identification and functional characterization of cation-chloride cotransporters in plants. Plant J 50(2):278–292

Cuin TA, Shabala S (2007) Amino acids regulate salinity-induced potassium efflux in barley root epidermis. Planta 225(3):753–761

Davenport R (2002) Glutamate receptors in plants. Ann Bot 90(5):549–557

Debez A, Saadaoui D, Ramani B, Ouerghi Z, Koyro HW, Huchzermeyer B, Abdelly C (2006) Leaf H+-ATPase activity and photosynthetic capacity of Cakile maritima under increasing salinity. Environ Exp Bot 57(3):285–295

Demidchik V, Essah PA, Tester M (2004) Glutamate activates cation currents in the plasma membrane of Arabidopsis root cells. Planta 219(1):167–175

Diédhiou CJ, Golldack D (2006) Salt-dependent regulation of chloride channel transcripts in rice. Plant Sci 170(4):793–800

Fecht-Bartenbach JV, Bogner M, Krebs M, Stierhof YD, Schumacher K, Ludewig U (2007) Function of the anion transporter AtCLC-d in the trans-Golgi network. Plant J 50(3):466–474

Feng ZT, Deng YQ, Zhang SC, Liang X, Yuan F, Hao JL, Zhang JC, Sun SF, Wang BS (2015) K+ accumulation in the cytoplasm and nucleus of the salt gland cells of Limonium bicolor accompanies increased rates of salt secretion under NaCl treatment using NanoSIMS. Plant Sci 238:286–296

Flowers TJ (1985) Physiology of halophytes. In: Biosalinity in action: bioproduction with saline water. Springer, Dordrecht, pp 41–56

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol:945–963

Flowers TJ, Flowers SA, Hajibagheri MA, Yeo AR (1990) Salt tolerance in the halophytic wild rice, *Porteresia coarctata* Tateoka. New Phytol 114(4):675–684

Gadsby DC (2009) Ion channels versus ion pumps: the principal difference, in principle. Nat Rev Mol Cell Biol 10(5):344–352

Galdiero S, Falanga A, Cantisani M, Tarallo R, Elena Della Pepa M, D'Oriano V, Galdiero M (2012) Microbe-host interactions: structure and role of gram-negative bacterial porins. Curr Protein Pept Sci 13(8):843–854

Garbarino J, DuPont FM (1988) NaCl induces a Na+/H+ antiport in tonoplast vesicles from barley roots. Plant Physiol 86(1):231–236

Glenn EP, Brown JJ, Blumwald E (1999) Salt tolerance and crop potential of halophytes. Crit Rev Plant Sci 18(2):227–255

Golldack D, Dietz KJ (2001) Salt-induced expression of the vacuolar H+-ATPase in the common ice plant is developmentally controlled and tissue specific. Plant Physiol 125(4):1643–1654

Guo S, Yin H, Zhang X, Zhao F, Li P, Chen S, Zhao Y, Zhang H (2006) Molecular cloning and characterization of a vacuolar H+-pyrophos-phatase gene, SsVP, from the halophyte Suaeda salsa and its overexpression increases salt and drought tolerance of Arabidopsis. Plant Mol Biol 60(1):41–50

Guo J, Li Y, Han G, Song J, Wang B (2018) NaCl markedly improved the reproductive capacity of the euhalophyte *Suaeda salsa*. Funct Plant Biol 45(3):350–361

Hajibagheri MA, Hall JL, Flowers TJ (1984) Stereological analysis of leaf cells of the halophyte *Suaeda maritima* (L.) Dum. J Exp Bot 35(10):1547–1557

Hajibagheri MA, Yeo AR, Flowers TJ (1985) Salt tolerance in *Suaeda maritima* (L.) Dum. Fine structure and ion concentrations in the apical region of roots. New Phytol 99(3):331–343

Hamada A, Shono M, Xia T, Ohta M, Hayashi Y, Tanaka A, Hayakawa T (2001) Isolation and characterization of a Na+/H+ antiporter gene from the halophyte *Atriplex gmelini*. Plant Mol Biol 46(1):35–42

Hassine AB, Ghanem ME, Bouzid S, Lutts S (2008) An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. J Exp Bot 59(6):1315–1326

Heginbotham L, Abramson T, MacKinnon R (1992) A functional connection between the pores of distantly related ion channels as revealed by mutant K+ channels. Sci 258(5085):1152–1155

Horie T, Hauser F, Schroeder JI (2009) HKT transporter-mediated salinity resistance mechanisms in Arabidopsis and monocot crop plants. Trends Plant Sci 14(12):660–668

Hurst AC, Grams TE, Ratajczak R (2004) Effects of salinity, high irradiance, ozone, and ethylene on mode of photosynthesis, oxidative stress and oxidative damage in the C3/CAM intermediate plant *Mesembryanthemum crystallinum* L. Plant Cell Environ 27(2):187–197

Husain S, von Caemmerer S, Munns R (2004) Control of salt transport from roots to shoots of wheat in saline soil. Funct Plant Biol 31(11):1115–1126

Hussain S, Khalid MF, Hussain M, Ali MA, Nawaz A, Zakir I, Fatima Z, Ahmad S (2018) Role of micronutrients in salt stress tolerance to plants. In: Plant nutrients and abiotic stress tolerance. Springer, Singapore, pp 363–376

Inan G, Zhang Q, Li P, Wang Z, Cao Z, Zhang H, Zhang C, Quist TM, Goodwin SM, Zhu J, Shi H (2004) Salt cress. A halophyte and cryophyte Arabidopsis relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. Plant Physiol 135(3):1718–1737

Jefferies RL (1973) The ionic relations of seedlings of the halophyte *Triglochin maritima* L. ion transport in plants, pp 297–321

Jennings BD (1976) The effects of sodium chloride on higher plants. Biol Rev 51(4):453–486

Kader MA, Lindberg S (2005) Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, *Oryza sativa* L. determined by the fluorescent dye *SBFI*. J Exp Bot 56 (422):3149–3158

Kant S, Kant P, Raveh E, Barak S (2006) Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na+ uptake in T. halophila. Plant Cell Environ 29(7):1220–1234

Karimi G, Ghorbanli M, Heidari H, Nejad RK, Assareh MH (2005) The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrata*. Biol Plantarum 49(2):301–304

Khalid MF, Hussain S, Ahmad S, Ejaz S, Zakir I, Ali MA, Ahmed N, Anjum MA (2019) Impacts of abiotic stresses on growth and development of plants. In: Plant tolerance to environmental stress. CRC Press, pp 1–8

Khalid MF, Hussain S, Anjum MA, Ahmad S, Ali MA, Ejaz S, Morillon R (2020) Better salinity tolerance in tetraploid vs diploid volkamer lemon seedlings is associated with robust antioxidant and osmotic adjustment mechanisms. J Plant Physiol 244:153071

Kurkova EB, Balnokin YV (1994) Pinocytosis and its possible role in ion-transport in the salt-accumulating organs of halophytes. Russ J Plant Physiol 41(4):507–511

Kuznetsov V, Shorina M, Aronova E, Stetsenko L, Rakitin V, Shevyakova N (2007) NaCl-and ethylene-dependent cadaverine accumulation and its possible protective role in the adaptation of the common ice plant to salt stress. Plant Sci 172(2):363–370

Lefevre I (2007) Investigation of three Mediterranean plant species suspected to accumulate and tolerate high cadmium and zinc levels. Morphological, physiological and biochemical characterization under controlled conditions. Doctoral dissertation, PhD thesis, Université catholique de Louvain

Li J, Jiang G, Huang P, Ma J, Zhang F (2007) Overexpression of the Na+/H+ antiporter gene from Suaeda salsa confers cold and salt tolerance to transgenic Arabidopsis thaliana. Plant Cell Tissue Organ Cult 90(1):41–48

Lin J, Li JP, Yuan F, Yang Z, Wang BS, Chen M (2018) Transcriptome profiling of genes involved in photosynthesis in *Elaeagnus angustifolia* L. under salt stress. Photosynthetica 56 (4):998–1009

Longpré JP, Lapointe JY (2011) Determination of the Na+/glucose cotransporter (SGLT1) turnover rate using the ion-trap technique. Biophys J 100(1):52–59

Lovelock CE, Ball MC (2002) Influence of salinity on photosynthesis of halophytes. In: Salinity: environment-plants-molecules. Springer, Dordrecht, pp 315–339

Maathuis FJ, Sanders D (2001) Sodium uptake in Arabidopsis roots is regulated by cyclic nucleotides. Plant Physiol 127(4):1617–1625

Maathuis FJ, Flowers TJ, Yeo AR (1992) Sodium chloride compartmentation in leaf vacuoles of the halophyte *Suaeda maritima* (L.) Dum. and its relation to tonoplast permeability. J Exp Bot 43 (9):1219–1223

MacKinnon R (2004) Potassium channels and the atomic basis of selective ion conduction (Nobel Lecture). Angew Chem Int Ed Engl 43(33):4265–4277

Maffeo C, Bhattacharya S, Yoo J, Wells D, Aksimentiev A (2012) Modeling and simulation of ion channels. Chem Rev 112:6250–6284

Maggio A, Reddy MP, Joly RJ (2000) Leaf gas exchange and solute accumulation in the halophyte *Salvadora persica* grown at moderate salinity. Environ Exp Bot 44(1):31–38

Mähler J, Persson I (2012) A study of the hydration of the alkali metal ions in aqueous solution. Inorg Chem 51(1):425–438

Marcum KB, Murdoch CL (1990) Salt glands in the Zoysieae. Ann Bot 66(1):1–7

Marcum KB, Murdoch CL (1992) Salt tolerance of the coastal salt marsh grass, *Sporobolus virginicus* (L.) Kunth. New Phytol 120(2):281–288

Marschner H (1995) Mineral nutrition in higher plants, 2nd edn. Academic Press, London

Mateos-Naranjo E, Redondo-Gómez S, Álvarez R, Cambrollé J, Gandullo J, Figueroa ME (2010) Synergic effect of salinity and CO_2 enrichment on growth and photosynthetic responses of the invasive cordgrass Spartina densiflora. J Exp Bot 61:1643–1654

Matoh T, Ishikawa T, Takahashi E (1989) Collapse of ATP-induced pH gradient by sodium ions in microsomal membrane vesicles prepared from Atriplex gmelini leaves: possibility of Na+/H+ antiport. Plant Physiol 89(1):180–183

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Munns R, Hare RA, James RA, Rebetzke GJ (2000) Genetic variation for improving the salt tolerance of durum wheat. Aust J Agric Res 51(1):69–74

Munns R, James RA, Läuchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot 57(5):1025–1043

Naidoo G, Rughunanan R (1990) Salt tolerance in the succulent, coastal halophyte, Sarcocornia natalensis. J Exp Bot 41(4):497–502

Nakamura A, Fukuda A, Sakai S, Tanaka Y (2006) Molecular cloning, functional expression and subcellular localization of two putative vacuolar voltage-gated chloride channels in rice (*Oryza sativa* L.). Plant Cell Physiol 47(1):32–42

Nassery H, Jones RL (1976) Salt-induced pinocytosis in barley and bean. J Exp Bot 27(2):358–367

Neales TF, Sharkey PJ (1981) Effect of salinity on growth and on mineral and organic constituents of the halophyte *Disphyma australe* (Soland.) JM Black. Funct Plant Biol 8(2):165–179

Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, Uchimiya H, Hashimoto M, Iba K (2008) CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature 452(7186):483–486

Niu X, Narasimhan ML, Salzman RA, Bressan RA, Hasegawa PM (1993) NaCl regulation of plasma membrane H+-ATPase gene expression in a glycophyte and a halophyte. Plant Physiol 103(3):713–718

Oh DH, Gong Q, Ulanov A, Zhang Q, Li Y, Ma W, Yun DJ, Bressan RA, Bohnert HJ (2007) Sodium stress in the halophyte *Thellungiella halophila* and transcriptional changes in a thsos1-RNA interference line. J Integr Plant Biol 49(10):1484–1496

Oh DH, Leidi E, Zhang Q, Hwang SM, Li Y, Quintero FJ, Jiang X, D'Urzo MP, Lee SY, Zhao Y, Bahk JD (2009) Loss of halophytism by interference with *SOS1* expression. Plant Physiol 151 (1):210–222

Olías R, Eljakaoui Z, Li J, De Morales PA, Marín-Manzano MC, Pardo JM, Belver A (2009) The plasma membrane Na+/H+ antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na+ between plant organs. Plant Cell Environ 32(7):904–916

Ownbey RS, Mahall BE (1983) Salinity and root conductivity: differential responses of a coastal succulent halophyte, Salicornia virginica, and a weedy glycophyte, Raphanus sativus. Physiol Plant 57(2):189–195

Pardo JM (2010) Biotechnology of water and salinity stress tolerance. Curr Opin Biotechnol 21 (2):185–196

Parida AK, Jha B (2010) Antioxidative defense potential to salinity in the euhalophyte Salicornia brachiata. J Plant Growth Regul 29(2):137–148

Parida AK, Das AB, Mittra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees 18(2):167–174

Parks GE, Dietrich MA, Schumaker KS (2002) Increased vacuolar Na+/H+ exchange activity in *Salicornia bigelovii* Torr. in response to NaCl. J Exp Bot 53(371):1055–1065

Qiu N, Lu C (2003) Enhanced tolerance of photosynthesis against high temperature damage in salt-adapted halophyte *Atriplex centralasiatica* plants. Plant Cell Environ 26(7):1137–1145

Ramani B, Reeck T, Debez A, Stelzer R, Huchzermeyer B, Schmidt A, Papenbrock J (2006) *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survive in saline habitats. Plant Physiol Biochem 44(5–6):395–408

Ramos J, López MJ, Benlloch M (2004) Effect of NaCl and KCl salts on the growth and solute accumulation of the halophyte *Atriplex nummularia*. Plant Soil 259(1–2):163–168

Ranf S, Wünnenberg P, Lee J, Becker D, Dunkel M, Hedrich R, Scheel D, Dietrich P (2008) Loss of the vacuolar cation channel, AtTPC1, does not impair Ca2+ signals induced by abiotic and biotic stresses. Plant J 53(2):287–299

Raschke K (1975) Stomatal action. Annu Rev Plant Physiol 26(1):309–340

Redondo-Gómez S, Mateos-Naranjo E, Davy AJ, Fernández-Muñoz F, Castellanos EM, Luque T, Figueroa ME (2007) Growth and photosynthetic responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. Ann Bot 100(3):555–563

Rozema J, Gude H, Bijl F, Wesselman H (1981) Sodium concentration in xylem sap in relation to ion exclusion, accumulation and secretion in halophytes. Acta Bot Neerl 30(4):309–311

Scholander PF, Hammel HT, Hemmingsen E, Garey W (1962) Salt balance in mangroves. Plant Physiol 37(6):722

Serrano R (1988) Structure and function of proton translocating ATPase in plasma membranes of plants and fungi. Biochim Biophys Acta 947(1):1–28

Shabala S, Cuin TA (2008) Potassium transport and plant salt tolerance. Physiol Plant 133 (4):651–669

Shabala S, Bose J, Hedrich R (2014) Salt bladders: do they matter? Trends Plant Sci 19 (11):687–691

Shevyakova NI, Rakitin VY, Stetsenko LA, Aronova EE, Kuznetsov VV (2006) Oxidative stress and fluctuations of free and conjugated polyamines in the halophyte *Mesembryanthemum crystallinum* L. under NaCl salinity. Plant Growth Regul 50(1):69–78

Shi H, Quintero FJ, Pardo JM, Zhu JK (2002) The putative plasma membrane Na+/H+ antiporter SOS1 controls long-distance Na+ transport in plants. Plant Cell 14(2):465–477

Skou JC (1998) The identification of the sodium pump. Biosci Rep 18(4):155–169

Smajgl A, Toan TQ, Nhan DK, Ward J, Trung NH, Tri LQ, Tri VP, Vu PT (2015) Responding to rising sea levels in the Mekong Delta. Nat Clim Chang 5(2):167–174

Song J, Wang B (2015) Using euhalophytes to understand salt tolerance and to develop saline agriculture: *Suaeda salsa* as a promising model. Ann Bot 115(3):541–553

Sottosanto JB, Saranga Y, Blumwald E (2007) Impact of AtNHX1, a vacuolar Na+/H+ antiporter, upon gene expression during short-and long-term salt stress in Arabidopsis thaliana. BMC Plant Biol 7(1):18

Staal M, Maathuis FJ, Elzenga JT, Overbeek JH, Prins HB (1991) Na+/H+ antiport activity in tonoplast vesicles from roots of the salt-tolerant *Plantago maritima* and the salt-sensitive *Plantago media*. Physiol Plant 82(2):179–184

Stetsenko LA, Rakitin VY, Shevyakova NI, Kuznetsov VV (2009) Organ-specific changes in the content of free and conjugated polyamines in *Mesembryanthemum crystallinum* plants under salinity. Russ J Plant Physiol 56(6):808–813

Storey R (1995) Salt tolerance, ion relations and the effect of root medium on the response of citrus to salinity. Funct Plant Biol 22(1):101–114

Storey R, Jones RGW (1979) Responses of *Atriplex spongiosa* and *Suaeda monoica* to salinity. Plant Physiol 63(1):156–162

Storey R, Pitman MG, Stelzer R (1983) X-ray micro-analysis of cells and cell compartments of Atripiex spongiosa: II. ROOTS. J Exp Bot 34(9):1196–1206

Su H, Golldack D, Zhao C (2002) The expression of HAK-type K+ transporters is regulated in response to salinity stress in common ice plant. Plant Physiol 129(4):1482–1493

Talke IN, Blaudez D, Maathuis FJ, Sanders D (2003) CNGCs: prime targets of plant cyclic nucleotide signalling? Trends Plant Sci 8(6):286–293

Tester M, Davenport R (2003) Na+ tolerance and Na+ transport in higher plants. Ann Bot 91 (5):503–527

Tyerman SD, Skerrett IM (1998) Root ion channels and salinity. Sci Hortic 78(1–4):175–235

Ueda A, Kanechi M, Uno Y, Inagaki N (2003) Photosynthetic limitations of a halophyte sea aster (*Aster tripolium* L) under water stress and NaCl stress. J Plant Res 116(1):63–68

Vera-Estrella R, Barkla BJ, García-Ramírez L, Pantoja O (2005) Salt stress in *Thellungiella halophila* activates Na+ transport mechanisms required for salinity tolerance. Plant Physiol 139(3):1507–1517

Viehweger K, Dordschbal B, Roos W (2002) Elicitor-activated phospholipase A2 generates lysophosphatidylcholines that mobilize the vacuolar H+ pool for pH signaling via the activation of Na+−dependent proton fluxes. Plant Cell 14(7):1509–1525

Volkov V, Amtmann A (2006) *Thellungiella halophila*, a salt-tolerant relative of Arabidopsis thaliana, has specific root ion-channel features supporting K+/Na+ homeostasis under salinity stress. Plant J 48(3):342–353

Volkov V, Wang B, Dominy PJ, Fricke W, Amtmann A (2003) *Thellungiella halophila*, a salt-tolerant relative of Arabidopsis thaliana, possesses effective mechanisms to discriminate between potassium and sodium. Plant Cell Environ 27(1):1–14

Wang B, Lüttge U, Ratajczak R (2001) Effects of salt treatment and osmotic stress on V-ATPase and V-PPase in leaves of the halophyte *Suaeda salsa*. J Exp Bot 52(365):2355–2365

Wang SM, Zhang JL, Flowers TJ (2007) Low-affinity Na+ uptake in the halophyte *Suaeda maritima*. Plant Physiol 145(2):559–571

Wegner LH, De Boer AH (1997) Two inward K+ channels in the xylem parenchyma cells of barley roots are regulated by G-protein modulators through a membrane-delimited pathway. Planta 203 (4):506–516

Xu YG, Liu R, Sui N, Shi W, Wang L, Tian C (2016) Changes in endogenous hormones and seed-coat phenolics during seed storage of two *Suaeda salsa* populations. Aust J Bot 64(4):325–332

Ye CJ, Zhao KF (2003) Osmotically active compounds and their localization in the marine halophyte eelgrass. Biol Plant 46(1):137–140

Yokoi S, Quintero FJ, Cubero B, Ruiz MT, Bressan RA, Hasegawa PM, Pardo JM (2002) Differential expression and function of *Arabidopsis thaliana* NHX Na+/H+ antiporters in the salt stress response. Plant J 30(5):529–539

Yuan F, Lyu MJ, Leng BY, Zhu XG, Wang BS (2016) The transcriptome of NaCl-treated Limonium bicolor leaves reveals the genes controlling salt secretion of salt gland. Plant Mol Biol 91(3):241–256

Zhang HX, Blumwald E (2001) Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat Biotech 19(8):765–768

Dual Role of Nitrogen: Essential Plant Mineral Element and Source of Inorganic Pollution

Dragana Jakovljević and Milan Stanković

Contents

Abstract

Increased demand for food products in modern agriculture is attributed to the application of chemical fertilizers which generally possess damaging environmental impact. Nitrogen is an essential mineral element required in large amounts for normal growth and development of plants, and nitrogen-based fertilizers are of particular importance since they may increase nitrogen use efficiency and consequently can lead to better plant yield and productivity. The regulation of nitrogen metabolism in plants is very complex and conditioned with both physiological processes in plants, as well as environmental factors. Although the use of nitrogen fertilizers resulting in significant increases in crop yield, the applied fertilizers are used by plants in small amounts, whereas more than 50% is lost by leaching, runoff, or microbial activity. In this chapter, we discuss main plant mechanisms regarding nitrogen absorption, translocation, and assimilation

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together with environmental factors affecting nitrogen metabolism with a special focus on the possibilities to overcome problems caused by nitrogen fertilizer usage.

Keywords

Absorption · Assimilation · Fertilization · Mineral nutrition · Nitrogen

Abbreviations

AMT	Ammonium transporters
GOGAT	Glutamate synthase
GS	Glutamine synthetase
HATS	High affinity transport system
LATS	Low affinity transport system
N	Nitrogen
NH_4^+	Ammonium ion
NiR	Nitrite reductase
NO_3^-	Nitrate ion
NR	Nitrate reductase
NRT	Nitrate transporters
NUE	Nitrogen use efficiency
NUpE	Nitrogen uptake efficiency
NUtE	Nitrogen utilization efficiency

1 Introduction

For the main processes of plant growth, development, and reproduction, availability of nutrients together with the absorption and conversion of solar energy are mandatory (Attaran et al. 2014; Jakovljević et al. 2017). The fundamental processes during which plants assimilate carbon and nitrogen into components necessary for growth and development are conditioned by the selective partition of primary and secondary metabolites between organelles, cells, tissues, and organs. The diversity of the produced metabolites is wide, as well as the pathways through which metabolites are incorporated, and the synthesis and incorporation are also partitioned between plant main structures (Tegeder and Weber 2006; de Wit et al. 2018).

Roughly, plant nutrient requirement can be assessed through the inorganic composition of the plants, since plant dry matter constitutes 10–20% of the fresh weight, while nearly 10% of the dry matter consists of mineral elements (Reddy and Raghavendra 2006). The low level of availability of a particular element in the soil or its increased concentration most often leads to nutritional stress. Continuous deficiency/excess of essential elements is usually accompanied by visual symptoms and morphological changes on the plant itself, which include changes in color, loss of chlorophyll, necrosis, etc. As sessile organisms, plants are very often limited with the unfavorable concentrations of an essential element in soil, and to survive in a nutritionally unfavorable environment, they need to adapt at the biochemical and physiological level and adjust development according to the available elements. Nitrogen (N), phosphorus (P), and potassium (K) are mandatory for plants for normal growth and development, however, soil supply of these elements is often limited in different areas of plant cultivation. To achieve better crop yield farmers usually increase the amount of fertilizers leading to damage to soil structure and nutrient status. In addition, the damaging environmental impact of nitrogen N-fertilizers on global nitrogen cycle is noticed (Robertson and Vitousek 2009; Araus et al. 2020).

Taking into consideration the amount of fertilizers applied in agricultural ecosystems together with pollution caused by increasing nitrogen-based fertilization, this chapter provides information about ecophysiological bases of absorption and assimilation of different forms of nitrogen, plant adaptations to the variations of nitrogen concentration, as well as potential solutions to overcome the problems caused by excessive intake of nitrogen-based pollutions.

2 Nitrogen as an Essential Mineral Element

The nitrogen (N) is, in the largest amount, stored in the atmosphere in the unreactive N_2 (gaseous) form (Stevens et al. 2011). In general, on continents and in the oceans, biological N_2 fixation (biological reduction of atmospheric N_2 to ammonium) is the starting point of biological N turnover. Although it is significantly higher on land than in oceans, the denitrification is almost identical on land and in the ocean. Additionally, the N cycle is particularly affected by industrial N_2 assimilation resulting in NH_3 production. The N fixation provides about 65% of the biosphere's available nitrogen (Lambers et al. 2008; Mokhele et al. 2012).

As a constituent of amino acids and nucleic acids, nitrogen is an essential component of plant cells at the structural, genetic, and metabolic levels and is involved in many processes of growth and development. Due to its key role in chlorophyll production, nitrogen is fundamental for the photosynthetic process; as part of various enzymatic proteins nitrogen regulates growth processes; contributes to the protection against parasites and diseases; and affects crop yield and biomass (Muñoz-Huerta et al. 2013).

2.1 Nitrogen Absorption

While the inorganic forms of nitrogen are produced by soil microorganisms and represent less than 5% of total nitrogen, nitrogen is present in organic forms at levels lower than 1% of the total soil volume (Araus et al. 2020). The content of organic and inorganic nitrogen sources in the soil is heterogeneous and dynamic, and depends on a different soil factors, such as temperature, pH, chemical properties, and the presence of microorganisms (López-Arredondo et al. 2013). Inorganic nitrogen is available in soil in two forms, as the NO_3^- (nitrate) ion and as the NH_4^+ (ammonium) ion and is absorbed by the roots. It is known that the soil water regimes affect the availability of nitrogen forms and nitrogen and water status have both synergistic and antagonistic effects of root architecture and stomata aperture (Araus et al. 2020). Besides the fact that the nitrate ions can very easily be found mainly in deeper layers of the soil, and that much more energy is required for these ions to be reduced compared to ammonia absorption, for most of the dry environment or agricultural soil plants, nitrate ions are major nitrogen sources. In contrast, ammonium ions are favorable in humid environments and forest habitats (Harrison et al. 2007; Christophe et al. 2011). In addition, since the concentration of nitrate ions in the soil varies over a wide range (from 10 μM to 100 μM), non-leguminous plants must adapt to the spatial and temporal fluctuations in the availability of this essential element (Miller et al. 2007; Christophe et al. 2011).

The regulation of nitrogen metabolism in plants is very complex and influenced by both physiological as well as metabolic processes such as nitrogen metabolite level, circadian rhythms, and sucrose synthesis and transport. Additionally, different plant species respond to nitrogen availability through the modification of root architecture, remobilization of nitrogen storage, and activity of the transport system (López-Arredondo et al. 2013). It is known that when it comes to nitrogen absorption plant roots possess multiple transport system, and one active and one passive transporter for ammonium absorption. These transport systems require mechanisms for root to shoot signaling as well as signal transduction within the root (Glass et al. 1999).

During the vegetative phase, meristematic tissue and developing organs require large amounts of nitrogen to synthesize and store amino acids that are incorporated into proteins. However, excessive quantities of ammonium have detrimental effects on plant growth and may lead to ammonium toxicity (Domínguez-Valdivia et al. 2008; Hachiya and Sakakibara 2017; Jakovljević et al. 2017). At the molecular level, the adaptation to variation in the amount of available nitrogen is based on the presence of nitrate and ammonium transporters whose affinities vary. According to Hachiya and Sakakibara (2017), the root nitrogen net influx consists of total nitrogen influx and total nitrogen efflux, and specific transporters of nitrate (NRT) and ammonium (AMT) contribute to the total nitrogen influx, except for high ammonium nutrition. Regarding the high affinity ammonium transporters (AMT1) the different plant species show regulation of the expression at the transcriptional level; the transporters are mainly induced in roots of plants deprived of nitrogen and downregulated after ammonium re-supply (Rogato et al. 2010). When the soil nitrogen concentration is below 1 mM, the high affinity transport system (HATS) is predominant, while the low affinity transport system (LATS) is predominant for nitrogen concentrations above 1 mM (Fig. 1). The main component of HATS is the plasma membrane proteins of the epidermis, cortex, and endodermis of the root, and their synthesis is regulated by the NRT2 gene family. Part of the LATS are proteins (controlled by the NRT1 gene family involved in the regulation of root development and auxin transport) that are located in the root epidermis, cortex, and endoderm. (Little et al. 2005; Hermans et al. 2006; Remans et al. 2006; Christophe et al. 2011;

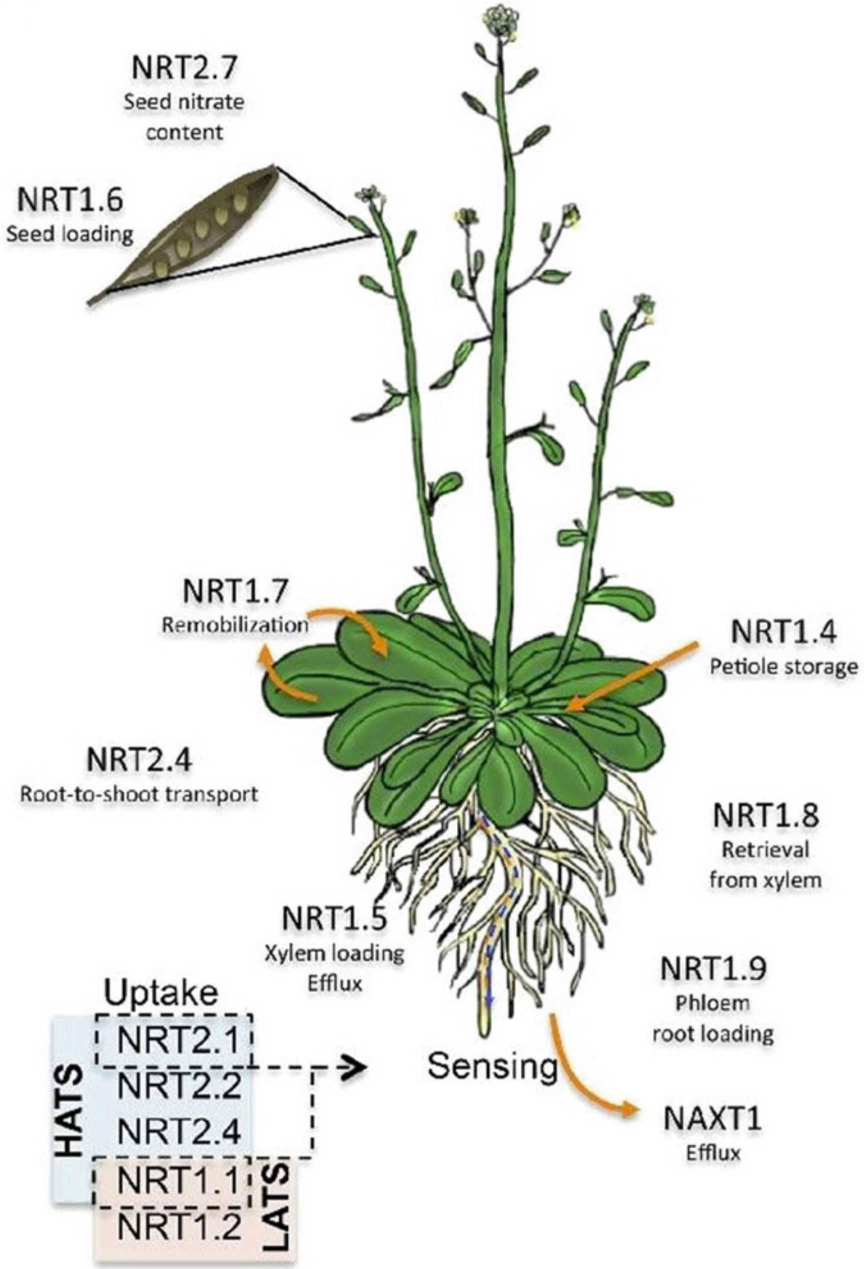

Fig. 1 Transporters and regulatory elements involved in nitrate metabolism according to López-Arredondo et al. (2013); by numerous transporters nitrate is absorbed through the root system and allocated to the different tissue

Bai et al. 2013). For a detailed explanation of the complex network of proteins included in nitrogen uptake, transport, and assimilation in plants see López-Arredondo et al. (2013); for the possible existence of a plant root derived mechanism for sensing the ammonium external concentration and achieving a convenient modulation of the root development in conditions of potentially toxic external ammonium concentration see Rogato et al. (2010).

2.2 Nitrogen Assimilation

For plant growth and development nitrogen assimilation into carbon skeletons represents a physiological process of the utmost importance. Inorganic nitrogen is assimilated into glutamate, glutamine, and asparagine—amino acids with an essential role as nitrogen-transport compounds in plants (Lea and Miflin 2003; Mokhele et al. 2012).

The nitrate assimilation takes place mainly in the plant roots, and it is dependent on the age and limitation of space for root growth (Marquez et al. 2007). According to Aslam et al. (2001) plants that exhibit low rates of nitrates in roots export absorbed nitrates to the shoots where it is reduced and incorporated into amino acids. Since the ammonium is the only reduced nitrogen form available to plants for assimilation into amino acids (Ruiz et al. 2007), absorbed nitrate needs to be further reduced to ammonium.

Once it enters the cell, the nitrate ion is subject to reduction. Nitrate reduction is always carried out in two steps—first to nitrite and then to ammonium ions by nitrate reductase (NR) and nitrite reductase (NiR). Since the reduction of nitrate to ammonium via NR and NiR requires eight moles of electrons per mole of nitrate, the ammonium utilization significantly decreases the energy consumption for organic nitrogen compound synthesis (Hachiya and Sakakibara 2017). The reduction of nitrate is dependent on the plant species, the amount of nitrate available, and the energy required. It is also tissue specific. Assimilated nitrates can be reduced in the root or in the aboveground organs, and further reduction and synthesis of organic compounds are done in leaves (Schjoerring et al. 2002). NR uses NADH as an electron donor in the nitrate reduction process, and this process takes place in the cytoplasm. The resulting nitrites are further reduced by NiR in chloroplasts of leaf cells or in root cells. Due to the highly reactive nature of nitrites, plant cells immediately transport them from the cytosol into chloroplasts in leaves and plastids in roots, and in these organelles, nitrites are further reduced to NH_4^+ via NiR. In leaves the electrons for reduction are derived from the reduced ferredoxin from the photosynthetic electron transport chain (ETC), while the root and leaf cells in the dark are supplied by electrons from respiratory metabolism (NADH) (Stitt 1999; Rosales et al. 2011).

Due to their high toxicity even at low concentrations, as well as the possible disruption of the respiratory process, ammonium ions must be assimilated into non-toxic organic components in the shortest amount of time. Toxic effects of ammonium are the consequence of proton extrusion, changes in cytosolic pH, as well as uncoupling of photophosphorylation in plants (Wang et al. 2007). The assimilation of ammonium takes place in root cells. NH_4^+ ions, formed either by nitrite reduction or by direct assimilation of ammonium, are immediately assimilated by the glutamine synthetase-glutamate synthase (GS/GOGAT) cycle. This system provides entry for reduced inorganic nitrogen into all plant nitrogen-containing organic compounds. GS catalyzes the ligation of ammonia and glutamate to glutamine, while GOGAT catalyzes the redox transfer of the glutamine amide group to α-ketoglutarate, forming two glutamate molecules (Dragićević et al. 2016). The pivotal role in ammonium assimilation have the GS which activity is critical and rate-limiting (Mokhele et al. 2012). The enzyme activity of the GS-GOGAT system is regulated by genes responsible for the synthesis of plastid and cytoplasmic glutamine synthetases and GOGAT. Assimilation is accompanied by the synthesis of organic acids, primarily α-ketoglutaric acid (which serves as an ammonium acceptor in the GOGAT system) and malate, which prevents alkalization. In addition, transcription of NIA and NII genes encoding proteins involved in the synthesis of these amino acids is significantly increased, as is the activity of the corresponding enzymes (Raab and Terry 1995; Stitt 1999; Schjoerring et al. 2002).

Glutamic acid (which always exists in plant cells in a certain amount) receives NH_4^+ ion and is converted to glutamine by ATP and GS enzymes. GOGAT then catalyzes the transition of the amino group from glutamine to α-ketoglutaric acid, producing two glutamic acid molecules. The glutamic acid formed is partially recovered and partly used to build nitrogen-containing compounds. These two reactions that are generally referred to as the GS/GOGAT cycle are nowadays accepted as the primary route of nitrogen assimilation in plants. Cytosolic GS1 and plastidic GS2 are two GS isoenzymes with different locations in subcellular compartments (Mokhele et al. 2012). In higher plants, GOGAT is represented in two different isoforms—NADH-dependent GOGAT (NADH-GOGAT) and ferredoxin-dependent GOGAT (Fd-GOGAT). NADH-GOGAT is, above all, active in non-photosynthetic tissue and young growing organs. The expression of genes responsible for the synthesis of Fd-GOGAT (GLU1 and GLU2) is tissue specific. GLU1 genes are dominant in leaves, whereas GLU2 genes are expressed in the root (Lam et al. 1996; Oliveira et al. 1997; Temple et al. 1998; Stitt 1999). The pathway of nitrogen metabolism and incorporation into organic compounds is presented in Fig. 2.

3 Environmental Factors Affecting Nitrogen Absorption and Assimilation

Despite the fact that certain critical level of nutrients is obligatory for plant normal growth and development, the mineral composition of plants shows wide variations and is under the influence of the plant age, the genetic constitution of the plant, chemical constituents of the soil, as well as climate conditions (Reddy and Raghavendra 2006). Under the conditions of natural field plants faced changing environments where nitrogen concentrations vary and may be limiting for growth

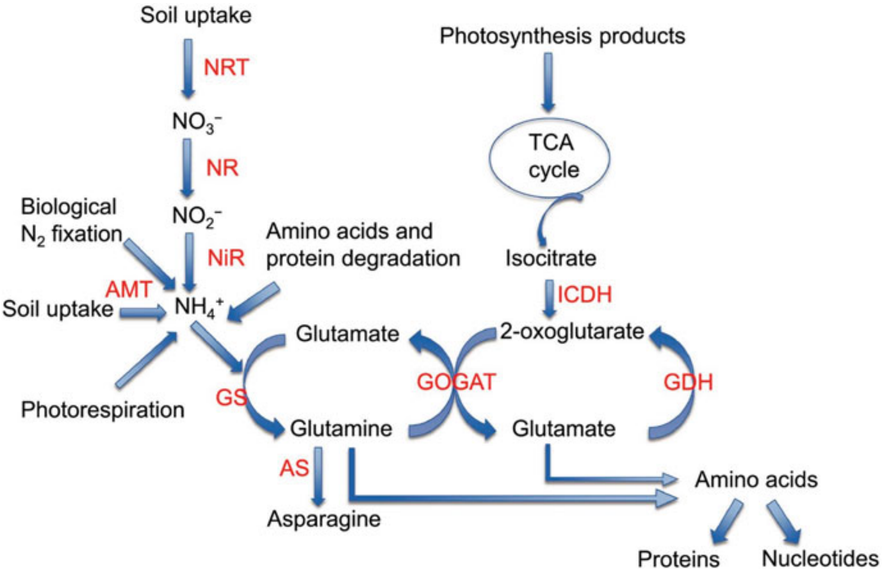

Fig. 2 Pathway of nitrogen assimilation and incorporation of nitrate or ammonia into amino acids and other molecules in higher plants according to Lu et al. (2016). NRT nitrate transporters, NR nitrate reductase, NiR nitrite reductase, AMT ammonium transporters, GS glutamine synthetase, GOGAT glutamate synthetase, AS asparagine synthetase, GDH glutamate dehydrogenase, ICDH isocitrate dehydrogenase

together with C/N ratio in the soil and within the plant, as well as rainfall, temperature, and soil type. Therefore, it is required for plants to maintain the optimum nitrogen and carbon for normal root and shoot development, photosynthetic rate, synthesis of amino acids, organic acids, and lipids (Kant et al. 2011). In general, nutrient stress is a complex phenomenon since may originate from either low levels or by the presence of excess concentrations of the element, whereas, in some cases, the presence of one element in excess concentrations may induce the deficiency of another element (Reddy and Raghavendra 2006). Inappropriate amount of nutrients directly affects photosynthetic apparatus through the functioning of major photosynthetic components (Kalaji et al. 2014; Jakovljević et al. 2017). According to Clay et al. (2006), nitrogen stress reduces chlorophyll production, and reduced chlorophyll content may lead to increased reflectance of photosynthetically active light and nitrogen-stressed plants appearing yellow. In addition, the photosynthetic apparatus is the main endogenous source of reactive oxygen species even during coordinated physiological processes and inappropriate functioning may result in cell death (Foyer and Shigeoka 2011). Therefore, adaptation to the conditions of limiting mineral nutrition is an important survival strategy (Kant et al. 2011).

Generally, a low level of nitrogen in the soil leads to the reduction of photosynthesis, root growth inhibition, suppression of plant lateral root initiation as well as leaf senescence. The high environmental concentration of nitrate inhibits lateral root elongation mainly due to the accumulation of this ion together with nitrogen metabolites inside the plants (Kant et al. 2011). Morphologically, nitrogen deficiency is accompanied by rapid plant growth inhibition. Due to the mobilization of nitrogen in older leaves, young leaves may not show symptoms of deficiency; however, within persistent deprivation older leaves become yellow and fall of the plant. Additionally, plants have woody stems since carbohydrates cannot be used in the synthesis of nitrogen-containing compounds (Briskin and Bloom 2010). It is known that ammonia excess has detrimental effects on plant physiology through the induction of stress and oxidative imbalance in plants (Jakovljević et al. 2017, 2019). Additionally, hazard effects of this ion can also be seen through the soil acidification and soil nutrient leaching, as well as water pollution.

The effects of different nitrogen forms are interesting for agriculture, particularly due to the potential of organic nitrogen to enhance nitrogen use efficiency (Franklin et al. 2017). Nitrogen use efficiency (NUE) can be defined as the yield of grain per unit of nitrogen (both natural and applied) available in the soil (López-Arredondo et al. 2013) and it may be concluded that increasing NUE of the crops is a possible strategy of reducing nitrogen fertilizer losses in agriculture. As is suggested by Garnett et al. (2009), plants can increase utilization efficiency (doing more with the less), or can increase uptake efficiency; plants developed with improvement in both cases will be the solution, however, improvement in either trait would be beneficial for the present. In any case, it is important to gain a better understanding of the function and regulation mechanism of the main components involved in N absorption, transport, assimilation, and signal transduction to improve the NUE in crops (Li et al. 2017).

The NUE with two components (nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE)) depends on morpho-physiological characteristics of plant species, as well as complex interactions between plant and environment. The relationships between plant growth, nitrogen dynamics, and nitrogen use efficiency for rapeseed (*Brassica napus* L.) are illustrated on Fig. 3.

4 Nitrogen Fertilizers and Environmental Pollution

Due to the natural depletion or leaching, nitrogen must be added to the soil (Mokhele et al. 2012). According to Kant et al. (2011) increase in crop productivity is associated with a 20-fold increase in the global use of nitrogen fertilizer applications during the past decades and it is expected to increase several times by 2050. Additionally, the large amount of fertilizers used in developed countries helps prevent fluctuating levels of nitrogen, however, as a consequence, a lot of fertilizers is wasted to the environment.

The most commonly used nitrogen fertilizer in agriculture is urea, about half of all used fertilizers for crop production (Witte 2011; Mokhele et al. 2012). It is taken by plants actively from the soil, and after uptake into the plant cells, the assimilation is catalyzed with urease (urea amidohydrolase) and urea amidolase which hydrolyze urea in the cytosol to CO_2 and NH_3 (Wang et al. 2007). The addition of urea, as well

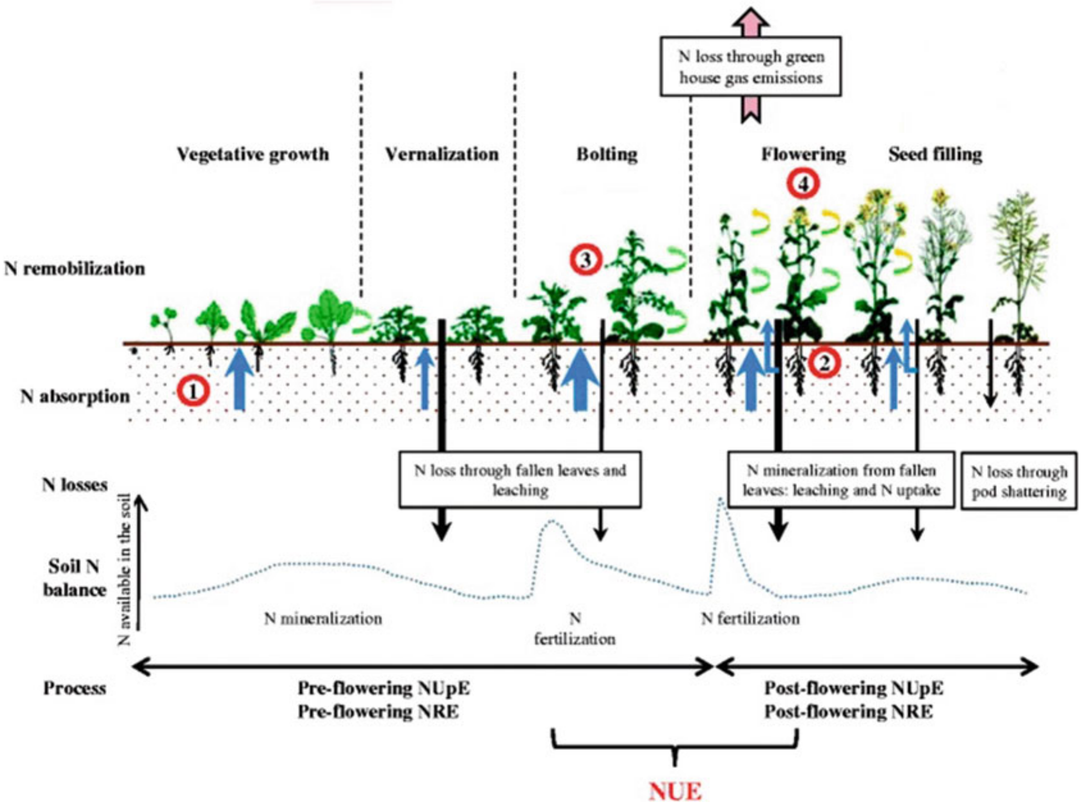

Fig. 3 Illustration of the relationship between *Brassica napus* L. growth, nitrogen dynamic, and yield over the crop cycle with the critical stages for the final establishment of NUE (according to Bouchet et al. 2016, with some modifications); blue arrows represent nitrogen uptake (width indicates the relative amount of absorbed nitrogen); black arrows represent nitrogen losses (width indicates the relative amount of nitrogen lost at a given point of time). NUE nitrogen use efficiency, NUpE nitrogen uptake efficiency, NRE nitrogen remobilization efficiency

as other nitrogen fertilizers, is the highest input cost for many crops. However, these compounds are highly mobile through the soil and crop plants can utilize only 30–40% of applied fertilizer leading to loss of more than 60% (Kant et al. 2011). The main processes of nitrogen fertilizer loss contributing to environmental pollution are combinations of nitrate leaching, soil denitrification, volatilization, and microbial consumption (Zebarth et al. 2009). The nitrate leaching contributes to eutrophication through the water contamination, while volatilized nitrogen contributes to global warming through the nitrous oxides releasing. Also, high nitrogen supply may become dangerous to human health through the high concentration of nitrogen forms in plant leaves (Muñoz-Huerta et al. 2013).

As mentioned, the reactive forms of nitrogen are utilized by organisms and naturally enter the ecosystem via biological N fixation, but human activities have more than doubled the input of nitrogen into the World's ecosystems over the last century (Stevens et al. 2011). According to Van Wijk et al. (2003), the response of ecosystems to increased nutrient ability is influenced by the plant characteristics and chemical and microbial immobilization. Stevens et al. (2011) noticed that although in Europe and parts of North America the deposition of NHx (ammonia and ammonium) and NOy (nitrate, nitric oxides and nitric acid) increased in the second half of twentieth century mainly due to industrial and agricultural activities, the significant differences are even seen at the continental scale. Figure 4 illustrates the main differences.

5 Concluding Remarks and Future Challenges

A marked increase in food production attributed to the application of chemical fertilizers results in climate change, environmental pollution, and biodiversity loss, which is an enormous environmental challenge of the twenty-first century since the application of nitrogen fertilizers will keep increasing with the growing demand for food (Godfray et al. 2010; Liu et al. 2013; Li et al. 2017). At the same time with nitrogen-based fertilizer underline of modern agriculture, the damaging environmental impacts including disruption of the global nitrogen cycle are already being left (Araus et al. 2020). Therefore, nitrogen fertilizer based pollution is a serious issue for many regions with highly concentrated agriculture, and in order to minimize the footprint of agricultural production there is a particular interest to develop technologies which allowing economical production with minimum applied nitrogen (Garnett et al. 2009).

One of the effective solutions may be the cultivation of plants tolerant to low nitrogen in order to reduce the amount of fertilizers, as well as to avoid excessive waste (Li et al. 2018; Jakovljević et al. 2019). The field management in general and reduction of nitrogen losses through improved field management are essential in order to optimize nitrogen losses. Some of the techniques involved are the better coordination of nitrogen/water status in the soil, the application of fertilizers in appropriate growing season and irrigation or rainfall time, as well as better matching of fertilizers with the particular crop and soil (Garnett et al. 2009; Araus et al. 2020).

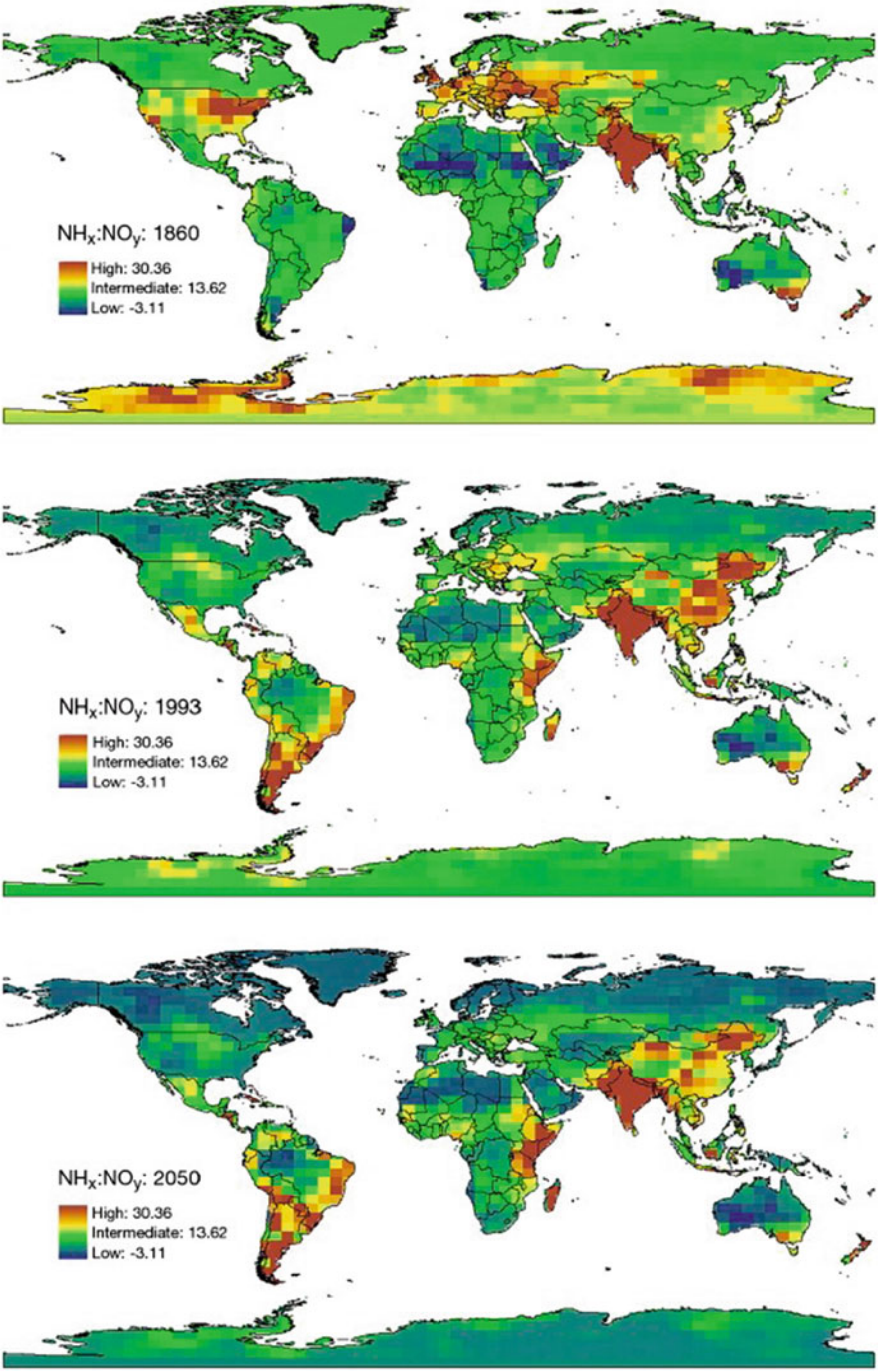

Fig. 4 Illustration of main differences in the NHx (ammonia and ammonium) and NOy (nitrate, nitric oxides, and nitric acid) inputs at the continental scale (accordin to Dentener 2006 and modified by Stevens et al. 2011)

Having in mind that with a 1% increase in NUE approximately US$1.1 billion could be saved, a promising way to deal with this crisis is an improvement in crops nitrogen use efficiency (Kant et al. 2011; Li et al. 2017). Potential targets of NUE improvement are nitrogen assimilatory enzymes and root morphology, since at the end of the growing season, particularly in rapidly leaching or in drying nitrogen depleted soils, root architecture may be of great importance (Garnett et al. 2009). Therefore, breeding plants with deeper root systems or with the ability to elongate roots into deeper soil layers may adapt plants to low nitrogen and low water conditions (Arai-Sanoh et al. 2014; Araus et al. 2020).

As is suggested by Díaz et al. (2019) many modern cultivars may be without genetic diversity opposite to their wild varieties, leading to an aggravated growth under low nitrogen environment. Consequently, researches focused on plant species with diverse phylogenetic origins and their adaptations will help to find a solution for plant survival in the dry and nutrient-poor environment (Araus et al. 2020). Continuous investigations are of great importance with a better understanding of soil environment, plant characteristics, and changes of these characteristics with the changing environment.

References

Arai-Sanoh Y, Takai T, Yoshinaga S, Nakano H, Kojima M, Sakakibara H, Kondo M, Uga Y (2014) Deep rooting conferred by DEEPER ROOTING 1 enhances rice yield in paddy fields. Sci Rep 4:1–6

Araus V, Swift J, Alvarez JM, Henry A, Coruzzi GM (2020) A balancing act: how plants integrate nitrogen and water signals. J Exp Bot. https://doi.org/10.1093/jxb/eraa054

Aslam M, Travis RL, Rains DW (2001) Differential effect of amino acids on nitrate uptake and reduction systems in barley roots. Plant Sci 160:219–228

Attaran E, Major IT, Cruz JA, Rosa BA, Koo AJ, Chen J, Kramer DM, He SY, Howe GA (2014) Temporal dynamics of growth and photosynthesis suppression in response to jasmonate signaling. Plant Physiol 165:1302–1314

Bai H, Euring D, Volmer K, Janz D, Polle A (2013) The nitrate transporter (NRT) gene family in poplar. PLoS One 8:72126

Bouchet A, Laperche A, Bissuel-Belaygue C, Snowdon R, Nesi N, Stahl A (2016) Nitrogen use efficiency in rapeseed. A review. Agron Sustain Dev 36:38. https://doi.org/10.1007/s13593-016-0371-0

Briskin DP, Bloom A (2010) Mineral nutrition. Plant physiology, 5th edn. Sinauer Associates, Sunderland, pp 107–130

Christophe S, Jean-Christophe A, Annabelle L, Alain O, Marion P, Anne-Sophie V (2011) Plant N fluxes and modulation by nitrogen, heat and water stresses: a review based on comparison of legumes and non legume plants. In: Abiotic stress in plants-mechanisms and adaptations. InTech, pp 79–118

Clay DE, Kim KI, Chang J, Clay SA, Dalsted K (2006) Characterizing water and nitrogen stress in corn using remote sensing. Agron J 98:579–587

de Wit M, George GM, Ince YÇ, Dankwa-Egli B, Hersch M, Zeeman SC, Fankhauser C (2018) Changes in resource partitioning between and within organs support growth adjustment to neighbor proximity in Brassicaceae seedlings. Proc Natl Acad Sci 115:9953–9961

Dentener FJ (2006) Global maps of atmospheric nitrogen deposition, 1860, 1993, and 2050. Data set. Oak Ridge National Laboratory Distributed Active Archive Center, Oak Ridge, TN. https://doi.org/10.3334/ORNLDAAC/830

Díaz FP, Latorre C, Carrasco- Puga G, Wood JR, Wilmshurst JM, Soto DC, Cole TL, Gutiérrez RA (2019) Multiscale climate change impacts on plant diversity in the Atacama Desert. Glob Chang Biol 25:1733–1745

Domínguez-Valdivia MD, Aparicio-Tejo PM, Lamsfus C, Cruz C, Martins-Loução MA, Moran JF (2008) Nitrogen nutrition and antioxidant metabolism in ammonium-tolerant and-sensitive plants. Physiol Plant 132:359–369

Dragićević M, Simonović A, Bogdanović M, Subotić A, Ghalawenji N, Dragićević I, Todorović S (2016) Differential regulation of GS-GOGAT gene expression by plant growth regulators in *Arabidopsis* seedlings. Arch Biol Sci 68:399–404

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100

Franklin O, Cambui CA, Gruffman L, Palmroth S, Oren R, Näsholm T (2017) The carbon bonus of organic nitrogen enhances nitrogen use efficiency of plants. Plant Cell Environ 40:25–35

Garnett T, Conn V, Kaiser BN (2009) Root based approaches to improving nitrogen use efficiency in plants. Plant Cell Environ 32:1272–1283

Glass ADM, Erner Y, Hunt T, Kronzucker HJ, Okamoto M, Rawat S, Silim S, Schjoerring JK, Siddiqi MY, Vidmar JJ, Wang MY, Zhuo D (1999) Inorganic nitrogen absorption by plant roots: physiology and molecular biology. In: Gissel-Nielsen G, Jensen A (eds) Plant nutrition – molecular biology and genetics. Kluwer, Dordrecht, pp 1–16

Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Hachiya T, Sakakibara H (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J Exp Bot 68:2501–2512. https://doi.org/10.1093/jxb/erw449

Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. Ecology 88:989–999

Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci 11:610–617

Jakovljević D, Stanković M, Bojović B, Topuzović M (2017) Regulation of early growth and antioxidant defense mechanism of sweet basil seedlings in response to nutrition. Acta Physiol Plant 39:243

Jakovljević D, Topuzović M, Stanković M (2019) Nutrient limitation as a tool for the induction of secondary metabolites with antioxidant activity in basil cultivars. Ind Crop Prod 138:111462

Kalaji HM, Oukarroum A, Alexandrov V, Kouzmanova M, Brestic M, Zivcak M, Samborska I, Cetner M, Allakhverdiev I, Goltsev V (2014) Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. Plant Physiol Biochem 81:16–25

Kant S, Bi YM, Rothstein SJ (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. J Exp Bot 62:1499–1509

Lam HM, Coschigano KT, Oliveira IC, Melo-Oliveira R, Coruzzi GM (1996) The molecular-genetics of nitrogen assimilation into amino acids in higher plants. Annu Rev Plant Biol 47:569–593

Lambers H, Chapin III FS, Pons TL (2008) Plant physiological ecology. Springer. New York

Lea PJ, Miflin BJ (2003) Glutamate synthase and the synthesis of glutamate in plants. Plant Physiol Biochem 41:555–564

Li H, Hu B, Chu C (2017) Nitrogen use efficiency in crops: lessons from Arabidopsis and rice. J Exp Bot 68:2477–2488

Li M, Xu J, Wang X, Fu H, Zhao M, Wang H, Shi L (2018) Photosynthetic characteristics and metabolic analyses of two soybean genotypes revealed adaptive strategies to low-nitrogen stress. J Plant Physiol 229:132–141

Little DY, Rao H, Oliva S, Daniel-Vedele F, Krapp A, Malamy JE (2005) The putative high-affinity nitrate transporter NRT2. 1 represses lateral root initiation in response to nutritional cues. Proc Natl Acad Sci U S A 102:13693–13698

Liu X, Zhang Y, Han W, Tang A, Shen J, Cui J, Vitousek P, Erisman JW, Goulding KWT, Christie P, Fangmeier A, Zhang F (2013) Enhanced nitrogen deposition over China. Nature 494:459–462

López-Arredondo DL, Leyva-González MA, Alatorre-Cobos F, Herrera-Estrella L (2013) Biotechnology of nutrient uptake and assimilation in plants. Int J Dev Biol 57:595–610

Lu J, Zhang L, Lewis RS, Bovet L, Goepfert S, Jack AM, Crutchfield JD, Ji H, Dewey RE (2016) Expression of a constitutively active nitrate reductase variant in tobacco reduces tobacco-specific nitrosamine accumulation in cured leaves and cigarette smoke. Plant Biotechnol J 14:1500–1510

Marquez AJ, Betti M, Garcia-Calderon M, Credali A, Diaz P, Monza J (2007) Primary and secondary nitrogen assimilation in Lotus japonicus and the relationship with drought stress. Lotus Newsl 37:71–73

Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM (2007) Nitrate transport and signalling. J Exp Bot 58:2297–2306

Mokhele B, Zhan X, Yang G, Zhang X (2012) Nitrogen assimilation in crop plants and its affecting factors. Can J Plant Sci 92:399–405

Muñoz-Huerta RF, Guevara-Gonzalez RG, Contreras-Medina LM, Torres-Pacheco I, Prado-Olivarez J, Ocampo-Velazquez RV (2013) A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. Sensors 13:10823–10843

Oliveira IC, Lam HM, Coschigano K, Melo-Oliveira R, Coruzzi G (1997) Molecular-genetic dissection of ammonium assimilation in *Arabidopsis thaliana*. Plant Physiol Biochem 35:185–198

Raab TK, Terry N (1995) Carbon, nitrogen, and nutrient interactions in *Beta vulgaris* L. as influenced by nitrogen source, NO3- versus NH4+. Plant Physiol 107:575–585

Reddy AR, Raghavendra AS (2006) Photooxidative stress. In: Rao MKV, Raghavendra AS, Reddy KJ (eds) Physiology and molecular biology of stress tolerance in plants. Springer, Dordrecht, pp 157–186

Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, Gojon A (2006) A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. Plant Physiol 140:909–921

Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: balancing the cost of an essential resource. Annu Rev Environ Res 34:97–125

Rogato A, D'Apuzzo E, Chiurazzi M (2010) The multiple plant response to high ammonium conditions: the *Lotus japonicus* AMT1; 3 protein acts as a putative transceptor. Plant Signal Behav 5:1594–1596

Rosales EPMF, Iannone M, Groppa DM, Benavides P (2011) Nitric oxide inhibits nitrate reductase activity in wheat leaves. Plant Physiol Biochem 49:124–130

Ruiz JM, Rivero R, Romero ML (2007) Comparative effect of Al, Se, and Mo toxicity on NO_3^- assimilation in sunflower (*Helianthus annuus* L.) plants. J Environ Manag 83:207–212

Schjoerring JK, Husted S, Mäck G, Mattsson M (2002) The regulation of ammonium translocation in plants. J Exp Bot 53:883–890

Stevens CJ, Manning P, Van den Berg LJ, De Graaf MC, Wamelink GW, Boxman AW, Bleeker A, Vergeer P, Arroniz-Crespo M, Limpens J, Lamers LPM, Bobbink R, Dorland E (2011) Ecosystem responses to reduced and oxidised nitrogen inputs in European terrestrial habitats. Environ Pollut 159:665–676

Stitt M (1999) Nitrate regulation of metabolism and growth. Curr Opin Plant Biol 2:178–186

Tegeder M, Weber APM (2006) Metabolite transporters in the control of plant primary metabolism. In: Plaxton WC, McManus MT (eds) Annual plant reviews, vol 22: Control of primary metabolism in plants. Blackwell, Oxford. https://doi.org/10.1002/9780470988640.ch4

Temple SJ, Vance CP, Gantt JS (1998) Glutamate synthase and nitrogen assimilation. Trends Plant Sci 3:51–56

Van Wijk MT, Williams M, Gough L, Hobbie SE, Shaver GR (2003) Luxury consumption of soil nutrients: a possible competitive strategy in above-ground and below-ground biomass allocation and root morphology for slow-growing arctic vegetation? J Ecol 91:664–676

Wang ZQ, Yuan YZ, Ou JQ, Lin QH, Zhang CF (2007) Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity. J Plant Physiol 164:695–701

Witte CP (2011) Urea metabolism in plants. Plant Sci 180:431–438

Zebarth BJ, Drury CF, Tremblay N, Cambouris AN (2009) Opportunities for improved fertilizer nitrogen management in production of arable crops in eastern Canada: a review. Can J Soil Sci 89:113–132

Synthesis and Regulation of Secondary Metabolites in Plants in Conferring Tolerance Against Pollutant Stresses

Muhammad Zia ur Rehman, Muhammad Ashar Ayub, Muhammad Umair, Zahoor Ahmad, Hinnan Khalid, Shafaqat Ali, and Muhammad Rizwan

Contents

Abstract

The soil environment is getting contaminated with heavy metals and other pollutants due to unattended anthropogenic and natural activities. Plants grown in such contaminated soils use different mechanisms to regulate and combat the deleterious effects of metal ions by producing several metabolites and antioxidants to combat oxidative damage. Among them, secondary metabolites actively participate in the alleviation of stress by acting as metal chelators or via direct scavenging of the reactive oxygen species. Plant-sourced secondary

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metabolites (SMs) are organic compounds that are highly inducible in stress experiencing plants. Though secondary metabolites are non-essential, but are directly involved in the healthy interaction of plants with the environment. As they strengthen the plants by making them adaptable to the environment and counteract the deleterious effects of pollutants. These SMs also act as basic immune-protector in medicinal plants for traditional medicine in different cultures of the world. The chemical structure of these metabolites mainly corresponds to the antioxidant behavior. Depending upon chemical structure, these metabolites can be classified into various types like phenolics, alkaloids, saponins, terpenes, lipids, carbohydrates, nitrogen, sulfur containing compounds, etc. Plant experiencing abiotic stress produces SMs from primary metabolites using the various pathways to help plants combat the stressed environment. Though mainly stress initiated, the production of these SMs in a plant is usually very less and varies among different plant species and affected by the strength of exogenous stimuli. Inorganic pollutants like mineral salts, gaseous toxins, heavy metals, pesticides, and aerosols are major factors involved in the initiation of SMs production in plants. Abiotic stress signals like methyl jasmonate, jasmonic acid, salicylic acid, calcium, polyamines, serotonin, abscisic acid, plant growth regulators, nutrient stress, and micro-drought serve as stimuli for SMs production. Cell culture technologies are a basic source of in vitro production of secondary metabolites for studying their effects on plants and for plant growth improvement purposes. This chapter addresses the nature and role of secondary metabolites in plant systems under abiotic stress conditions.

Keywords

Secondary metabolites (SMs) · Heavy metals · Pollutants

Abbreviations

ABA	Abscisic acid
Al	Aluminum
APX	Ascorbate peroxidase
As	Arsenic
AsA	Ascorbate
BRs	Brassinosteroids
C	Carbon
CAT	Catalase
Cd	Cadmium
CK	Cytokinin
Co	Cobalt
Cr	Chromium
Cu	Copper
GA	Gibberellic acid
GPX	Guaiacol peroxidase

GR	Glutathione reductase
GSH	Glutathione
GSL	Glucosinolates
H_2O_2	Hydrogen peroxide
Hg	Mercury
HMs	Heavy metals
IAA	Indole acetic acid
kDa	Kilodalton
Ni	Nickel
O	Oxygen
O_3	Ozone
Pb	Lead
POX	Peroxidase
ROS	Reactive oxygen species
SA	Salicylic acid
SMs	Secondary metabolites
SOD	Superoxide dismutase
UV	Ultraviolet
Zn	Zinc

1 Introduction

Agricultural and atmospheric contamination with heavy metal species and other inorganic pollutants like alkali salts is a major issue regarding food security and human health. Environmental risks linked with inorganic pollutants vary largely owing to complex interactions at extracellular and intracellular levels (Saha et al. 2017). Salts of the alkali group affect the physicochemical properties of the soil resulting in the interruption of the plant–soil–water relationships, nutrient use efficiency, bioavailability, and cycling. Toxic metal ions interact with soil colloids more strongly than salts depending on their speciation and elemental nature. Even at their low concentration and less mobility in the soil, they disrupt the metabolic processes by affecting the physiology of the plants. Heavy metals or trace elements are the group of metalloids having density and atomic number greater than 5 g cm^{-3} and 20, respectively (Alloway 2011). Some of these trace elements (zinc, copper, nickel, molybdenum, manganese, iron) are essential for the structural and biochemical processes in plants like proper growth, tissue development, electron transport, redox reactions, and many other metabolic processes (Andresen et al. 2018). While non-essential heavy metals including lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), etc. have been found to be toxic for plants growth with no known biological function along with food chain contamination even at minute concentrations (Shahid et al. 2017). Variability in spatial distribution and contamination of soils with heavy metals is owing to anthropogenic and natural sources, while the rapid increase in pollutants from last some decades in some ecosystems is typically linked with unmanaged anthropogenic activities (Sarma et al. 2011; Street

2012; Xiong et al. 2016a, b). Industrial effluents, mining, metallurgy, chemical fertilizers, urbanization, and transportation tend to increase the mass of pollutants in the environmental matrices (Megateli et al. 2009; De Lurdes Dinis and Fiúza 2010). The ultimate sink of these pollutants is the soil where they degrade the soil properties and badly affect the growth of plants. These toxic pollutants get absorbed into plants via root system, translocate to the aerial portion causing metabolic dysfunction and food chain contamination (Zheljazkov et al. 2006, 2008a, b; Baye and Hymete 2009; Chaiyarat et al. 2010; Li et al. 2010; Carrubba and Scalenghe 2012; Ebrahim et al. 2012; Shahid et al. 2017). Plants use different strategies to cope with these inorganic pollutants including physiological, morphological, genetic, and biochemical mechanisms (Sharma and Dietz 2009; Schreck et al. 2012).

Exposure to heavy metals results in overproduction of the reactive oxygen species, which are then detoxified by different transcriptional responses and epigenetic modifications (Dutta et al. 2018). Induction of heavy metals persuades the production of secondary metabolites (SMs) in the plants which help in mitigating their toxic effects. Primary metabolites like amino acids, carbohydrates, and lipids serve as base materials and are involved in the production of secondary metabolites (Hatami and Ghorbanpour 2016). Plant secondary metabolites have the least essential role in the functioning of the plants; however, they serve for the adaptive and defense interaction of the plant with its environment (Ramakrishna and Ravishankar 2011). About 100,000 types of secondary metabolites have been discovered so far with weight <150 kDa and contributing $<1\%$ of the plant total dry matter (Oksman-Caldentey and Inzé 2004). Depending on their biosynthetic pathways, they have been classified into nitrogen compounds (alkaloids) and nitrogen-deficient (terpenes and phenolics) (Kasote et al. 2015; Pagare et al. 2015). Accumulation of secondary metabolites in the plant tissues is often subjected to different types of stresses and signal molecules and gets regulated by many plant factors like evolution and genetic behavior, growing conditions, climate, mineral elements, and pollutants concentrations (Street 2012; Hatami and Ghorbanpour 2016; Yang et al. 2018). The induced stress of heavy metals and other inorganic molecules leads to the production of secondary metabolites through stimulation of the defensive system of the plants (Asgari Lajayer et al. 2017). SMs also add in the tastes, odors, toxins, and colors in plants (Mazid et al. 2011; Pagare et al. 2015) and are the distinctive sources of food additives, flavors, pharmaceutically active substances, and industrial biochemicals (Heitefuss 2011; Ramakrishna and Ravishankar 2011). Chemicals involved in the synthesis of SMs are calcium, salicylic acid, abscisic acid, jasmonates, polyamines, and nitric oxides (Tuteja and Sopory 2008). Plant secondary metabolites can also be synthesized using plant tissue cultures and it is a subject of much interest in research owing to their large applications (Gonçalves and Romano 2018). But their considerable yield and extraction present challenges that need to be solved yet as part of biological and phyto-chemical investigations. Successful production and extraction begin with cautious selection and preparation of the plants used. During this process, it is necessary to minimize the contamination that may get extracted with concerned compounds.

2 Classification of Secondary Metabolites

Among various organic molecules that are being synthesized by plants in response to stress signals is a special class called secondary metabolites. They are not necessary for cell life but have a role in making the plant adaptable to its surroundings. Having a unique structure with a carbon skeleton is the basic property of the SMs (Pagare et al. 2015). These SMs can be classified into various classes depending upon their chemical structure, composition, solubility, or pathway through which they are synthesized (McMurry 2014). Based upon structural composition SMs can be classified into enzymatic and non-enzymatic (antioxidants) (Hasanuzzaman et al. 2019). These antioxidants are very important in combating stresses in plants making them an integral part of plant biochemistry (Kapoor et al. 2019). Among enzymatic antioxidants, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase, catalase, superoxide dismutase, glutathione peroxidase, peroxiredoxin, and glutathione-transferase are well known (Noctor et al. 2014; Hasanuzzaman et al. 2017a). While ascorbate, carotenoids, flavonoids, glutathione, and tocopherols are non-enzymatic antioxidants (Gill and Tuteja 2010). Based upon the biosynthetic pathway of SMs, another classification has been proposed in which SMs are divided into three major groups: terpenoids, phenolics, and nitrogenous/sulfur compounds. Terpenoids represent the biggest group of SMs in plants (Nagegowda 2010). They are biosynthesized via common origin from glycolytic or acetyl Co-A intermediates. Most of the diverse terpenes' structures produced by plants are in their defense against feeding deterrents and are toxic in nature. Terpenes have been classified in accordance with the number of 5-carbon units present in their structure. Hence, it includes hemi (C-5), mono (C-10), sesqui (C-15), di (C-20), sester (C-25), tri (C-30), and tetra (C-40) terpenes (Nagegowda and Gupta 2020). Pyrethroid (terpenoid) occurring in the leaves of chrysanthemum species shows strong insecticidal effects and is an ingredient of many commercial insecticides owing to its low environmental persistency. In gymnosperms plants, commonly found terpenes are limonene, α-pinene, and myrcene. Similarly, sesquiterpenes including costunolides, abscisic acid; diterpenes including abietic acid, phorbol; tetraterpenes including carotenoids family and many high molecular weight polyterpenes have been reported as beneficial SMs in abiotic stress mitigation (Pagare et al. 2015).

A large variety of phenolic compounds are also produced in the plants as SMs for defense purposes (Colak et al. 2019; Jiang et al. 2019). These compounds contain phenolic acids, lignin, anthocyanidin (Taiz and Zeiger 2006), coumarin derived by shikimic acid pathways (Brooker et al. 2008), furano, psoralene (Ali et al. 2008), flavonoids, and isoflavonoids (Sreevidya 2006; Lake et al. 2009; Saviranta et al. 2010). Phenolic metabolites specially phenylpropanoids and flavonoids get oxidize by peroxidases and help in scavenging H_2O_2, phenolic/POX system (Michalak 2006). Several phenolic SMs have been reported in plants for stress tolerance like ascorbate peroxidase (Esmaeilzadeh et al. 2017), ascorbic acid (Maleki et al. 2017), catalase, dehydroascorbic acid, cytosolic dehydroascorbate reductase, glutathione reductase, glutathione, glutathione disulfide, monodehydroascorbate,

monodehydroascorbate reductase, peroxidase, and superoxide dismutase (Michalak 2006; Blokhina et al. 2003; Kovacik et al. 2009a, b). Nitrogen-containing SMs include alkaloids, defensins, GSL, and N-glycosides, while sulfur-containing SMs include phytoalexins, allicin, S-glycosides, and thionins (Saito 2004; Grubb and Abel 2006; Halkier and Gershenzon 2006; Figueiredo et al. 2008; Street 2012). Some nitrogen-containing SMs also include phenolic acids (Taiz and Zeiger 2006).

3 Production of Secondary Metabolites in Plants

Most of the secondary metabolites are produced in plants by three pathways, via the shikimate pathway, polyketide pathway, and isoprenoid pathway. After the formation of the basic skeletons, SMs get modified according to the species-specific compounds. Aromatic compounds are mainly sourced from the shikimate pathway. While phenylpropanoid pathway is involved in the synthesis of lignin, anthocyanins, and flavonoids which are major SMs in stress conditions including heavy metal and alkali salts pollution (Pagare et al. 2015). Phenylpropanoid is one of the central metabolic pathways in terms of carbon flux as about 20% of the total metabolism in a plant cell goes through this pathway. Fundamental to SM products in this pathway is the enzyme phenylalanine ammonia-lyase which changes phenylalanine into trans-cinnamic acid through non-oxidative deamination. Isoprenoid pathway is another important pathway in this regard including terpenoids production which is 1/3 of the plants SMs (Pagare et al. 2015).

The production and concentration of the SMs in plants are dependent on the soil environment and plant resistance. Exposure to stress like inorganic pollutants and HMs can produce reactive oxygen species in the plants which are damaging to the cell functions. They can limit plant growth and productivity severely (Pandey et al. 2017). Expressions of certain genes get an increase in response to the ROS (Tuteja 2007; Nakashima et al. 2009; Roy 2016). These genes are involved in the production of SMs to combat with the ROS and help in their scavenging. A stress signal is responded when recognized at the cellular level in the form of SMs formation as a protective function. Edreva et al. (2007) reported phenyl amides and polyamines accumulation in the tobacco and bean plants under abiotic stress conditions suggesting the role of these SMs as antioxidants. Similarly, accumulation of anthocyanin is induced by several environmental stress signal including wounds, drought, nutrient deficiency, high-intensity light, blue light, UV-rays, and pathogen attack (Winkel-Shirley 2001; Truong et al. 2018; Hu et al. 2020; Zhang et al. 2020; Zheng et al. 2020).

Salts usually incur both osmotic and ionic stress resulting in the increase or decrease of specific SMs in plants (Mahajan and Tuteja 2005). Truong et al. (2018) reported enhanced production of anthocyanins under low nitrate and high salt stress. Contrary to this, Daneshmand et al. (2009) described that salt stress reduced anthocyanins concentration in salt-sensitive plant species. Similarly, Petrusa and Winicov (1997) explained that salt tolerant plant alfalfa quickly doubled its root proline contents, while salt-sensitive plant’s rate of increase in proline contents was slow. Torre-González et al. (2018) reported proline accumulation linked with salt tolerance in *Solanum lycopersicum* L. Pedranzani et al. (2003) also reported endogenous jasmonic acid accumulation in tomato plants under salt stress. Methyl jasmonate and salicylic acid are well-known elicitors for a number of SMs including terpenoid, alkaloids, and phenolic phytoalexins in medicinal plants (Singh and Dwivedi 2018). Polyphenol synthesis often occurs owing to biotic or abiotic stresses (Muthukumarasamy et al. 2000; Popović et al. 2016). Increasing salinity has also been reported for high polyphenol contents in many plants (Parida and Das 2005). Navarro et al. (2006) described high levels of phenolic compounds in moderately saline soil in red pepper and have been reported many times as salinity response. Plant polyamines also get involved in the salinity response. Mutlu and Bozcuk (2007) reported changes inbound and free polyamine concentration in the roots of sunflower due to salinity.

Heavy metals also influence the production of SMs as they are toxic in nature and cause the production of ROS leading to increase the SMs production via specific signals. A number of studies are present that describes the role of HMs in inducing the plant defense system. Copper exhibited the stimulation for the production of betalains in *Beta vulgaris* (Trejo-Tapia et al. 2001). Silver and cadmium enhanced the production of two tropane alkaloids (hyoscyamine and scopolamine) in *Brugmansia candida* (Angelova et al. 2006). Lanthanum has been found involved in the production of taxol in the *Taxus* species. A decrease in putrescine has been observed in the leaf disks of sunflower (Jacobsen et al. 1992). But there was no effect of chromium exposure on the spermidine and spermine level in leaves except putrescine which increased with increasing Cr level and exposure time via leaves. Lin and Kao (1999) also reported an increase in the level of putrescine in the leaves of rice on exposure to Cu, while the centration of spermine decreased. Production of SMs in plants varies largely depending on the stress conditions, HMs, salt types, and species of plants.

4 Roles of Secondary Metabolites in Plants

Secondary metabolites have a prominent role in the protection of plants against abiotic as well as biotic stress factors although they are considered insignificant for development and growth processes (Schafer and Wink 2009; Ncube and Staden 2015). It is thought that most of the known SMs are involved in the chemical defensive system of the plants from millions of years as plants are suffering from the invaders (Wink 1999). High concentrations of SMs result in more resistance in plants, while their production is costly for the plants leading to a decrease in their growth and reproduction (Siemens et al. 2002). Plants get damaged when the amount of ROS exceed than the antioxidant or detoxification mechanisms capacity. So, plants have developed extensive protective systems to get rid of ROS in the form of SMs. They can stop the oxidation process by blocking the detrimental oxidation chain reaction to save plants (Sgherri et al. 2003; Karuppanapandian et al. 2011). Low molecular weight antioxidants like vitamin E, vitamin C, and phenolic acids, etc. are considered most important under inorganic pollutant stress (Kasote et al. 2015). They are also necessary for the defense of cellular components and are substantial scavenger of many ROS but unable to cope with metastable hydroperoxides like reducing radicals (Chaudiere and Ferrari-iliou 1999). In this regard superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase, and catalase participate in ascorbate glutathione cycle, a highly established detoxification system (Noctor 2002; Hasanuzzaman et al. 2019). Ascorbate peroxidase is an important peroxidase to detoxify H_2O_2 acting both in chloroplasts and cytosol using ascorbic acid as a reductant in the ascorbate glutathione cycle (Smirnoff 2000).

Isoflavonoids are well-known SMs for their role in defense against ROS (Posmyk et al. 2009). ROS are the major stress-causing agents under heavy metal and other salt pollution. Antioxidant enzymes including superoxide dismutase, catalase, proline oxidase, ascorbate peroxidase, glutathione peroxidase, and glutathione reductase are the most effective agents for scavenging ROS under metal stress. Phenolic SMs are the substrate for these peroxidase enzymes and found to be the first line of defense regarding various stress factors including HMs and other pollutants (Novak et al. 2004; Posmyk et al. 2009). Glutathione (GSH) is a major form of organic sulfur and also serves as an effective antioxidant in stress conditions (Kang and Kim 2007). Trichomes a specialized type of cells show high enzymatic activities for the synthesis of many phytochelatins and GSH required for the detoxification of the HMs (Tian et al. 2017). GSH directly takes part as an antioxidant in mitigating oxidative stress as well as a reducing agent for other antioxidants like ascorbic acid and scavenge O_3 produced by ROS (Nocito et al. 2002). It also helps plants in detoxification of cytotoxins and xenobiotics by sequestering them to the vacuole (Hasanuzzaman et al. 2017b).

Phenolic compounds are potential antioxidants produced by plants against heavy metal stress (Colak et al. 2019). A lot of studies have been reported about metals induced production of peroxidases and phenolic compounds in plants. The ability of phenolic compounds to alleviate HMs stress is owing to their high propensity to chelate metals. They own carboxyl and hydroxyl groups which make them capable of binding copper and iron (Jung et al. 2003). Yang and Pan (2013) have reported high exudation of phenolic compounds by roots of *Helianthus annuus* L. exposed to HMs. While Moran et al. (1997) described that chelating ability is due to the high nucleophilic property of the aromatic rings rather than specifically due to chelating groups in the molecules. There is another mechanism involved in the antioxidant nature of the phenolic compounds. Heavy metal ions produce lipid alkoxyl-radicals by the hemolytic breakdown of O–O bonds and decomposition of lipid hydroperoxides, which induce free radical-chain oxidation. Phenolic SMs trap these lipid alkoxyl-radicals and help in the inhibition of lipid peroxidation. It depends on the structure, position, and number of hydroxyl groups of the molecule (Milic et al. 1998). Arora et al. (2000) described that phenolic SMs especially flavonoids also have the ability to modify the peroxidation kinetics through lipid packing order. They help in the stabilization of the membranes by reducing their fluidity, restrict peroxidative reactions, and decrease the diffusion of free radicals

(Arora et al. 2000; Blokhina et al. 2003). In addition to their protein binding ability, procyanidins and flavonols interact with membrane phospholipids via hydrogen bonding with polar heads of phospholipids (Verstraeten et al. 2003). Hence, these compounds can get accumulated at the surfaces of the membranes both inside and outside of the cell. This suggests that flavonoids help in maintaining the integrity of the membranes by inhibiting the access of damaging species to the hydrophobic expanse of the bilayer along with others that can affect the rheology of the membrane and can initiate oxidative damage to the cell.

5 Advances in Synthesis of Secondary Metabolites

There are a lot of ways of production of secondary metabolites like plant-based, tissue culture, and strategies like traditional and metabolic engineering (Gonçalves and Romano 2018). With increasing consumer demand for safe product provision, plant-derived product demand is on all-time rise (Lucera et al. 2012). Though chemosynthesis can be used to produce various simple plant products with simple chemical structure but producing SMs like alkaloids via this process is not economical (Stevenson and Szczeklik 2006; Greger 2017). Environmental constraints and restrictions can also limit the extraction of some plant products from naturally grown plants (Yue et al. 2014). Plant farming for the extraction of SMs is a very time-consuming process and plant tissue culture seems to be more appropriate in this regard as it can facilitate mass production of SMs (Isah et al. 2017). Plant culture-based production of SMs is comparably neat, pesticide/herbicide and microbe contamination free system with more efficiency (Verpoorte et al. 2002; Murthy et al. 2014; Ochoa-Villarreal et al. 2016) making them suitable for commercial scale production (Kolewe et al. 2008). In tissue culture-based production of SMs, undifferentiated plant tissue callus is preferred as plant cell is considered as a totipotent in its mechanical and biochemical machinery (Yue et al. 2014). Tissue culture allows us the production of various novel plant-based SMs whose production from native plants was otherwise difficult (Ochoa-Villarreal et al. 2016; de Pádua et al. 2012). Tissue culture has been successfully used to produce SMs like artemisinin (Baldi and Dixit 2008), ajmalicine (Ten Hoopen et al. 2002), taxol (Patil et al. 2014; Sharma and Zafar 2016), resveratrol (Farag and Hassan 2004), and ginsenosides. For improvement in the production of secondary metabolites, nutrient precursor feeding and stimuli provision can boost yield, while other metabolic engineering approaches are also viable to use.

6 Inorganic Pollutant Stress and SMs Production in Plants

The SMs are widely produced by plant species to combat stress and strengthening defense mechanisms (Isah 2019). Among various abiotic stresses, heavy metals are very much potent and persistent in modern-day agriculture. Plants tend to cope with this stress via the production of various SMs in real-time out of which plant hormones are very important. Under abiotic stress, out of all SMs, phytohormones are very important which tend to regulate mineral homeostasis for plants. Phytohormones production (triggered by abiotic stress) results in the regulation of enzymatic activities and SMs production which is helpful in stress mitigation (Wani et al. 2016). Under heavy metal stress, plant tends to regulate the production of abscisic acid (ABA) which acts as a signaling compound regulating gene expression for stress mitigation in plants (O'Brien and Benková 2013). Heavy metals like Cd, Hg, Cu, As turns ABA gene expression on causing a surge in endogenous ABA concentration (Bücker-Neto et al. 2017). Another phytohormone, auxin, or indole acetic acid (IAA) is also helpful in increasing plant growth under normal and stress conditions (Kazan 2013). Similarly, gibberellic acid (GA) is also involved in plant adaption to provide resistance towards various abiotic stresses like HMs (Maggio et al. 2009). Cytokinin (CK) hormone is involved in inter-hormonal signaling under heavy metal stress and sometimes works antagonistically to ABA (Ha et al. 2012). Another phytohormone, salicylic acid (SA) production in plants is also observed to be a defensive response towards heavy metal stress (Rivas-San Vicente and Plasencia 2011) as not only it can detoxify HMs stress but also enhance the activities of antioxidants (Metwally et al. 2003). Ethylene production is enhanced under minute HMs stress, but severe HMs toxicity tends to decrease ethylene production (Gora and Clijsters 1989).

Besides phytohormones, trace metals also alter the composition of other SMs like Ni inhibiting anthocyanin in plants (Hawrylak et al. 2007). A decrease in anthocyanin levels is due to inhibition in the activity of l-phenylalanine ammonia-lyase (Krupa et al. 1996). Copper and Cadmium (Cu and Cd) have been reported to increase shikonin in *Lithospermum* callus cultures upon toxic accumulation (Mizukami et al. 1977). Similarly, Cu and Co stress have been reported to enhance betalains production in *Beta vulgaris* (Trejo-Tapia et al. 2001). Various other heavy metals have been reported to affect the production of SMs which is primarly plant's defensive response against HMs stress. Similarly, heavy metal toxicity also alters SMs production in the medicinal plants where these compounds are responsible for the medicinal properties of plants. A heavy metal derived inactivation of plant metabolites is due to the loss of specific enzymes or damage to biochemical cycles involved in SMs production (Pandey et al. 2007). Nickel toxicity in *Hypericum perforatum* has shown a 15–20 folds' decrease in hyperforin production. While induction of phenolic compounds has been reported in Ni, Al, and Cu toxicity in wheat, maize, and Mascarene Island leaf flower (Winkel-Shirley 2002; Michalak 2006). A chapter summarized by Nasim and Dhir (2009) has reviewed several types of research on the effect of heavy metals on SMs production by medicinal plants and various plant species have been shown to do so.

Heavy metals aim to activate ROS production in plant species upon toxic concentration leading to cellular degradation of biochemical machinery. To cope with ROS production, plants tend to accumulate several antioxidant SMs like flavonoids, lignin, phenolic acids, tocopherol, stilbenes, tannins, and organic acids (Hou et al. 2003). Other antioxidants are enzymatic (catalase, (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR)) and non-enzymatic (glutathione (GSH), ascorbate (AsA), carotenoids) that act as the scavengers of ROS (Michalak 2006; Rastgoo et al. 2011; Sharma et al. 2012). Various studies have reported the regulation of antioxidants by heavy metal stimuli. Bankajj et al. (2016) reported an increased activity of GPX while a decreased activity of CAT and APX in *Atriplex halimus* under Cu stress. Cadmium toxicity in *Kosteletzkya virginica* has caused a net increase in endogenous GSH, tocopherol, SA and GR activities (Han et al. 2012).

7 Exogenous Applications of SMs for Combating Stress

Under abiotic stress like salinity and HM, plants tend to produce various SMs (metabolites, phytohormones, and antioxidants) like IAA, SA, GA, ethylene, CK, brassinosteroids, ascorbates, glutathione, and crotonids. Exogenous application of some of these SMs is helpful for plant to counter abiotic stresses as well.

Phytohormones are essential to plant biochemical machinery products to counter abiotic stress, improve plant growth, and enhance plant defense mechanisms (Sytar et al. 2018). Bali et al. (2019) reported that exogenous application of jasmonic acid activates the production of essential SMs in tomato tissues, triggers stress suppression genes, and detoxifies Pb toxicity. Similarly, exogenous application of auxins to *Arabidopsis thaliana* has shown a remarkable decrease in Cu (Peto et al. 2011; Yuan et al. 2013), Cd (Yuan and Huang 2015), and As (Srivastava et al. 2012) uptake and toxicities. Similarly, the exogenous application of ABA decreased Cd root–shoot translocation in *Arabidopsis thaliana* (Perfus-Barbeoch et al. 2002). Another important class of phytohormones BRs is also helpful in HMs derived stress mitigation in the plant via increasing antioxidants defense as reported for Zn (Arora et al. 2010), Pb (Rady and Osman 2012), and Cr (Choudhary et al. 2011) toxicity. Salicylic acid (SA) has also been reported to have a mitigative effect on barley plants experiencing Cd toxicity (Tamás et al. 2015).

Among various SMs, glutathione is one of the most cited and reported non-enzymatic antioxidants and has a nullification effect on HM toxicity if applied exogenously. Glutathione exogenous application has shown stress mitigation effects against Ni (Khan and Khan 2014; Khan et al. 2016), Cd (Khan et al. 2015, 2016), As (Sakai et al. 2010; Dixit et al. 2016), Zn (Barrameda-Medina et al. 2014; Khan and khan 2014), Cu (Mostofa et al. 2015), Al (Ruiz et al. 2006), and Pb (Yuan and Huang 2015).

8 Summary and Prospects

Plants are considered to be more capable of tolerating the heavy metals and salts pollution in the environment than other organisms. Oxidative metabolism is one of the major mechanisms involved in the tolerance behavior of plants. They have salinity tolerance strategies which also help in conferring the heavy metals toxicity. The organization of enzymatic and non-enzymatic pathways of the antioxidant system helps the plants in providing defense against inorganic pollutants. Phenolic, terpenoid, nitrogen, sulfur, etc. compounds increase tolerance of the plants against various stress elicitors. The accumulation of osmoprotectants, metallothioneins, phytochelatins, etc. regulates the plant's normal metabolism. So, a better understanding of these secondary metabolites pathways, processes, and formations along with their precursor can help to mitigate the inorganic pollutant stress as well as other stress factors on plants. Exogenous application of these SMs and their precursors need a thorough understanding and comprehensive study to evaluate their effects on plant growth. Many studies are present indicating the effects of SMs on plants but controversy is present about their exogenous application and plant-specific effects. Some cases are present where plants showed a decrease in growth on SMs overexpression which needs further research to explore the benefits of SMs regarding their optimum concentration. It is a vast area of research that can help scientists in dealing with heavy metals and salinity stress for plants. Exogenous application or elicitation genes understanding of the SMs can be a novel option in conferring stress in plants.

References

Ali ST, Mahmooduzzafar-Abdin MZ, Iqbal M (2008) Ontogenetic changes in Foliar features and psoralen content of *Psoralea corylifolia* Linn. Exposed to SO_2 stress. J Environ Biol 29:661–668

Alloway BJ (2011) Heavy metals in soil, 3rd edn. Wiley, New York

Andresen E, Peiter E, Küpper H (2018) Trace metal metabolism in plants. J Exp Bot 69:909–954

Angelova Z, Georgiev S, Roos W (2006) Elicitation of plants. Biotechnol Biotechnol Equip 20:72–83. https://doi.org/10.1080/13102818.2006.10817345

Arora A, Byrem TM, Nair MG, Strasburg GM (2000) Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys 373:102–109. https://doi.org/10.1006/abbi.1999.1525

Arora P, Bhardwaj R, Kumar Kanwar M (2010) 24-epibrassinolide induced antioxidative defense system of *Brassica juncea* L. under Zn metal stress. Physiol Mol Biol Plants 16:285–293. https://doi.org/10.1007/s12298-010-0031-9

Asgari Lajayer B, Ghorbanpour M, Nikabadi S (2017) Heavy metals in contaminated environment: destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. Ecotoxicol Environ Saf 145:377–390. https://doi.org/10.1016/j.ecoenv.2017.07.035

Baldi A, Dixit VK (2008) Yield enhancement strategies for artemisinin production by suspension cultures of *Artemisia annua*. Bioresour Technol 99:4609–4614. https://doi.org/10.1016/j.biortech.2007.06.061

Bali S, Jamwal VL, Kohli SK, Kaur P, Tejpal R, Bhalla V, Ohri P, Gandhi SG, Bhardwaj R, Al-Huqail AA, Siddiqui MH, Ali HM, Ahmad P (2019) Jasmonic acid application triggers detoxification of lead (Pb) toxicity in tomato through the modifications of secondary metabolites and gene expression. Chemosphere 235:734–748. https://doi.org/10.1016/j.chemosphere.2019.06.188

Bankajj I, Sleimi N, Gómez-Cadenas A, Pérez-Clemente RM (2016) NaCl protects against Cd and Cu-induced toxicity in the halophyte *Atriplex halimus*. Spanish J Agric Res 14:e0810. https://doi.org/10.5424/sjar/2016144-10117

Barrameda-Medina Y, Montesinos-Pereira D, Romero L, Blasco B, Ruiz JM (2014) Role of GSH homeostasis under Zn toxicity in plants with different Zn tolerance. Plant Sci 227:110–121

Baye H, Hymete A (2009) Lead and cadmium accumulation in medicinal plants collected from environmentally different sites. Bull Environ Contam Toxicol 84:197–201. https://doi.org/10.1007/s00128-009-9916-0

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91:179–194

Brooker N, Windorski J, Blumi E (2008) Halogenated coumarins derivatives as novel seed protectants. Commun Agric Appl Biol Sci 73:81–89

Bücker-Neto L, Paiva ALS, Machado RD, Arenhart RA, Margis-Pinheiro M (2017) Interactions between plant hormones and heavy metals responses. Genet Mol Biol 40:373–386. https://doi.org/10.1590/1678-4685-GMB-2016-0087

Carrubba A, Scalenghe R (2012) The scent of Mare Nostrum: medicinal and aromatic plants in Mediterranean soils. J Sci Food Agric 92:1150–1170. https://doi.org/10.1002/jsfa.5630

Chaiyarat R, Suebsima R, Putwattana N, Kruatrachue M, Pokethitiyook P (2010) Effects of soil amendments on growth and metal uptake by *Ocimum gratissimum* grown in Cd/Zn-contaminated soil. Water Air Soil Pollut 214:383–392. https://doi.org/10.1007/s11270-010-0430-0

Chaudiere J, Ferrari-Iliou R (1999) Intracellular antioxidants: from chemical to biochemical mechanisms. Food Chem Toxicol 37:949–962. https://doi.org/10.1016/s0278-6915(99)00090-3

Choudhary SP, Kanwar M, Bhardwaj R, Gupta BD, Gupta RK (2011) Epibrassinolide ameliorates Cr (VI) stress via influencing the levels of indole-3-acetic acid, abscisic acid, polyamines and antioxidant system of radish seedlings. Chemosphere 84:592–600. https://doi.org/10.1016/j.chemosphere.2011.03.056

Colak N, Torun H, Gruz J, Strnad M, Ayaz FA (2019) Exogenous N-Acetylcysteine alleviates heavy metal stress by promoting phenolic acids to support antioxidant defence systems in wheat roots. Ecotoxicol Environ Saf 181:49–59

Daneshmand F, Arvin MJ, Kalantari KM (2009) Physiological responses to NaCl stress in three wild species of potato in vitro. Acta Physiol Plant 32:91–101. https://doi.org/10.1007/s11738-009-0384-2

De Lurdes Dinis M, Fiúza A (2010) Exposure assessment to heavy metals in the environment: measures to eliminate or reduce the exposure to critical receptors. In: Environmental heavy metal pollution and effects on child mental development, pp 27–50

de Pádua RM, Meitinger N, Filho JDDS, Waibel R, Gmeiner P, Braga FC, Kreis W (2012) Biotransformation of 21-O-acetyl-deoxycorticosterone by cell suspension cultures of *Digitalis lanata* (strain W.1.4). Steroids 77:1373–1380. https://doi.org/10.1016/j.steroids.2012.07.016

Dixit G, Singh AP, Kumar A, Mishra S, Dwivedi S, Kumar S, Trivedi PK, Pandey V, Tripathi RD (2016) Reduced arsenic accumulation in rice (*Oryza sativa* L.) shoot involves sulfur mediated improved thiol metabolism, antioxidant system and altered arsenic transporters. Plant Physiol Biochem 99:86–96

Dutta S, Mitra M, Agarwal P, Mahapatra K, De S, Sett U, Roy S (2018) Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. Plant Signal Behav 13:8

Ebrahim AM, Eltayeb MH, Khalid H, Mohamed H, Abdalla W, Grill P, Michalke B (2012) Study on selected trace elements and heavy metals in some popular medicinal plants from Sudan. J Nat Med 66:671–679. https://doi.org/10.1007/s11418-012-0630-6

Edreva AM, Velikova VB, Tsonev TD (2007) Phenylamides in plants. Russ J Plant Physiol 54:287–301. https://doi.org/10.1134/s1021443707030016

Esmaeilzadeh M, Karbassi A, Bastami KD (2017) Antioxidant response to metal pollution in Phragmites australis from Anzali wetland. Mar Pollut Bull 119:376–380

Farag AM, Hassan MA (2004) Purification, characterization and immobilization of a keratinase from *Aspergillus oryzae*. Enzym Microb Technol 34:85–93. https://doi.org/10.1016/j.enzmictec.2003.09.002

Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (2008) Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr J 23:213–226. https://doi.org/10.1002/ffj.1875

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gonçalves S, Romano A (2018) Production of plant secondary metabolites by using biotechnological tools. In: Vijayakumar R, Raja SSS (eds) Secondary metabolites - sources and applications. IntechOpen. https://doi.org/10.5772/intechopen.76414

Gora L, Clijsters H (1989) Effect of copper and zinc on the ethylene metabolism in *Phaseolus Vulgaris* L. In: Biochemical and physiological aspects of ethylene production in lower and higher plants, pp 219–228

Greger H (2017) Phytocarbazoles: alkaloids with great structural diversity and pronounced biological activities. Phytochem Rev 16:1095–1153. https://doi.org/10.1007/s11101-017-9521-5

Grubb CD, Abel S (2006) Glucosinolate metabolism and its control. Trends Plant Sci 11:89–100. https://doi.org/10.1016/j.tplants.2005.12.006

Ha S, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2012) Cytokinins: metabolism and function in plant adaptation to environmental stresses. Trends Plant Sci 17:172–179. https://doi.org/10.1016/j.tplants.2011.12.005

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333. https://doi.org/10.1146/annurev.arplant.57.032905.105228

Han R-M, Lefèvre I, Albacete A, Pérez-Alfocea F, Barba-Espín G, Díaz-Vivancos P, Quinet M, Ruan CJ, Hernández JA, Cantero-Navarro E, Lutts S (2012) Antioxidant enzyme activities and hormonal status in response to Cd stress in the wetland halophyte *Kosteletzkya virginica* under saline conditions. Physiol Plant 147:352–368

Hasanuzzaman M, Nahar K, Anee TI, Fujita M (2017a) Exogenous silicon attenuates cadmium-induced oxidative stress in *Brassica napus* L. by modulating AsA-GSH pathway and glyoxalase system. Front Plant Sci 8:1061. https://doi.org/10.3389/fpls.2017.01061

Hasanuzzaman M, Nahar K, Anee TI, Fujita M (2017b) Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiol Mol Biol Plants 23:249–268

Hasanuzzaman M, Bhuyan MHMB, Anee TI, Parvin K, Nahar K, Al Mahmud J, Fujita M (2019) Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants 8:384

Hatami M, Ghorbanpour M (2016) Retracted chapter: changes in phytochemicals in response to rhizospheric microorganism infection. In: Microbial-mediated induced systemic resistance in plants, pp 1–14

Hawrylak B, Matraszek R, Szymańska M (2007) Response of lettuce (*Lactuca Sativa* L.) to selenium in nutrient solution contaminated with nickel. Veg Crop Res Bull 67:63–70. https://doi.org/10.2478/v10032-007-0031-7

Heitefuss R (2011) Functions and biotechnology of plant secondary metabolites, 2nd edn, annual plant reviews, vol 39. J Phytopathol 159:72–72

Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY, Lee MH (2003) Free radical-scavenging activity of Taiwanese native plants. Phytomedicine 10:170–175. https://doi.org/10.1078/094471103321659898

Hu J, Fang H, Wang J, Yue X, Su M, Mao Z, Zou Q, Jiang H, Guo Z, Yu L, Feng T, Lu L, Peng Z, Zhang Z, Wang N, Chen X (2020) Ultraviolet B-induced MdWRKY72 expression promotes anthocyanin synthesis in apple. Plant Sci 292:110377

Isah T (2019) Stress and defense responses in plant secondary metabolites production. Biol Res 52:39. https://doi.org/10.1186/s40659-019-0246-3

Isah T, Umar S, Mujib A, Sharma MP, Rajasekharan PE, Zafar N, Frukh A (2017) Secondary metabolism of pharmaceuticals in the plant in vitro cultures: strategies, approaches, and limitations to achieving higher yield. Plant Cell Tissue Organ Cult 132:239–265. https://doi.org/10.1007/s11240-017-1332-2

Jacobsen S, Hauschild MZ, Rasmussen U (1992) Induction by chromium ions of chitinases and polyamines in barley (*Hordeum vulgare* L.) and rape (*Brassica napus* L. ssp. oleifera). Plant Sci 84:119–128. https://doi.org/10.1016/0168-9452(92)90125-6

Jiang S, Han S, He D, Cao G, Fang K, Xiao X, Yi J, Wan X (2019) The accumulation of phenolic compounds and increased activities of related enzymes contribute to early defense against walnut blight. Physiol Mol Plant Pathol 108:101433

Jung C, Maeder V, Funk F et al (2003) Release of phenols from *Lupinus albus* L. roots exposed to Cu and their possible role in Cu detoxification. Plant and Soil 252:301–312. https://doi.org/10.1023/a:1024775803759

Kang SY, Kim YC (2007) Decursinol and decursin protect primary cultured rat cortical cells from glutamate-induced neurotoxicity. J Pharm Pharmacol 59:863–870. https://doi.org/10.1211/jpp.59.6.0013

Kapoor D, Singh S, Kumar V, Romero R, Prasad R, Singh J (2019) Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene 19:100182

Karuppanapandian T, Moon JC, Kim C, Manoharan K, Kim W (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci 5:709–725

Kasote DM, Katyare SS, Hegde MV, Bae H (2015) Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 11:982–991

Kazan K (2013) Auxin and the integration of environmental signals into plant root development. Ann Bot 112:1655–1665. https://doi.org/10.1093/aob/mct229

Khan MIR, Khan NA (2014) Ethylene reverses photosynthetic inhibition by nickel and zinc in mustard through changes in PSII activity, photosynthetic nitrogen use efficiency, and antioxidant metabolism. Protoplasma 251:1007–1019

Khan MIR, Nazir F, Asgher M, Per TS, Khan NA (2015) Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. J Plant Physiol 173:9–18

Khan NA, Asgher M, Per TS, Masood A, Fatma M, Khan MIR (2016) Ethylene potentiates sulfur-mediated reversal of cadmium inhibited photosynthetic responses in mustard. Front Plant Sci 7:1628

Kolewe ME, Gaurav V, Roberts SC (2008) Pharmaceutically active natural product synthesis and supply via plant cell culture technology. Mol Pharm 5:243–256. https://doi.org/10.1021/mp7001494

Kovacik J, Klejdus B, Hedbavny J, Stork F, Backor M (2009a) Comparison of cadmium and copper effect on phenolic metabolism, mineral nutrients and stress-related parameters in *Matricaria chamomilla* plants. Plant and Soil 320:231–242

Kovacik J, Klejdus B, Backor M (2009b) Phenolic metabolism of *Matricaria chamomilla* plants exposed to nickel. J Plant Physiol 166:1460–1464

Krupa Z, Baranowska M, Orzol D (1996) Can anthocyanins be considered as heavy metal stress indicator in higher plants. Acta Physiol Plant 18:147–151

Lake JA, Field KJ, Davey MP, Beerling DJ, Lomax BH (2009) Metabolomic and physiological responses reveal multi-phasic acclimation of *Arabidopsis thaliana* to chronic UV radiation. Plant Cell Environ 32:1377–1389. https://doi.org/10.1111/j.1365-3040.2009.02005.x

Li QS, Cai SS, Mo CH, Chu B, Peng LH, Yang FB (2010) Toxic effects of heavy metals and their accumulation in vegetables grown in a saline soil. Ecotoxicol Environ Saf 73:84–88. https://doi.org/10.1016/j.ecoenv.2009.09.002

Lin CC, Kao CH (1999) Excess copper induces an accumulation of putrescine in rice leaves. Bot Bull Acad Sin 40:213–218

Lucera A, Costa C, Conte A, Del Nobile MA (2012) Food applications of natural antimicrobial compounds. Front Microbiol 3:287. https://doi.org/10.3389/fmicb.2012.00287

Maggio A, Barbieri G, Raimondi G, De Pascale S (2009) Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity. J Plant Growth Regul 29:63–72. https://doi.org/10.1007/s00344-009-9114-7

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158. https://doi.org/10.1016/j.abb.2005.10.018

Maleki M, Ghorbanpour M, Kariman K (2017) Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. Plant Gene 11:247–254

Mazid, M., Khan, T.A. and Mohammad, F., 2011. Role of secondary metabolites in defense mechanisms of plants. Biology and medicine, 3(2), pp.232-249.

McMurry JE (2014) Organic chemistry with biological applications. Cengage Learning

Megateli S, Semsari S, Couderchet M (2009) Toxicity and removal of heavy metals (cadmium, copper, and zinc) by *Lemna gibba*. Ecotoxicol Environ Saf 72:1774–1780. https://doi.org/10.1016/j.ecoenv.2009.05.004

Metwally A, Finkemeier I, Georgi M, Dietz K-J (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol 132:272–281. https://doi.org/10.1104/pp.102.018457

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish J Environ Stud 15:523–530

Milic BL, Djilas SM, Čanadanović-Brunet JM (1998) Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system. Food Chem 61:443–447. https://doi.org/10.1016/s0308-8146(97)00126-x

Mizukami H, Konoshima M, Tabata M (1977) Effect of nutritional factors on shikonin derivative formation in *Lithospermum callus* cultures. Phytochemistry 16:1183–1186. https://doi.org/10.1016/s0031-9422(00)94356-5

Moran JF, Klucas RV, Grayer RJ, Abian J, Becana M (1997) Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. Free Radic Biol Med 22:861–870. https://doi.org/10.1016/s0891-5849(96)00426-1

Mostofa MG, Hossain MA, Fujita M, Tran LSP (2015) Physiological and biochemical mechanisms associated with trehalose-induced copper-stress tolerance in rice. Sci Rep 5:11433. https://doi.org/10.1038/srep11433

Murthy HN, Lee E-J, Paek K-Y (2014) Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant Cell Tissue Organ Cult 118:1–16. https://doi.org/10.1007/s11240-014-0467-7

Muthukumarasamy M, Gupta SD, Pannerselvam R (2000) Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. Biol Plant 43:317–320

Mutlu F, Bozcuk S (2007) Salinity-induced changes of free and bound polyamine levels in sunflower (*Helianthus annuus* L.) roots differing in salt tolerance. Pak J Bot 39:1097–1102

Nagegowda DA (2010) Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation. FEBS Lett 584:2965–2973. https://doi.org/10.1016/j.febslet.2010.05.045

Nagegowda DA, Gupta P (2020) Advances in biosynthesis, regulation, and metabolic engineering of plant specialized terpenoids. Plant Sci 294:110457

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiol 149:88–95

Nasim SA, Dhir B (2009) Heavy metals alter the potency of medicinal plants. Rev Environ Contam Toxicol:139–149

Navarro J, Flores P, Garrido C, Martinez V (2006) Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chem 96:66–73. https://doi.org/10.1016/j.foodchem.2005.01.057

Ncube B, Staden JV (2015) Tilting plant metabolism for improved metabolite biosynthesis and enhanced human benefit. Molecules 20:12698–12731

Nocito FF, Pirovano L, Cocucci M, Sacchi GA (2002) Cadmium-induced sulfate uptake in maize roots. Plant Physiol 129:1872–1879. https://doi.org/10.1104/pp.002659

Noctor G (2002) Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. J Exp Bot 53:1283–1304. https://doi.org/10.1093/jexbot/53.372.1283

Noctor G, Mhamdi A, Foyer CH (2014) The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol 164:1636–1648. https://doi.org/10.1104/pp.113.233478

Novak K, Lisa L, Skrdleta V (2004) Rhizobial nod gene-inducing activity in pea nodulation mutants: dissociation of nodulation and flavonoid response. Physiol Plant 120:546–555. https://doi.org/10.1111/j.0031-9317.2004.0278.x

O'Brien JA, Benková E (2013) Cytokinin cross-talking during biotic and abiotic stress responses. Front Plant Sci 4:451. https://doi.org/10.3389/fpls.2013.00451

Ochoa-Villarreal M, Howat S, Hong SM, Jang MO, Jin YW, Lee EK, Loake GJ (2016) Plant cell culture strategies for the production of natural products. BMB Rep 49:149–158. https://doi.org/10.5483/bmbrep.2016.49.3.264

Oksman-Caldentey K-M, Inzé D (2004) Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. Trends Plant Sci 9:433–440. https://doi.org/10.1016/j.tplants.2004.07.006

Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK (2015) Secondary metabolites of plants and their role: overview. Curr Trends Biotechnol Pharm 9:293–304

Pandey S, Gupta K, Mukherjee AK (2007) Impact of cadmium and lead on *Catharanthus roseus* – a phytoremediation study. J Environ Biol 28:655–662

Pandey P, Irulappan V, Bagavathiannan MV, Senthil-Kumar M (2017) Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front Plant Sci 8:537

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324–349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Patil RA, Lenka SK, Normanly J, Walker EL, Roberts SC (2014) Methyl jasmonate represses growth and affects cell cycle progression in cultured Taxus cells. Plant Cell Rep 33:1479–1492. https://doi.org/10.1007/s00299-014-1632-5

Pedranzani H, Racagni G, Alemano S, Miersch O, Ramirez I, Pena-Cortes H, Taleisnik E, Machado-Domenech E, Abdala G (2003) Salt tolerant tomato plants show increased levels of jasmonic acid. Plant Growth Regul 412:149–158

Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548. https://doi.org/10.1046/j.1365-313X.2002.01442.x

Peto A, Lehotai N, Lozano-Juste J, León J, Tari I, Erdei L, Kolbert Z (2011) Involvement of nitric oxide and auxin in signal transduction of copper-induced morphological responses in *Arabidopsis* seedlings. Ann Bot 108:449–457. https://doi.org/10.1093/aob/mcr176

Petrusa LM, Winicov I (1997) Proline status in salt tolerant and salt sensitive alfalfa cell lines and plants in response to NaCl. Plant Physiol Biochem 35:303–310

Popović BM, Štajner D, Ždero-Pavlović R, Tumbas-Šaponjac V, Čanadanović-Brunet J, Orlović S (2016) Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (*Populus spp*.). Plant Physiol Biochem 105:242–250

Posmyk MM, Kontek R, Janas KM (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicol Environ Saf 72:596–602. https://doi.org/10.1016/j.ecoenv.2008.04.024

Rady MM, Osman AS (2012) Response of growth and antioxidant system of heavy metal-contaminated tomato plants to 24-epibrassinolide. Afr J Agric Res 7:3249–3254. https://doi.org/10.5897/ajar12.079

Ramakrishna A, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav 6:1720–1731. https://doi.org/10.4161/psb.6.11.17613

Rastgoo L, Alemzadeh A, Afsharifar A (2011) Isolation of two novel isoforms encoding zinc- and copper-transporting P1B-ATPase from Gouan (*Aeluropus littoralis*). Plant Omics J 4:377–383

Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot 62:3321–3338. https://doi.org/10.1093/jxb/err031

Roy S (2016) Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. Plant Signal Behav 11:e1117723

Ruiz JM, Rivero RM, Boron LR (2006) Increases synthesis of glutathione in sunflower plants subjected to aluminum stress. Plant Soil 279:25–30

Saha JK, Selladurai R, Coumar MV, Dotaniya ML, Kundu S, Patra AK (2017) Major inorganic pollutants affecting soil and crop quality. In: Soil pollution - an emerging threat to agriculture. Environmental Chemistry for a Sustainable World. Springer, pp 75–104

Saito K (2004) Sulfur assimilatory metabolism. The long and smelling road. Plant Physiol 136:2443–2450. https://doi.org/10.1104/pp.104.046755

Sakai Y, Watanabe T, Wasaki J, Senoura T, Shinano T, Osaki M (2010) Influence of arsenic stress on synthesis and localization of low-molecular-weight thiols in *Pteris vittata*. Environ Pollut 158:3663–3669

Sarma H, Deka S, Deka H, Saikia RR (2011) Accumulation of heavy metals in selected medicinal plants. Rev Environ Contam Toxicol:63–86

Saviranta NMM, Julkunen-Tiitto R, Oksanen E, Karjalainen RO (2010) Leaf phenolic compounds in red clover (*Trifolium pratense* L.) induced by exposure to moderately elevated ozone. Environ Pollut 158:440–446. https://doi.org/10.1016/j.envpol.2009.08.029

Schafer H, Wink M (2009) Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. Biotechnol J 4:1684–1703. https://doi.org/10.1002/biot.200900229

Schreck E, Foucault Y, Sarret G, Sobanska S, Cécillon L, Castrec-Rouelle M, Uzu G, Dumat C (2012) Metal and metalloid foliar uptake by various plant species exposed to atmospheric industrial fallout: mechanisms involved for lead. Sci Total Environ 427–428:253–262. https://doi.org/10.1016/j.scitotenv.2012.03.051

Sgherri C, Cosi E, Navari-Izzo F (2003) Phenols and antioxidative status of *Raphanus sativus* grown in copper excess. Physiol Plant 118:21–28. https://doi.org/10.1034/j.1399-3054.2003.00068.x

Shahid M, Dumat C, Khalid S, Schreck E, Xiong T, Niazi NK (2017) Foliar heavy metal uptake, toxicity and detoxification in plants: a comparison of foliar and root metal uptake. J Hazard Mater 325:36–58. https://doi.org/10.1016/j.jhazmat.2016.11.063

Sharma SS, Dietz K-J (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50. https://doi.org/10.1016/j.tplants.2008.10.007

Sharma K, Zafar R (2016) Optimization of methyl jasmonate and β-cyclodextrin for enhanced production of taraxerol and taraxasterol in (*Taraxacum officinale* Weber) cultures. Plant Physiol Biochem 103:24–30. https://doi.org/10.1016/j.plaphy.2016.02.029

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012:1–26. https://doi.org/10.1155/2012/217037

Siemens DH, Garner SH, Mitchell-Olds T, Callaway RM (2002) Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. Ecology 83:505. https://doi.org/10.2307/2680031

Singh A, Dwivedi P (2018) Methyl-jasmonate and salicylic acid as potent elicitors for secondary metabolite production in medicinal plants: a review. J Pharmacogn Phytochem 7:750–757

Smirnoff N (2000) Ascorbic acid: metabolism and functions of a multi-facetted molecule. Curr Opin Plant Biol 3:229–235. https://doi.org/10.1016/s1369-5266(00)00069-8

Sreevidya V (2006) Metabolic engineering of rice with soybean isoflavone synthase for promoting nodulation gene expression in rhizobia. J Exp Bot 57:1957–1969. https://doi.org/10.1093/jxb/erj143

Srivastava S, Srivastava AK, Suprasanna P, D'Souza SF (2012) Identification and profiling of arsenic stress-induced microRNAs in *Brassica juncea*. J Exp Bot 64:303–315. https://doi.org/10.1093/jxb/ers333

Stevenson D, Szczeklik A (2006) Clinical and pathologic perspectives on aspirin sensitivity and asthma. J Allergy Clin Immunol 118:773–786. https://doi.org/10.1016/j.jaci.2006.07.024

Street RA (2012) Heavy metals in medicinal plant products — an African perspective. S Afr J Bot 82:67–74. https://doi.org/10.1016/j.sajb.2012.07.013

Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2018) Phytohormone priming: regulator for heavy metal stress in plants. J Plant Growth Regul 38:739–752. https://doi.org/10.1007/s00344-018-9886-8

Taiz L, Zeiger E (2006) Plant physiology, vol 82, 4th edn. Sinauer Associates Publishers, Sunderland, pp 278–279. https://doi.org/10.1086/523151

Tamás L, Mistrík I, Alemayehu A, Zelinová V, Bočová B, Huttová J (2015) Salicylic acid alleviates cadmium-induced stress responses through the inhibition of Cd-induced auxin-mediated reactive oxygen species production in barley root tips. J Plant Physiol 173:1–8. https://doi.org/10.1016/j.jplph.2014.08.018

Ten Hoopen HJG, Vinke JL, Moreno PRH, Verpoorte R, Heijnen JJ (2002) Influence of temperature on growth and ajmalicine production by *Catharanthus roseus* suspension cultures. Enzym Microb Technol 30:56–65. https://doi.org/10.1016/s0141-0229(01)00456-2

Tian N, Liu F, Wang P, Zhang X, Li X, Wu G (2017) The molecular basis of glandular trichome development and secondary metabolism in plants. Plant Gene 12:1–12

Torre-González AD, Montesinos-pereira D, Blasco B, Ruiz JM (2018) Influence of the proline metabolism and glycine betaine on tolerance to salt stress in tomato (*Solanum lycopersicum* L.) commercial genotypes. J Plant Physiol 231:329–336

Trejo-Tapia G, Jimenez-Aparicio A, Rodriguez-Monroy M, De Jesus-Sanchez A, Gutierrez-Lopez G (2001) Influence of cobalt and other microelements on the production of betalains and the growth of suspension cultures of *Beta vulgaris*. Plant Cell Tissue Organ Cult 67:19–23. https://doi.org/10.1023/a:1011684619614

Truong HA, Lee WJ, Jeong CY, Trịnh CS, Lee S, Kang CS, Cheong YK, Hong SW, Lee H (2018) Enhanced anthocyanin accumulation confers increased growth performance in plants under low nitrate and high salt stress conditions owing to active modulation of nitrate metabolism. J Plant Physiol 231:1–48

Tuteja N (2007) Mechanisms of high salinity tolerance in plants. Methods Enzymol:419–438

Tuteja N, Sopory SK (2008) Chemical signaling under abiotic stress environment in plants. Plant Signal Behav 3:525–536. https://doi.org/10.4161/psb.3.8.6186

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1:13–25. https://doi.org/10.1023/a:1015871916833

Verstraeten SV, Keen CL, Schmitz HH, Fraga CG, Oteiza PI (2003) Flavan-3-ols and procyanidins protect liposomes against lipid oxidation and disruption of the bilayer structure. Free Radic Biol Med 34:84–92. https://doi.org/10.1016/s0891-5849(02)01185-1

Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176. https://doi.org/10.1016/j.cj.2016.01.010

Wink M (1999) Functions of plant secondary metabolites and their exploitation in biotechnology. In: Annual plant reviews, vol 3. CRC Press, Boca Raton

Winkel-Shirley B (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol 126:485–493. https://doi.org/10.1104/pp.126.2.485

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol 5:218–223. https://doi.org/10.1016/s1369-5266(02)00256-x

Xiong T, Austruy A, Pierart A, Shahid M, Schreck E, Mombo S, Dumat C (2016a) Kinetic study of phytotoxicity induced by foliar lead uptake for vegetables exposed to fine particles and implications for sustainable urban agriculture. J Environ Sci 46:16–27. https://doi.org/10.1016/j.jes.2015.08.029

Xiong T, Dumat C, Pierart A, Shahid M, Kang Y, Li N, Bertoni G, Laplanche C (2016b) Measurement of metal bioaccessibility in vegetables to improve human exposure assessments:

field study of soil–plant–atmosphere transfers in urban areas, South China. Environ Geochem Health 38:1283–1301. https://doi.org/10.1007/s10653-016-9796-2

Yang J, Pan X (2013) Root exudates from sunflower (*Helianthus annuus* L.) show a strong adsorption ability toward Cd (II). J Plant Interact 8:263–270

Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q (2018) Response of plant secondary metabolites to environmental factors. Molecules 24:762

Yuan HM, Huang X (2015) Inhibition of root meristem growth by cadmium involves nitric oxide-mediated repression of auxin accumulation and signalling in *Arabidopsis*. Plant Cell Environ 39:120–135. https://doi.org/10.1111/pce.12597

Yuan HM, Xu HH, Liu WC, Lu YT (2013) Copper regulates primary root elongation through pin1-mediated auxin redistribution. Plant Cell Physiol 54:766–778. https://doi.org/10.1093/pcp/pct030

Yue W, Ming Q, Lin B, Rahman K, Zheng CJ, Han T, Qin LP (2014) Medicinal plant cell suspension cultures: pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. Crit Rev Biotechnol 36:215–232. https://doi.org/10.3109/07388551.2014.923986

Zhang Y, Wang G, Li L, Li Y, Zhou B, Yan H (2020) Identification and expression analysis of BrTT8 during anthocyanin biosynthesis and exposure to abiotic stress in turnip (*Brassica rapa* subsp. rapa ‘Tsuda’). Sci Hortic 268:109332

Zheljazkov VD, Craker LE, Xing B (2006) Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. Environ Exp Bot 58:9–16. https://doi.org/10.1016/j.envexpbot.2005.06.008

Zheljazkov VD, Jeliazkova EA, Kovacheva N, Dzhurmanski A (2008a) Metal uptake by medicinal plant species grown in soils contaminated by a smelter. Environ Exp Bot 64:207–216. https://doi.org/10.1016/j.envexpbot.2008.07.003

Zheljazkov VD, Craker LE, Xing B, Nielsen NE, Wilcox A (2008b) Aromatic plant production on metal contaminated soils. Sci Total Environ 395:51–62. https://doi.org/10.1016/j.scitotenv.2008.01.041

Zheng T, Li Y, Lei W, Qiao K, Liu B, Zhang D, Lin H (2020) SUMO E3 ligase SIZ1 stabilizes MYB75 to regulate anthocyanin accumulation under high light conditions in *Arabidopsis*. Plant Sci 292:110355

Bicarbonate Toxicity and Elevated pH in Plants: Metabolism, Regulation and Tolerance

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and Malay Kumar Adak

Contents

Abstract

Bicarbonate (HCO_3^-) in the lime soil may be accepted as an alternative carbon source in plant system. Few plant species, particularly semi-aquatic or aquatic have their specific pathways to exercise the HCO_3^- in photosynthetic reactions. The accumulation of HCO_3^- is quite efficient over a threshold value in elevation of pH. Apart from the carbon dioxide concentration mechanism (CCM) in plants, HCO_3^- concentration is a pH indicator. In CO_2 depleted ecosystem for cyanobacteria, micro algae and even in few aquatic macrophytes, HCO_3^- is a readily available source for CO_2 concentration mechanism as DIC (Dissolved

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Inorganic Carbon). Through the biological membrane of those species the uptake of HCO_3^- is posed with any doubtful transporter. Therefore, metabolic fates of HCO_3^- with reference to pH tolerance are a worth of study. The most important regulatory paths of HCO_3^- tolerance are discussed with reactions of hydration of CO_2. Along with carbonic anhydrase, phosphoenolpyruvate carboxylase and concentration gradient of CO_2/HCO_3^- would be a choice for a plant for stomatal regulation under drought stress. This review may cover the soil alkalinity through dissolved HCO_3^- as well as induced osmotic stress in plants with various aspects of physiology. The knowledge gap of uptake, movement through cellular spaces and metabolism in crops in alkaline soil may open up the possibilities of CCM and HCO_3^- resistance related traits.

Keywords

Bicarbonate toxicity · Alkaline soil · Dissolved inorganic carbon · Carbon dioxide concentration mechanism · Aquatic species

Abbreviations

AMP	Adenosine monophosphate
AQP	Aquaporins
ATP	Adenosine triphosphate
CAM	Crassulacean acid metabolism
RuBisCO	Ribulose-1,5-bisphosphate carboxylase

1 Introduction

The terrestrial crops/plants regardless of carbon dioxide concentration mechanism (CCM) universally accept gaseous CO_2 as a predominant source for primary metabolite biosynthesis. Even in aquatic species dissolved CO_2 also would be a source for carbon, however, in different forms, an inorganic salt (HCO_3^-) being the predominant. The vegetation on earth is principally based on light mediated chemical transformation of inorganic carbon and water into reduced *vis-à-vis* organic residues (Deirmendjian et al. 2019). The reduced carbon in turn is oxidized into CO_2 and energy for the heterotrophic organisms. The terrestrial photosynthetic plants beside their atmospheric CO_2, the main source of inorganic carbon are also benefited by dissolved CO_2 in aqueous medium as carbonic acids (H_2CO_3) (Rubio and Fernández 2019). This carbonic acid undergoes hydrolysis into bicarbonate (HCO_3^-) and carbonate (CO_3^{-2}). Sea water is the richest source of inorganic carbon as HCO_3^- through the course of evolution. The HCO_3^- concentration in ocean gradually increased due to atmospheric CO_2 elevation that manifests into acidification as well as more solubility of carbonate under acidic condition. Photosynthetic algae and fresh water plant species are more accessed of this abundant HCO_3^- to make use of organic carbon (Jull et al. 2019). In soil the lime stones are the predominant source of HCO_3^- (Devaraj et al. 2018). A higher HCO_3^- soil is now observed over their CO_2 gas which is based on CO_3^{-2} solubility in constant rate in soil solution (Terés et al. 2019). Even plant biological based activity is facilitated by hydration of CO_2 from air as well as the respiratory reactions in underground plant parts, microorganisms and other related soil fauna by HCO_3^-. The CO_2 hydration reaction in soil is facilitated by carbonic anhydrase on the micro-fauna like algae and cyanobacteria (Maheshwari et al. 2019).

The cyanobacterial and allied groups are the most efficient users of HCO_3^- in their aqueous environment for photosynthesis (Beck et al. 2019). On the contrary, the terrestrial or land plants are in general affected with high HCO_3^- concentration that may be detrimental for those with special reference to the calcifuges species sensitive. It is particularly with calciferous soil where the alkaline pH may hinder the essential nutrients like Fe (iron) and few micronutrients as those come on precipitate in the form of carbonate (Zanin et al. 2019). The chlorosis of leaves under such lime soil often leads to low productivity, predominantly in sensitive species. Among the crops, few horticultural species like *Citrus*, deciduous fruit trees, grapes and few pulses are affected with low Fe assimilation since the soil precipitate as HCO_3^- species of Fe. phytosiderophore would be the most common those are related by adventitious roots of many members of Gramineae (Lynch 2019). Still, cereal crops like *Oryza sativa*, *Zea mays*, *Hordeum vulgare* and *Triticum aestivum* are often affected with some micronutrient deficiency on such calciferous soil.

2 Mobilization of HCO_3^- into Plant Tissues

HCO_3^- is the anion which finds some resistance for permeability to the cell membrane of lipid bi-layer. There is specific transporter for HCO_3^- transport over the membrane or transmembrane diffusion of CO_2 which is converted to HCO_3^- using the specific enzyme like carbonic anhydrase (Su et al. 2019). Rapid diffusion of CO_2 through plant cell membrane through the lipid bi-layer sometimes possesses very specific channel gated proteins. AQP and other related proteins could be few of those to carry CO_2 over the membrane (Torralbo et al. 2019). People suggest a permeability of 3.6 cm/s over the membrane for each HCO_3^- molecules to cross. It is not possible for CO_2 which is transported through AQP or other transporter proteins (Pawłowicz and Masajada 2019). On the cell membrane, CO_2 diffusion is offered with some resistance that is depended on membrane discretion such as composition and its functioning. Few AQP in specific plant cells can enhance the CO_2 diffusion into stomatal guard cell as well as related mesophyll tissues. In Cyanophyceae and higher algae, spermatophytes report the relative distribution of CO_2 diffusion path through the biological membrane which follows downhill non-energetic to transfer of CO_2 (Lines and Beardall 2018). HCO_3^- being a charged residue is offered to electro potential variance/gradient which drives the plasma membrane ion transport. These are existing negative electrochemical potential on the inner surface of plant cell. The uphill HCO_3^- movement is odd to be with any energy devised link. In membranes of Cyanophyceae, few microalgae and few species of Rhodophyceae the

HCO_3^- pumps are reported in less frequent (Roleda and Hurd 2019). Specific ion channels occur in those cells where efflux of HCO_3^- is followed with a downhill movement. In *Arabidopsis thaliana* L., the hypocotyls cells are possessed with specific anion channel with their moderate selectivity for HCO_3^-. Those anion channels are co-transporters for nitrate, sulphate, malate, chloride, etc. Typically, *Chlamydomonas sp.* with their inbuilt acquisition of specific anion channels within inner membrane of chloroplast gives the access for HCO_3^- (Mishra et al. 2018). Therefore, for CCM, anion channels for HCO_3^- are distinguished features or even it play a biomarker for HCO_3^- abundance in microalgae.

In most of the cases metabolism of HCO_3^- is well understood when aquatic photosynthetic organisms are concerned. There has been a good citation of occurrence of HCO_3^- in the perspective of ecological and physiological levels. The dissolved CO_2 in the water system is absorbed by the plants with a slow diffusion rate, particularly, in unstirred layers (Gwak et al. 2019). There reported three possible factors for HCO_3^- metabolism in aquatic plants. Those are to establish the mechanism of function dependant CO_2 assimilation rate (from HCO_3^-) with changes in pH. The kinetic relationship for CO_2 to HCO_3^- in aqueous system and finally its experimental modalities is found out with the discriminatory rates of ^{12}C and ^{13}C in CO_2 and HCO_3^- fixation. From the taxon level there recorded various groups of aquatic photosynthetic organisms where cryptogam species like bryophytes and early vascular species like pteridophytes are the exceptional. Still, in few Chlorophyceae (*Chara* spp.) and higher species the fates of HCO_3^- in flax as well as OH^- efflux are spatially separated. As, for example, in *Chara* spp. the polar leaves are more efficient in this separation for HCO_3^- and OH^-. Regardless of aquatic species there revealed few distinct mechanisms for HCO_3^- assimilation (Hang and Wu 2019). This includes an H^+/HCO_3^- symport that increases the acidification with HCO_3^- from external environment into CO_2. This reaction is coupled in a reversible manner in conjugation of HCO_3^--CO_2 (Williams et al. 2019). The typical enzyme in this reverse reaction is carbonic anhydrase. In this reaction the cellular paths like transfer cells or symplast (plasmalemma) are most important and found in the polar leaves where HCO_3^- sensitivity becomes a key factor for a transmembrane pass into CO_2 fixation plants, particularly, for harsh habit species. Those often face with the constraint of prolonged drought. In particular lime soil the drought condition becomes severe with alkaline pH when plants have to regulate their stomata and *vis-à-vis* restricts gaseous carbon in photosynthesis (Donzia et al. 2018). HCO_3^- in such lime soil acts as an inorganic carbon to support the photosynthesis. The most popular method is the carbon isotopes ($^{13}C/^{14}C$) technique to decipher the metabolic consumption of HCO_3^- carbon. Few plants have shown their choice over HCO_3^- carbon than gaseous one for active photosynthesis. The inter-conversion of HCO_3^- into dry matter/biomass is facilitated by activity of carbonic anhydrase and thus this enzyme becomes a physiological marker to set the biomass production for energy. Still, the behaviour of carbonic anhydrase in different plant species has not been yet completely deciphered with its discriminating nature of activity. But it is hypothesized that carbonic anhydrase activity and its over accumulation under drought stress in production of water (H_2O) and CO_2

from HCO_3^- and its equilibrium in soil could be a factor for plant sustain. A number of plant species within C_3, C_4, C_3-C_4 intermediate have shown insights for physiological behaviour of carbonic anhydrase under stress conditions where limitations of CO_2 are a key factor. Likewise few plant species were analysed in biomass, through biofuel energy productions are noted in *Euphorbia lathyris*, *Orychophragmus violaceus*, *Brassica juncea etc.* Still the additional informations requires where biomass production is also compartmentalized into biofuel under constrains of environmental stress which may collaborate metabolism of HCO_3^- as a function with carbonic anhydrase activity. Carbon is most important for maintenance of a standard equilibrium between plasma membrane and aquatic bodies. HCO_3^- is not discriminated from molecular CO_2 or even un-disassociated carbonic acids over their root membrane in transport. The utilization of HCO_3^- in photosynthetic carbon source must involve pump and that is supported by ample evidences. Therefore, hydrolysis of ATP is occured to facilitate the DIC transportation and hence, undoubtedly HCO_3^- acts as a DIC source for carbon fixation and the active transport mechanism. CO_2 when dissolved in water follows the normal laws and regulations for solubility. The partition coefficient value with regard to solubility of CO_2 refers the distribution ratio of CO_2 dissolved in water to air (Prelovšek et al. 2018). It is almost reached with the values as unity within the temperature gradient (10–20 °C) that reaches the values of 12.3 μM. On the other hand in water the values within 10.8 μM, still, dissolved CO_2 may have to face either ionized or non-ionized states. So, the concentration of dissolved CO_2 in water is trailed by fair significant amount of the same at air. The availability of CO_2 from HCO_3^- is pH dependent, equilibrium constant with respect to water, total alkalinity, etc. (Krüger et al. 2019). The quantity of dissolved CO_2 in water is still validated according to soil types as well as the variation is quite significant of aquatic macrophytes/microphytes of same ecosystem. HCO_3^- absorption and utilization by plant is a significant slow process of both CO_2 and O_2. Both the gaseous CO_2 and O_2 are trailed by several folds, however, less in diffusion through water than air. For ideal photosynthetic conditions where gaseous CO_2 as only the inorganic carbon source is acquired with low tissue concentration for CO_2 but high of O_2 poses a severe problem (Saha et al. 2018a, b). The later is focused with a subdued photosynthetic rate coupled with high rates of photorespiration (Dusenge et al. 2019). The importance of slow diffusion rates under any stress even in turbid water solution as compared to unstirred environment of plants sets strategy to check the photorespiration in case of higher plants as well as aquatic plants (Saha et al. 2019). The boundary layer of water on leaf surface may play an important role herein to limit any physical entry of the gases through leaf surfaces. Therefore, HCO_3^- toxicity becomes a factor to focus the CO_2 concentration in soil water ecosystem. Therefore, from the past couple of years, the molecular mechanism of HCO_3^- metabolism and its impact on ecological niche dominating aquatic plants species have been significantly increased for ecologists and plant physiologists. The productive exchange of HCO_3^- excluding (CO_2 exclusion mechanism) in plant tissues to soil water becomes more an area of research where carbon sequestering is considered. The aquatic species *Elodea sp.* and *Potamogeton sp.* showed the calcium carbonate conversion to release CO_2 and thereby changes of the pH become a bio-monitoring index for leaves. The researchers are more focused to find out the role of carbonic anhydrase when the HCO_3^- and molecular CO_2 cross the cell membrane (Effendi and Ng 2019). Carbonic anhydrase even now becomes the key factor not only for aquatic macrophytes but also an enzymatic protein for the C_4 species to trigger the initial carboxylation to PEP-Carboxylase (PEP-C). In fact, carbonic anhydrase mediated release of CO_2 *vis-à-vis* HCO_3^- becomes a substrate for phosphoenolpyruvate carboxylase to initiate C_4 pathways (Jurić et al. 2019). Now, it is already confirmed that HCO_3^- utilization is initially energy dependent pumping process. Therefore, it is still in confusion that HCO_3^- acts as a DIC source for energy harnessing metabolism in aquatic plants. The actual chemical form crossing the mesophyll membrane is yet to ascertain for this carboxylation process. The distribution coefficient of CO_2 dissolved in water and that in air is around 1.0 within 10–20 °C. Therefore, the molar concentration of CO_2 is almost equal between water and ambient air. The equilibrium concentration of CO_2 dissolved in water records 10.8 μM, which corresponds the value of 12.3 μM irrespective of CO_2 (300 μL/L) in air. Still, the CO_2 may be present both as ionized and non-ionized form in water. Therefore, HCO_3^- utilization and its slow diffusion rate in an aqueous medium must be taken for consideration. This diffusion rate is 1/1000-fold less than air when CO_2 is compared with water for dissolution. Therefore, CO_2 as sole source of DIC for photosynthesis is hindered not only by low intercellular concentration but also high partial pressure of O_2 (Carmi et al. 2019). This gives another constrains for photorespiratory loss. DIC through the boundary layer resistance is the key factor for slow diffusion. The boundary layer resistance with a thick layer of air on leaf surface may be maximum but not zero (0) at any time depending on size of the leaves. The unstirred layer of air sets the diffusion barrier for direct gaseous entry of CO_2 through the stomata. The photosynthetic rate (μM $CO_2/dm^2/h$) depends on the water turbulence (for aquatic species) and thereby thickness of the unstirred layer is bothered. The constant for boundary layer conductance is variable in submerged or aquatic macrophytes. The ambient CO_2 concentration and photosynthetic rate are approximately linear within a stretch of ranges and that mark the indicative limitations for diffusion (Zheng et al. 2019). For the depletion of CO_2 in the boundary layers, the pH is increased and causes shifting of CO_2 to HCO_3^- as DIC. Under this condition CO_2 utilization is totally based on HCO_3^- to increase the rate of photosynthesis. Under condition of limited CO_2 assimilation HCO_3^- may alleviate the constraints for gaseous CO_2 and DIC is the only source for photosynthesis in submerged/aquatic plant species.

3 Physiological Evidences for Utilization of Bicarbonate in Aquatic Plants

On net photosynthetic gain, i.e. CO_2 utilization, it does not significantly varied with HCO_3^- absorption by leaf tissues, still, HCO_3^- utilization may result in release of OH^- along with CO_2 assimilation. The OH^- is often neutralized by H^+ influx into the mesophyll tissue. Therefore, plants successfully utilizing HCO_3^- are not much different from other CO_2 scrubbing species like C_4 variants (Poschenrieder et al. 2018). Still, the efficiency of C_4 plants is not dependent on the pH and that may differ from other HCO_3^- utilizer which is relied on high pH. Physiologically few pathways are accomplished for HCO_3^- utilization which depends on the pH of the media and rate of photosynthesis. This is principally based on the findings out of DIC dissolving compensation points which is related to the pH. Another possible approach is with the determination of equilibrium constant between DIC and CO_2. This method is dependent on kinetic reaction of carbonic anhydrase, the principal enzyme for HCO_3^- metabolism and mostly the unicellular algae and few microorganisms are most targeted species to be deciphered by their DIC utilization. The most advance and reliable physiological technique that establishes HCO_3^- is the isotopic discrimination between HCO_3^- and CO_2. It is the value of proportion for $\Delta^{13}C/\Delta^{12}C$ which predicts the $\Delta^{13}C$ values almost more than 10% positive than the sum of dissolved CO_2. It is well admitted that this method is well standardized in laboratory scale but showing a number of complication and fluctuations adhered to field condition. This procedure is dependent on availability of carbon isotopes like ^{13}C and ^{12}C following very small proportion of the earliest as compared to later. (Thompson et al. 2018). The DIC as a source may determine the ratio between ^{13}C and ^{12}C in sample (Zhao et al. 2019).

4 HCO_3^- Elevated pH, Its Impact for Net Photosynthesis

The availability of HCO_3^- in aqueous solution and its proper utilization affecting the rate of photosynthesis may directly be measured by radioactive or labelled carbon fixation or generation of oxygen. A variable response of labelled carbon fixation and O_2 generation against different pH values could be a direct indication of HCO_3^- effect. Technically, the comparison of the rate of photosynthesis with the observed values for CO_2 and HCO_3^- under distinct pH would prove the HCO_3^- utilization (Zemedkun et al. 2019). Classically using *Potamogeton* sp. recorded a plateau formation within pH 8 at the maximum concentration of salt. The relationship of HCO_3^- utilization at alkaline pH is low and that indicates for the utilization of DIC. This technique may encompass the avoidance for diffusion out of free CO_2 from aqueous solution at acidic pH. This is particularly happened when CO_2 of that solution may cross the same for equilibrium concentration with air. This is still to remember that light may not be a limiting factor for photosynthesis at acidic pH values. Undoubtedly, it is understood that HCO_3^- utilization always remains lower values than direct use of CO_2 in photosynthesis at identical concentration (Nayak et al. 2018). So, light limitations would be a factor to regulate photosynthesis at acidic pH, however, a high value of HCO_3^- at the higher alkaline pH values.

The buffer system in maintenance of a constant pH is essential to evaluate the rate of photosynthesis under varying pH. It is well admitted that buffer can lower the rate of HCO_3^- utilization but increase the CO_2, so it is recommended to use the effects of buffering at low and high pH or escaping buffer as a whole for the exclusively submerged aquatic macrophytes. In another technique regarded as pH drift technique, the plant species are exposed to light in alkaline solution of HCO_3^- in a closed/sealed container. Therefore, the changes in CO_2 values due to the gaseous CO_2 or HCO_3^- utilization and the simultaneous upliftment of pH were recorded. The experiment is continued till the further changes of pH and designed compensation point wise. For the aquatic macrophytes, particularly, under submerged condition this method relies on the conductivity of DIC and its possible indication by changes of pH values. Thus, the toxicity of HCO_3^- would be otherwise documented for which the values of compensation point are varied in C_3 and C_4 plant in air. This HCO_3^- compensation concentration value is highly variable and same in C_3 and C_4 plant species.

Therefore, the HCO_3^- metabolism in aquatic plants is manifested in nature. Those have already been established from different aquatic plant species. In spite of sole source of DIC, few marine habitats not experiencing a wider range of pH and HCO_3^- on their territory is rather uniform. The HCO_3^- utilization for DIC optimizes the maximum growth rates in macroalgae and few sea grasses (Poschenrieder et al. 2018). Under benthic condition the algae report an inducible mechanism for HCO_3^- utilization that requires the sole carbon source under limited CO_2 condition. The other species also utilizes HCO_3^- under condition of nitrogen (N_2) limitation (Molina and Covarrubias 2019). Still, algal photosynthetic rate is not necessarily superseded the rate of CO_2 extraction from HCO_3^-. Physiologically CO_2 enrichment of HCO_3^- utilization would suffice the optimum concentration inhibiting photorespiration. In complementation, it also regulates the loss of ammonia which acts as a source of N_2 during photo-oxidation for conversion of glycine into serine (Busch et al. 2018). In brief, these all may circumvent the maintenance of growth even under sub-optimal regime of CO_2 utilizing HCO_3^- pump in few microalgae (Yin et al. 2019).

5 Molecular Mechanism of DIC Utilization

Undoubtedly, it is proven that there exists no necessarily general pathways to exercise blue green algae and bacteria may have some new variation in their mode of HCO_3^- accumulation than rest of angiosperms and species of *Chara* (Kim et al. 2019). The later species and angiosperms also are quite efficient with their few species like *Elodea*, *Potamogeton* with polarity which is a basic and central position for HCO_3^- fixation exists. These are varied with other aquatic species like *Myriophyllum spicatum*, *Vallisneria spiralis* where no such polarity in use of HCO_3^- is claimed. Regardless of these two approaches are proposed in molecular mechanism of HCO_3^- utilization. In one approach the reversible conversion of HCO_3^- into CO_2/HCO_3^- is the basic reaction in a co-transport path where cellular/symplastic spaces are used to let enter H^+/HCO_3^- over the cell membrane. This was more supported on subsequent mechanism in *Potamogeton*, *Elodea* and *Chara* adopting the co-transport mechanism. Still, the cross talks are the barrier in acceptance for higher green plants with extracellular HCO_3^- to CO_2 conversion. The most contradiction for higher plants is initially based on the difference of pH with quite low value surrounding the abaxial leaf face (Knoll and Schreiber 2000). This is also extrapolated with the non-linear acidification with the concentration of HCO_3^-. Due to acidic pH the CO_2 would be very high in proportion to HCO_3^- on to leaf surface. The DIC assimilation could also be inhibited with the effects of buffer and that may influence negatively the H^+/HCO_3^- co-transporter. In few cases, extracellular HCO_3^- utilization is also found in some non-polar species. The cell membrane when folded, particularly, on transfer cell may circumvent to localize the acidic pH favouring HCO_3^- absorption. It is undoubtedly established that the major point through symplastic co-transport (for extracellular conversion) is the lower/acidic pH on to ventral surface of leaf. The precise values of such lower pH were derived with an established decisive role for assimilation in different species of *Chara*. Through H^+/HCO_3^- co-transport is more optimized under higher pH, but for extracellular conversion, the lower pH becomes the key factor (Wang et al. 2018). This has more clearly been elucidated with the detection of microelectrodes for cell wall pH. More so, the buffers inhibit the reaction of those pK values in *Chara* sp. for H^+/HCO_3^- co-transport. Specific structures of plasma lemma zone and their frequencies reported a fair correlation for HCO_3^- absorption in *Chara corallina*. Carbonic anhydrase, the key enzyme optimum in activity under lower pH has also been evident with the use of inhibitors like ethoxyzolamide. Collectively, these results are in better support for external conversion of CO_2 from DIC which makes entry by simple diffusion according to gradient over the cell membrane. In case of blue green alga the process is some different. HCO_3^- is competent to be transported over the plasma membrane. The most striking difference is not to get any carbonic anhydrase activity (e.g. *Anabaena* sp.). Few inhibitors for carbonic anhydrase may reduce the rate of HCO_3^- uptake. Few positive charged metals or cations appear to play in facilitation of HCO_3^- uptake as found in blue green algae. In few cases H^+ pump is available to be induced by Na^+ efflux (Zhang et al. 2017). Therefore, active transport in terms of pump is also a factor to metabolize HCO_3^- in those species.

6 The Factors Influencing and Regulation of DIC Utilization

There recorded a distinct variation on uptake and utilization of HCO_3^- in different plant groups those mostly focused with specific mode of mechanisms. Within the different species of same genus the variations still exist with regard to pH, ambient temperature, HCO_3^- concentration, etc. (Wasli et al. 2018). The growth and condition are also factors for a species within the same genus in DIC use. In marine microalgae the low DIC value would be the factor to induce HCO_3^-, whereas the green macrophytes record no as such reports for an optimum value of DIC (Iñiguez et al. 2016). Light and CO_2 compensation point as a factor of photorespiration would be another aspect for higher plants that varies with growth condition (Saha et al. 2018a, b). Aquatic macrophytes like *Hydrilla verticillata* has a distinct correlation for the utilization of HCO_3^- (Dhir 2015). With the illustration of C_4 weeds the organic acids (C_4) and HCO_3^- utilization would be the factor as found in *Elodea* sp. The allelopathic mode of utilization may set the other factors for induction of

HCO_3^- utilization through release of organic residues from vegetation and deposition of inorganic chemicals into environment (Gupta et al. 2020).

As a whole within a community or ecological niche the different physico-chemical input, ontogenic stages, presence of elicitors/inhibitors and their utilization of DIC following metabolism are rather complicated. This is more diversified, non-lenient, inconsistent process without stipulated pathways, however, flexible yet, modified to the demands of the plant species. So, more insights to decipher the regulation at the generic level would be appropriate to add the photosynthetic reserves with HCO_3^- utilization. Therefore, it would be more imperative to justify the regulatory mechanism with relation to luminance under aquatic environment.

7 Prospects and Remediatory Achievement Against Bicarbonate Toxicity

The wider variations of HCO_3^- utilization are highly dependent on the solubility of salts in varying degrees and genotypic potential of the plants for its metabolism. Within the same genus a significant variation would be a reliable index for HCO_3^- adsorption by roots, its translocation to shoots and downstream breakdown into smaller units. With the active participation of carbonic anhydrase, undoubtedly, plants may arise a possibility for an alternative source of HCO_3^-. This is also based on the ages and growth conditions of the tissue for the adaptation to HCO_3^- toxicity. The use of HCO_3^- becomes a factor for its inclusion under specific condition like concentration of DIC below its optimum level. In contrast, macrophytes are less organized through its research in actual land condition. Still, it may vary the ability of HCO_3^- use in few aquatic plants. Likewise, the *Potamogeton* sp. the light sensitivity is more important in favouring the HCO_3^- transportation across membrane. In common weeds like *Myriophyllum* and *Hydrilla*, the C_3 and C_4 intermediates appear to be a sensitive species to respond HCO_3^- accumulation under low compensation point. This may give the mileage for C_4 species as insensitive to elevated CO_2 in the atmosphere. In another grass species such as *Elodea*, the generation of C_4 acids through anaplerotic pathways would suffice the loss of CO_2 in photorespiration and other anabolic metabolism. Other factors, particularly, those are allelopathic in nature secreted by the aquatic species can also affect the HCO_3^- utilization. In water bodies, cyclic AMP could be the another chemical that affects the HCO_3^- absorption in plants. Therefore, utilization of HCO_3^- by plants species at different ecological niches is highly diverged that opens a wider aspect of environmental toxicity and its interaction with plants specifically, in phototrophs. HCO_3^- is contributory in carbon concentration mechanism for the species in CO_2 depleted habitats (Cyanophyceae, few microalgae), macrophytes including weeds under polluted area. In higher terrestrial plants, particularly, in C_4 and CAM groups the affectivity is more advanced by such a varied assimilation. Therefore, plants response to alkalinity in excess would be more interesting for their approval in accommodation to agronomic as well as plant biological process. In future, accumulation of considerable knowledge of HCO_3^- as well as its metabolism may opine the selection pressure of tolerant crop species. The progress of genetic engineering for essential genes in transfer of CCM from efficient species like cyanobacteria to higher crop species is welcome in breeding programme under lime soil. Finally, if the key roles of regulatory genes are identified there may be ample possibility in application of reduction measures to lime toxicity in agricultural soil.

8 Conclusion

In crop habitat it is the high alkaline soil that offers dissolution of bicarbonate in a high rate that causes an adverse effect. Draught and salt affected soil would be ideal to deposit more bicarbonate ions readily absorbed by plants. From the point of plant response it includes: a limitations of photosynthetic carbon assimilation under alkaline pH, increased activity of carbonic anhydrase, shifting of metabolic fluxes mostly those of anaploratic reactions, etc. Whatever the cases might be a tolerant species must be possessing with adjustment of alkaline pH by over expressed carbonic anhydrase activity. In aquatic species this would aid special mechanism to assimilate dissolved CO_2. Therefore under elevated CO_2 factor in the atmosphere, the capacity for bicarbonate use would be physiological indices vis-a-vis bioindicator in lime rich soil in agriculture system and a few well adapted plant species in acquisition of carbon source towards biomass production have been also discussed. Any additional methods for screening of those types with increasing anhydrase activity are yet to hail to meet the constraints of alkaline toxicity. The insight may enrich the ecological connotation of photosynthetic bicarbonate consumption reimbursing a slow deliver of CO_2 that may generate high intercellular carbon pool in those species.

References

Beck R, Xu M, Zhan S, Johansen R, Liu H, Tong S, Yang B, Shu S, Wu Q, Wang S, Berling K (2019) Comparison of satellite reflectance algorithms for estimating turbidity and cyanobacterial concentrations in productive freshwaters using hyperspectral aircraft imagery and dense coincident surface observations. J Great Lakes Res 45(3):413–433

Busch FA, Sage RF, Farquhar GD (2018) Plants increase CO2 uptake by assimilating nitrogen via the photorespiratory pathway. Nat Plants 4(1):46–54

Carmi I, Kronfeld J, Moinester M (2019) Sequestration of atmospheric carbon dioxide as inorganic carbon in the unsaturated zone under semi-arid forests. Catena 1(173):93–98

Deirmendjian L, Anschutz P, Morel C, Mollier A, Augusto L, Loustau D, Cotovicz LC Jr, Buquet D, Lajaunie K, Chaillou G, Voltz B (2019) Importance of the vegetation-groundwater-stream continuum to understand transformation of biogenic carbon in aquatic systems–a case study based on a pine-maize comparison in a lowland sandy watershed (Landes de Gascogne, SW France). Sci Total Environ 661:613–629

Devaraj N, Chidambaram S, Gantayat RR, Thivya C, Thilagavathi R, Prasanna MV, Panda B, Adithya VS, Vasudevan U, Pradeep K, Paramaguru P (2018) An insight on the speciation and genetical imprint of bicarbonate ion in the groundwater along K/T boundary, South India. Arab J Geosci 11(12):291

Dhir B (2015) Status of aquatic macrophytes in changing climate: a perspective. J Environ Sci Technol 8(4):139

Donzia SK, Yeo H, Kim HK (2018) Design and evaluation of soil pH IoT sensor attribute for rice agriculture in Central Africa. In: International conference on applied computing and information technology. Springer, Cham, pp 37–47

Dusenge ME, Duarte AG, Way DA (2019) Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. New Phytol 221(1):32–49

Effendi SS, Ng IS (2019) The prospective and potential of carbonic anhydrase for carbon dioxide sequestration: a critical review. Process Biochem 23:55–65

Gupta DK, Chatterjee S, Mitra A, Voronina A, Walther C (2020) Uranium and plants: elemental translocation and phytoremediation approaches. In: Uranium in plants and the environment. Springer, Cham, pp 149–161

Gwak G, Kim DI, Kim J, Zhan M, Hong S (2019) An integrated system for CO_2 capture and water treatment by forward osmosis driven by an amine-based draw solution. J Membr Sci 581:9–17

Hang H, Wu Y (2019) Effect of bicarbonate stress on carbonic anhydrase gene expressions from Orychophragmus violaceus and *Brassica juncea* seedlings. Pol J Environ Stud 28(3): 1135–1143

Iñiguez C, Carmona R, Lorenzo MR, Niell FX, Wiencke C, Gordillo FJ (2016) Increased temperature, rather than elevated CO2, modulates the carbon assimilation of the Arctic kelps Saccharina latissima and Laminaria solidungula. Mar Biol 163(12):248

Jull AT, Burr GS, Leonard AG, Fitzgerald J, Cheng L, Cruz R, Biddulph D (2019) Dissolved inorganic and organic carbon in an ephemeral fresh water stream in southern Arizona. Radiocarbon 61(5):1531–1539

Jurić I, Hibberd JM, Blatt M, Burroughs NJ (2019) Computational modelling predicts substantial carbon assimilation gains for C3 plants with a single-celled C4 biochemical pump. PLoS Comput Biol 15(9):e1007373

Kim GY, Roh K, Han JI (2019) The use of bicarbonate for microalgae cultivation and its carbon footprint analysis. Green Chem 21(18):5053–5062

Knoll D, Schreiber L (2000) Plant–microbe interactions: wetting of ivy (Hedera helix L.) leaf surfaces in relation to colonization by epiphytic microorganisms. Microb Ecol 40(1):33–42

Krüger A, Wiechert J, Gätgens C, Polen T, Mahr R, Frunzke J (2019) Impact of CO2/HCO3– availability on anaplerotic flux in pyruvate dehydrogenase complex-deficient *Corynebacterium glutamicum* strains. J Bacteriol 201(20):387–319

Lines T, Beardall J (2018) Carbon acquisition characteristics of six microalgal species isolated from a subtropical reservoir: potential implications for species succession. J Phycol 54(5):599–607

Lynch JP (2019) Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. New Phytol 223(2):548–564

Maheshwari N, Kumar M, Thakur IS, Srivastava S (2019) Cloning, expression and characterization of β-and γ-carbonic anhydrase from Bacillus sp. SS105 for biomimetic sequestration of CO2. Int J Biol Macromol 131:445–452

Mishra S, Joshi B, Dey P, Pathak H, Pandey N, Kohra A (2018) CCM in photosynthetic bacteria and marine alga. J Pharmacogn Phytochem 7(6):928–937

Molina J, Covarrubias JI (2019) Influence of nitrogen on physiological responses to bicarbonate in a grapevine rootstock. J Soil Sci Plant Nutr 19(2):305–312

Nayak M, Suh WI, Lee B, Chang YK (2018) Enhanced carbon utilization efficiency and FAME production of Chlorella sp. HS2 through combined supplementation of bicarbonate and carbon dioxide. Energ Conver Manage 156:45–52

Pawłowicz I, Masajada K (2019) Aquaporins as a link between water relations and photosynthetic pathway in abiotic stress tolerance in plants. Gene 687:166–172

Poschenrieder C, Fernández JA, Rubio L, Pérez L, Terés J, Barceló J (2018) Transport and use of bicarbonate in plants: current knowledge and challenges ahead. Int J Mol Sci 19(5):1352

Prelovšek M, Šebela S, Turk J (2018) Carbon dioxide in Postojna Cave (Slovenia): spatial distribution, seasonal dynamics and evaluation of plausible sources and sinks. Environ Earth Sci 77(7):289

Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. Phycologia 58(5):552–562

Rubio L, Fernández JA (2019) Seagrasses, the unique adaptation of angiosperms to the marine environment: effect of high carbon and ocean acidification on energetics and ion homeostasis. In: Halophytes and climate change: adaptive mechanisms and potential uses, vol 14, p 89

Saha I, De AK, Sarkar B, Ghosh A, Dey N, Adak MK (2018a) Cellular response of oxidative stress when sub1A QTL of rice receives water deficit stress. Plant Sci 5(3):84–94

Saha I, De AK, Ghosh A, Sarkar B, Dey N, Adak MK (2018b) Preliminary variations in physiological modules when sub1A QTL is under soil-moisture deficit stress. Am J Plant Sci 9 (4):732–744

Saha I, Sarkar B, Ghosh A, De AK, Adak MK (2019) Abscisic acid induced cellular responses of sub1A QTL to aluminium toxicity in rice (*Oryza sativa* L). Ecotoxicol Environ Saf 15 (183):109600

Su Z, Ho D, Merrill AR, Lipkowski J (2019) In situ electrochemical and PM-IRRAS studies of colicin E1 ion channels in the floating bilayer lipid membrane. Langmuir 35(25):8452-8459

Terés J, Busoms S, Perez Martín L, Luís-Villarroya A, Flis P, Álvarez-Fernández A, Tolrà R, Salt DE, Poschenrieder C (2019) Soil carbonate drives local adaptation in Arabidopsis thaliana. Plant Cell Environ 42(8):2384–2398

Thompson HA, White JR, Pratt LM (2018) Spatial variation in stable isotopic composition of organic matter of macrophytes and sediments from a small Arctic lake in west Greenland. Arct Antarct Alp Res 50(1):S100017

Torralbo F, González-Moro MB, Baroja-Fernández E, Aranjuelo I, González-Murua C (2019) Differential regulation of stomatal conductance as a strategy to cope with ammonium fertilizer under ambient versus elevated CO_2. Front Plant Sci 10:597

Wang T, Gu CB, Wang SX, Kou P, Jiao J, Fu YJ (2018) Simultaneous extraction, transformation and purification of psoralen from fig leaves using pH-dependent ionic liquid solvent based aqueous two-phase system. J Clean Prod 172:827–836

Wasli H, Jelali N, Silva AM, Ksouri R, Cardoso SM (2018) Variation of polyphenolic composition, antioxidants and physiological characteristics of dill (Anethum graveolens L.) as affected by bicarbonate-induced iron deficiency conditions. Ind Crop Prod 126:466–476

Williams NJ, Seipp CA, Brethomé FM, Ma YZ, Ivanov AS, Bryantsev VS, Kidder MK, Martin HJ, Holguin E, Garrabrant KA, Custelcean R (2019) CO2 capture via crystalline hydrogen-bonded bicarbonate dimers. Chem 5(3):719–730

Yin D, Wang Z, Wen X, Ding Y, Hou X, Geng Y, Li Y (2019) Effects of carbon concentration, pH, and bubbling depth on carbon dioxide absorption ratio in microalgae medium. Environ Sci Pollut R 26(32):32902–32910

Zanin L, Tomasi N, Cesco S, Varanini Z, Pinton R (2019) Humic substances contribute to plant iron nutrition acting as chelators and biostimulants. Front Plant Sci 10:675

Zemedkun D, Alaparmak H, Apte S (2019) The effect of herbicides on the rates of photosynthesis and respiration in spinach leaves. J High School Sci 16:7518

Zhang Y, Wang Y, Sa G, Zhang Y, Deng J, Deng S, Wang M, Zhang H, Yao J, Ma X, Zhao R (2017) Populus euphratica J3 mediates root K+/Na+ homeostasis by activating plasma membrane H+-ATPase in transgenic Arabidopsis under NaCl salinity. Plant Cell Tiss Org Cult (PCTOC) 131(1):75–88

Zhao K, Zhang M, Xu J (2019) Carbon reliance on dissolved inorganic carbon of junior and matured reed beds indicated by stable isotope analysis. J Anim Plant Sci 29(4):971–977

Zheng Y, Li F, Hao L, Yu J, Guo L, Zhou H, Ma C, Zhang X, Xu M (2019) Elevated CO_2 concentration induces photosynthetic down-regulation with changes in leaf structure, non-structural carbohydrates and nitrogen content of soybean. BMC Plant Biol 19(1):255

Antioxidant Defense Systems and Remediation of Metal Toxicity in Plants

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Contents

Abstract

Over the past years, knowledge concerning bioremediation of heavy metals via fungi and bacteria has been extensively developed. Globally, there has been a notable improvement in the level of several toxic metals in different environments

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as well as soils due to industrial usage (anthropogenic activities) and causing a severe affair to plants and human health as well. Plants growing in such a contaminated environment show a decrease in plant growth, development, and yield; thereby, there is a high-priority to cope with the toxicity of heavy metals. Several heavy metals have been examined to give rise to oxidative injury in crops by the generation of freely available toxic O_2 radicals. In the arrangement to tackle with the toxicity of heavy metals or to keep alive the level of some vital metals inside the range of physiological processes, the plant has evolved a wide range of multiplex mechanisms for metal tolerance. Plant and microorganisms possess various mechanisms for the bioremediation of contaminated environments, including soils. Different microorganisms have been favorably employed to decrease the toxic effects of heavy metals. Nevertheless, the critical action is to sectionalize and accumulate heavy metals in plant tissues; and antioxidant defense system plus enzymatic antioxidants (SOD, CAT, APX, GR, POD, GSTs, GPx, MDHAR, and DHAR) and non-enzymatic antioxidants (ASA, GSH, carotenoids, and tocopherols) have been declared. Additionally, chelation has emerged as prospect mechanisms, which widely control the metal resistance in crops via retaining the low level of freely available metal ions in the cytoplasm. Chelation of metals can be carried out by thiol compounds (GSH, PCs, and MTs), and non-thiol compounds (organic acids, amino acids, and their derivatives). Together, GSH plays a vital role in the bioremediation process as a chelating agent, due to its high kinship of metals, and it acts as a forerunner for PCs. Under metal stress, ROS and antioxidant defense systems generate signaling, where GSH can affect the cellular pathways associated with the acclimation and repair process to tackle with oxidative damage caused by metal stress. In this chapter, we have reviewed the recent advancement in the decisive role of antioxidant defense systems in the bioremediation system along with chelation of metals in plants.

Keywords

Antioxidant defense · Bioaccumulation · Biosorption · Bioremediation · Chelation · Glutathione · Heavy metals · Metal homeostasis · Oxidative stress · ROS · Transgenic plants

1 Introduction

Environmental pollution is gradually increasing every day at a terrifying rate. Technological advancements, urbanization, and industrialization are the main contributors to pollution. Due to rapid industrializations, the level of toxic metals in the environment is exceeded and pollute not only air but also land and drinking water. Heavy metals, which are emitted from industries, are toxic, become persistent in the environment, and cause serious health problems. Enough level of metals is needed for proper biological functioning of animals and plants, but their elevated level causes metabolic interference. Cadmium (Cd), selenium (Se), lead (Pb), chromium (Cr), mercury (Hg), nickel (Ni), gold (Ag), arsenic (As), zinc (Zn), manganese (Mn), and uranium (U) act as most toxic metals, and their presence in the soil causes problems for plants. The plant growth, photosynthetic activities, and enzymatic activities become reduced (Nematian and Kazemeini 2013; Raza et al. 2019a; Salim and Raza 2020; Verma and Kuila 2019; Hasanuzzaman et al. 2020a; Raza et al. 2020a,b,c; Raza 2020). The eradication of harmful and toxic substances from the polluted environment is necessary for survival. Bioremediation is used for cleaning up the environmental site, which is contaminated due to hazardous and chemical pollutants. Living organisms like bacteria, microbes, fungi, and crops are used for degrading the hazardous compounds into less toxic compounds. Changing the pH of contaminants, redox responses, and contaminants adsorption from the adulterated site or environment are the basic principles of bioremediation (Jain and Arnepalli 2019). The process of bioremediations is redox-based, in which the microbiology and chemistry of water are modified by the addition of reagents into it, which helps in the degradation and extraction of contaminants. The harmful compounds become transferred to less toxic or inert substances through redox reactions (Tandon and Singh 2016). Through this process, few metals like Hg, Cr, Se, and As are converted into innocuous forms (Ojuederie and Babalola 2017).

The organisms used for bioremediation, environmental pressures prevailing at the origin of contamination, and the pollutants degree of that environment affect the effectiveness of the process of bioremediation (Azubuike et al. 2016). Microbes and plants are widely used for bioremediation. In microbial bioremediation, microorganisms are used to degrade the pollutants through its metabolic process into innocuous forms through the redox reactions (Jan et al. 2014). The process of remediation in which plants are used is called phytoremediation. The pollutants from the soil environment of any kind are extracted and accumulated into plants and eradicate them from the soil. The success of phytoremediation depends upon the contaminated soil, the metal concentration in it, and the metal accumulation in plants as biomass to eradicate them and make the contaminated site less toxic (Tak et al. 2013; Raza et al. 2020a).

The reactive oxygen species (ROS) and its by-products are catalytically transformed into non-toxic and stable compounds with the help of enzymes known as antioxidant enzymes. The process is an essential mechanism of defense against the cell damage induced by oxidative stress. In plants, antioxidant systems are present naturally and have a role in the removal of toxicity produced by ROS (Ojuederie and Babalola 2017; Hasanuzzaman et al. 2020b). Antioxidants have their role in the scavenging of ROS (Bulbovas et al. 2014). Superoxide dismutase (SOD) caused dismutation of superoxide ($O_2^{\bullet -}$) and in result produce oxygen and hydrogen peroxide (Gratão et al. 2012). Catalase (CAT), subsequently, causes the detoxification of hydrogen peroxide into water. This process is also carried out by several other peroxidases, which include ascorbate peroxidase (APX). Glutathione (GSH), a non-enzymatic enzyme, in reduced form is responsible for toxic metals cellular detoxification and hydrogen peroxide scavenging (Rehman and Anjum 2011). In the cell, the balance of GSH depends upon glutathione reductase (GR) enzyme, as it reduces the oxidized GSH. GSH donates thiol to

GST (glutathione *S*-transferase enzyme) which catalyzed the glutathiones conjugation with a different electrophilic substrate. The selected electrophiles are usually associated with the breakdown of secondary products and xenobiotic compounds (Ghelfi et al. 2011).

Antioxidant systems (enzymatic and non-enzymatic antioxidants) are efficient enough to play a vital role in achieving the detoxification or scavenging of excess ROS. Interior cell, SOD, GPX, CAT, and enzymes from the cycle of AsA-GSH, such as MDHAR, APX, GR, and DHAR are included in enzymatic antioxidants, while GSH, AsA, phenolics, tocopherols, and carotenoids are non-enzymatic antioxidants. Many workers report that in plants, the enzymatic activities of defense systems of antioxidants increased in order to combat oxidative stresses, which are modulated by environmental factors. Upkeep of a high ability of an antioxidant to scavenge the harmful ROS has been connected to the high resistance level of crops to metal toxicity (He et al. 2011; Islam et al. 2016; Singh et al. 2016b; Hasanuzzaman et al. 2017, 2020b).

However, against heavy metals, the plants use their physical barriers, which include all morphological structures, as their defense first line. Cuticles, trichomes, cell wall, and the mycorrhizal symbiosis, proved themselves as barriers whenever plant faces the stress of heavy metals (Emamverdian et al. 2015). If any case, pollutants cross these barriers, then the internal cellular mechanism of defense restrains the harmful effects of those pollutants or heavy metals (Silva and Matos 2016). The free radicals cause crops to lessen undesirable effects of heavy metals, the generation of enzymatic (CAT, SOD, GR) and non-enzymatic antioxidants (GSH, ascorbate, tocopherols, and alkaloids), as they remove free radicals (Ojuederie and Babalola 2017). The mechanism of defense used by plants is phytochelatin synthases synthesis, metallothioneins production, and proline production. The enzyme phytochelatin synthase binds with heavy metals when they are in excess amount (Chaudhary et al. 2018; Jadoon and Malik 2018; Raza et al. 2020a).

Nevertheless, both enzymatic and non-enzymatic antioxidants prevent the impact of ROS, and these antioxidant enzymes diffuse free radicals; hence, the oxidative stress risk becomes restricted. ROS also becomes inactivated at a cellular and molecular level. On the other hand, their low concentrations interrupt the radical chain reaction due to which the oxidative processes become delayed or inhibited (Hasanuzzaman et al. 2020b). Antioxidants can easily chelate metals ions, which generate ROS. The generation of a complex metal ion is called chelation. In the metal ion complex, the metal ions bind to uncharged or charged donor of electron known as a ligand. The ideal chelator should have the following properties: high water solubility, biotransformation resistance, the property of reaching to a metal storage site, the ability to restrict the chelation at the body fluids pH level and formation of complexes of metals less toxic to metal ions. The ideal chelator of heavy metal can quickly enter into a cell, from metal complex, with the help of metallothionein, easily chelate heavy metal. Moreover, it is noticed that chelating agent can easily bind to positively charged ions so, an effective and safe method for treatment of metal toxicity is required which can easily be fulfilled with antioxidants which exhibit all the above-mentioned chelating properties

(Flora 2009; Anjum et al. 2015; Al Mahmud et al. 2017). In this chapter, we have discussed the recent advancement in the role of antioxidant defense systems in the bioremediation system along with chelation of metals.

2 Mechanisms of Bioremediation

The bioremediation can be defined as a low-cost alternate biological process for detoxification of environmental hazards and contaminants (Wasi et al. 2011; Banerjee et al. 2015). A series of mechanisms associated with bioremediation processes have been presented in several studies (Ji and Silver 1995; Wasi et al. 2008; Dwivedi 2012). Figure 1 describes the generalized mechanisms of metal bioremediation. The detoxification and removal of metals by aids of microorganisms have been attributed to various reactions (involving siderophores and bio-emulsifiers) and processes, which include complexation, methylation, and oxidation-reduction (Wasi et al. 2008, 2013). Under high concentrations of heavy metals, the microorganism has developed numerous mechanisms to detoxify the harmful metals. These detoxifying mechanisms may be extra- or intra-cellular and highly dependent on specific metal types (Wasi et al. 2008). Nevertheless, Gadd (2010) reviewed the application of individual microbes to detoxify the toxicity of the metal in detail. The application of microorganism for the breakdown of pesticides and the process associated with this mechanism have been well investigated (Mai et al. 2001; Nawaz et al. 2011).

Under optimal availability of nutrients and favorable environmental conditions, the bacteria can incorporate the simple organic substances into their cells and able to

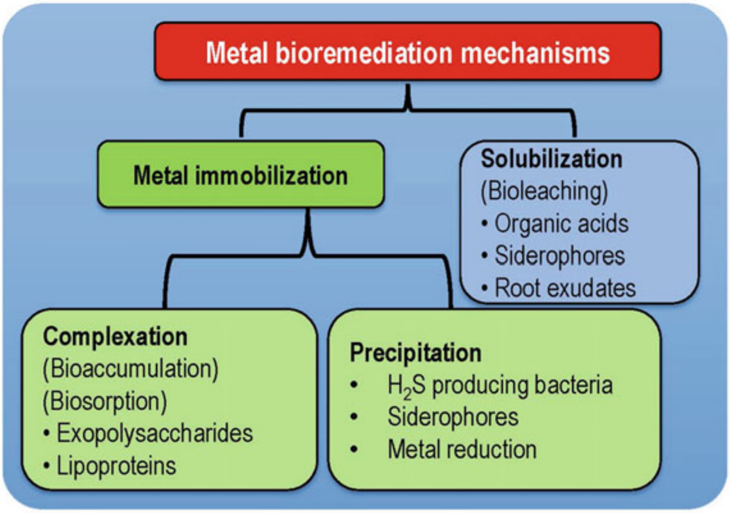

Fig. 1 Generalized mechanisms of metal bioremediation

oxidize them radially (Dwivedi 2012). Immobilization of bacterial cells under bioremediation to detoxify the pollutants from the environments is also examined in different studies (Cho et al. 2000; Das and Chandran 2011; Singh et al. 2011; Wasi et al. 2011). The connection of bacteria in the metabolism of 2,4-D is also well documented by many researchers (Fulthorpe et al. 1996; Han et al. 2015; Wasi et al. 2013). In another research, Yap et al. (1999) also reported the detoxification of phenols by *C. testosteroni* strain.

Bioremediation with algae also possessed a suitable method, since for removal of pollutants, oxygen is not required even under light conditions (Doshi et al. 2008; Dwivedi 2012; Megharaj et al. 2003). The author reported that uptake of metals under bioremediation with algae occurs through two processes, adsorption and intracellular uptake (Dwivedi 2012). During adsorption, metal ions are quickly absorbed by the surface of a cell and then transported to the cytoplasm by chemisorption process. The synthesis of phytochelatins is also reported in marine alga (Tsuji et al. 2002). Under metal toxicity, Scarano and Morelli (2002) characterize the formation of Cd and Pb phytochelatins complexes. The researchers also reported that, in green algae, some metals are sequestered in polyphosphate bodies, which provide a "storage pool" for metals, and this phenomenon is also reported for the detoxification of these metals.

3 Reactive Oxygen Species

ROS are produced due to unavoidable aerobic metabolisms consequences and play an essential part in the regulation of cell survival, cell distinction, cell death, cell signaling, and the production of inflammation-correlated factors. Free radicals and non-free radicals are included in ROS. Hydroxyl radicals and superoxide anion ($^{\bullet}OH$ and $O_2^{\bullet-}$) are free radicals, whereas singlet oxygen and hydrogen peroxide are non-free radicals. Highly reactive ROS is produced by molecular oxygen reduction in a stepwise manner either by the reaction of electron transfer or exposure of high energy. Cellular homeostasis disruption in plants due to environmental factors like salt, drought, metal toxicity, UV-radiation, and pathogen attack leads to the ROS production in enhanced ways (Abdal Dayem et al. 2017; Mishra et al. 2011; Hasanuzzaman et al. 2020b).

Moreover, a cell is in the stage of "oxidative stress" when ROS level surpasses mechanism of defense. Due to environmental stresses, the increased ROS production threatens cells and guide to cell death because of proteins oxidation, lipids peroxidation, nucleic acids damage, hampering of enzymes, and the activation of pathways leads to programmed cell death (Srivastava and Dubey 2011; Hasanuzzaman et al. 2020b). In a various cellular process, e.g., tolerance of environmental stresses, besides the destructive activities of ROS, it is also contributed as second messengers. The balance among the generation and scavenging of ROS is very delicate, and it governs the behavior of ROS either as signaling or as damaging molecules. ROS has multifunctional roles, due to which cell must manage the ROS level tightly. It will help them to bypass oxidative injury, and they will not abolish (Flora and Pachauri 2010; Hasanuzzaman et al. 2020b).

Nevertheless, against the attack of free radical antioxidant defense systems are developed in cells. GSH plays a key part in cell protection from oxidative injury, g-glutamyl-cysteinyl glycine. Firstly it targets mostly antioxidant enzymes, which are responsible for the removal of superoxide and peroxidases radical such as CAT, SOD, and GPx. Lead is involved in the inhibition of the synthesis of heme. The example of a heme-containing enzyme is CAT, and lead decreases its activity. $O_2^{\bullet -}$ is dismutase by SOD and then for its activity, it requires zinc and copper. In this reaction, copper ions played their functional by going through alternate oxidation, and zinc stabilizes the enzyme (Ercal et al. 2001). Free radicals formation, which is induced by arsenic, was mentioned first by Yamanaka et al. (1990). Superoxide anion and radical of dimethyl arsenic are formed by the reaction of molecular oxygen with dimethylarsine. The trivalent arsenic form is known as dimethylamine, and it is also an in vivo metabolite of dimethyl arsenic acid. "Dimethylarsenic peroxyl radical" is formed by the addition of one more molecular oxygen molecule onto dimethyl-arsenic radical. Due to the involvement of transition metals and cellular iron, the generation of hydroxyl radicals occurs during reactions.

Worldwide, heavy metals cause stress in terrestrial ecosystems, and it increases due to extensive industrialization. Heavy metals impart deleterious effects on soil due to which it becomes accumulated in the crops and affect their productions (Shahid et al. 2015; Hasanuzzaman et al. 2020a; Raza et al. 2020a,c). Heavy metals influence the molecular and physiological activities of plants, and their growth becomes retarded (Hassan et al. 2017; Raza et al. 2019b). The essential metals which play essential role in biological processes are Zn, Mo, Ni, Cu, Mn, and Cu (Shahid et al. 2015), whereas the existence of toxic metals like Pb, As, Cr, Hg, and Cd with them causes the reduction of crops productivity (Pierart et al. 2015). They cause metabolic disorders and morphological abnormalities due to plants yields become reduced. Due to these abnormalities, ROS are produced, which in turn disrupts the cell redox homeostasis. Heavy metals by dislodging the amino acids obstruct the normal functioning of plants. In this case, the bonds are formed, which connect sulfhydryl groups and heavy metals (Krumova et al. 2016). The proper functioning of molecules of cells becomes hindered by heavy metals and the functioning of enzymes, respiration, and photosynthesis processes become suppressed (Hossain et al. 2012). The metal that is redox-active (Fe, Mn, Cu, and Cr) due to direct generation of oxidative stress in crops affect the structure of DNA, damage chloroplast and also the accessory pigments due to which ROS is produced and destroyed the cell (Martins et al. 2016). Moreover, non-redox metals also produce oxidative stress, which eventually causes the production of ROS. In plants, this imbalance becomes the primary source of toxicity of heavy metals. To cope with all these problems, defense mechanisms such as binding of metals to phytochelatins, antioxidants activation, and metals sequestration into vacuoles have evolved (Shahid et al. 2015).

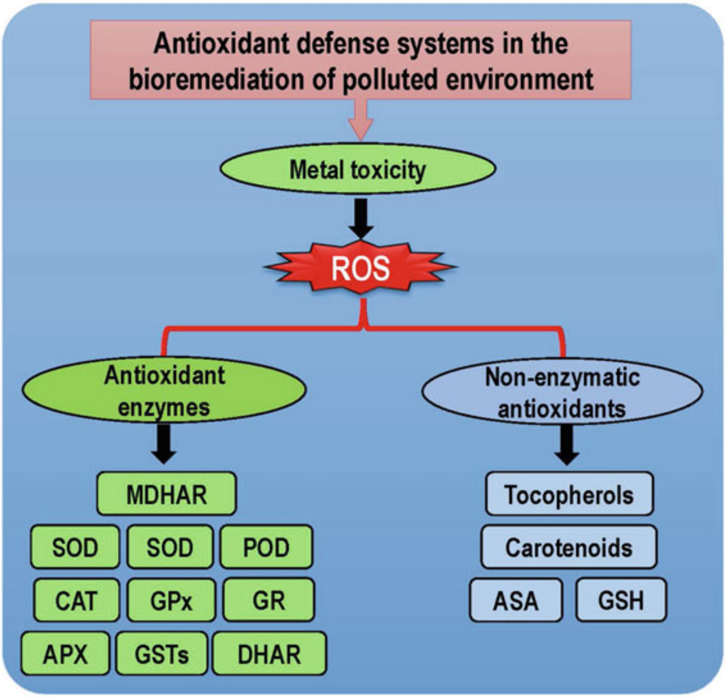

Fig. 2 Antioxidant defense systems involved in the bioremediation. Abbreviations have been described in the text

4 Role of Antioxidant Defense Systems in the Bioremediation

Environmental contaminants including metals toxicity promote the adverse effects on seed germination, photosynthetic production, and thus leading to reduce the biomass production in higher plants (Gill and Tuteja 2010b; Liu et al. 2012a; Hasanuzzaman et al. 2012, 2020a; Raza et al. 2020a,b,c). Furthermore, environment contaminants caused oxidative stress that results in enhancing the generation of ROS in plant cells. Below such circumstances, plants activate antioxidant defense systems, comprising enzymatic compounds, like CAT, APx, GR, MDHAR, DHAR, and GSTs (Fig. 2; Hasanuzzaman et al. 2012; Rahman et al. 2016; Hasanuzzaman et al. 2020b). Recently, bioremediation by the aid of plants, micro-organism, and algae can restore the contaminated environment. The available data indicate that bioremediation can regulate the antioxidant defense mechanism in plants and thus contributing to plant defense under stressed environmental condition (Creus et al. 1998; Mulligan et al. 2001; Pandey et al. 2017; Wu et al. 2010). However, Table 1 shows the information about antioxidant enzymes and their

Table 1 Information about antioxidant enzymes and their catalyzed reactions

Enzyme	EC number	KO definition	Reaction (IUBMB)
Superoxide dismutase (SOD)	1.15.1.1	K04564	$2O_2^{\bullet -} + 2H^+ <=> 2H_2O_2 + O_2$
Catalase (CAT)	1.11.1.6	K03781	$2H_2O_2 <=> O_2 + 2H_2O$
Ascorbate peroxidase (APX)	1.11.1.11	K00434	1. Ascorbate + $H_2O_2 <=>$ DHA + $2H_2O$ 2. 2Ascorbate + $H_2O_2 <=>$ 2MDA + $2H_2O$ 3. 2MDA < => DHA + Ascorbate
Glutathione reductase (GR/NADPH)	1.6.4.2 (changed to 1.8.1.7)	K00383	2GSH + $NADP^+ <=>$ GSSG + NADPH + H^+
Peroxidase (POD)	1.11.1.7	K19511	Reduced acceptor + $H_2O_2 <=>$ acceptor + $2H_2O$
Glutathione S-transferases (GSTs)	2.5.1.18	K00799	1. XR + GSH < => HX + R-S-GSH 2. RCN + GSH < => HCN + R-S-GSH 3. HSO4R + GSH < => O_4S^{-2} + R-S-GSH
Glutathione peroxidase (GPx)	1.11.1.9	K00432	H_2O_2 + 2GSH < => GSSG + $2H_2O$
Monodehydroascorbate reductase (MDHAR/NADH)	1.6.5.4	K08232	2Ascorbate + $NAD^+ <=>$ NADH + 2MDA + H^+
Dehydroascorbate reductase (DHAR)	1.8.5.1	K21888	DHA + 2GSH < => GSSG + Ascorbate

Source: https://www.brenda-enzymes.org/index.php; https://www.kegg.jp/kegg/kegg2.html
EC number enzyme commission number, KO KEGG Orthology, IUBMB International Union of Biochemistry and Molecular Biology, DHA dehydroascorbate, MDA monodehydroascorbate, GSSG glutathione disulfide, GSH glutathione, NADP nicotinamide adenine dinucleotide phosphate, HX halide, RX organic halide, RCN nitrile, HCN hydrogen cyanide, HSO_4R sulfuric monoester

catalyzed reactions; and Table 2 indicates a summary of some recent activities of antioxidant enzymes and non-enzymatic antioxidants under different bioremediation techniques.

4.1 Antioxidant Enzymes

4.1.1 Superoxide Dismutase

Under stress conditions, field-grown crops, surrounded by various types of microorganism, showed improved development and yield (Creus et al. 1998). Under high concentrations of salt, application of arbuscular mycorrhizal fungi (AMF)

Table 2 Examples of some recent studies about the activities of enzymatic and non-enzymatic antioxidants under different bioremediation techniques

Bioremediation techniques	Observations	References
Activities of antioxidant enzymes		
Bacteria (*S. bovis* and *S. ruminantium*)	Significant activities of SOD, GSHPx, and GR were observed under $HgCl_2$ stressed conditions	Lenártová et al. (1998)
Bioremediation with fungus (*Mucor hiemalis*)	Significantly increased the CAT, GR, and GSTs activities were reported	Hoque (2003) and Hoque et al. (2007)
Use of macrophyte (*Potamogeton pusillus*) as bioremediation	POD, GPs, and GR activities were reported to be enhanced	Monferrán et al. (2009)
Use of Indian mustard under Hg stress	Enhanced activeness of SOD, CAT, APX, and GR were examined under Hg stress	Ansari et al. (2009)
Remediation with bacterial strains (*Ochrobactrum* and *Bacillus* sp.)	The increased activeness of SOD, CAT, and GPs enzymes have been noticed under the toxicity of various heavy metals	Pandey et al. (2013)
Fungal bioremediation (*Aspergillus foetidus*)	The activities of CAT and GR have been investigated to be enhanced under Cd stress	Chakraborty et al. (2014)
Bacterial strain (Enterobacter cloacae)	Increased CAT and SOD activeness were reported under Cd stress	Banerjee et al. (2015)
Bioremediation with filamentous fungi (*Penicillium chrysogenum* XJ-1)	Increased SOD, CAT, GR under various Cd levels	Xu et al. (2015)
Bioremediation with bacterial strains (NBRIEAG-6, NBRIEAG-8, and NBRIEAG-9)	An increase in APX and CAT activities were reported in rice under As stress	Singh et al. (2016b)
Bacterial bioremediation (*Providencia vermicola*)	The increased activities of CAT, APX, and SOD under Cu stress were noted	Islam et al. (2016)
Inoculation of *S. nigrum* L. with *M. circinelloides*	The increased SOD activity was reported under Pb-contaminated soils	Sun et al. (2017)
Bioremediation with fungal strains (*Trichoderma hamatum* and *Rhizopus arrhizus*)	The increased activities of SOD (in *T. hamatum*), CAT (in *R. arrhizus*), GST and POX activities (in both species) were reported	Russo et al. (2019b)
Bioremediation with fungal species (*T. harzianum* and *P. simplicissimum*)	Significantly increased activities of CAT and GST (in both of the tested fungi), and SOD (in *P. simplicissimum*) were reported under HCH stress	Russo et al. (2019a)
Bioremediation of endophytic fungus (*Aspergillus tubingensis*)	Activities of CAT and SOD enzymes increased significantly under antimony treatments	Meghnous et al. (2019)

(continued)

Table 2 (continued)

Bioremediation techniques	Observations	References
Bioremediation with algae (*Chlorella vulgaris*)	An increase in SOD and CAT activities was reported on exposure to produced water concentrations	Calderón-Delgado et al. (2019)
Activates of non-enzymatic antioxidants		
Application of yeast	Activities of carotenoids increased significantly under metals toxic environments	Bhosale and Gadre (2001)
Bioremediation with pseudometallophytes	The increased activities of tocopherols were reported	Epelde et al. (2010)
Fungal bioremediation (*Aspergillus foetidus*)	The activities of tocopherols have been reported to be increased	Epelde et al. (2010)
Bioremediation of *Callitriche cophocarpa Sendtn*	A decrease of carotenoids was reported under Cr stress	Augustynowicz et al. (2010)
Bioremediation with *Microbacterium* sp.	Significantly decreased activities of GSH enzyme were reported	Liu et al. (2012b)
Bioremediation with photosynthetic bacteria	Significantly recovered the carotenoids under contaminated environments	Idi et al. (2015) and Pattanamanee et al. (2012)
Bacterial bioremediation (*S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*)	An increase in carotenoids content was reported	Radhika and Kannahi (2014)
Bioremediation with microalgae	An increase in carotenoids contents was reported	Raeesossadati et al. (2014)
Bioremediated with *P. chrysogenum-10*	Increased ascorbate and GSH activities were reported in plant roots	García-Sánchez et al. (2014)
Microbial bioremediation	Increased GSH activities	Xu et al. (2015)
Bacterial inoculation (*P. vermicola*)	Significant activities of ascorbate was observed under Cu stress conditions	Islam et al. (2016)
Bioremediation with bacterial strains (*Staphylococcus* sp., *Brevibacillus* sp., and *S. arlettae*)	Reduced GSH activity was recorded in rice under As stress	Singh et al. (2016b)

remarkably improves the activeness of antioxidant systems, i.e., SOD, which helps in elevating the harmful impacts of oxidative stress in *E. aphylla* and *P. trifoliate* (Alqarawi et al. 2014; Wu et al. 2010). Induction of SOD also observed in mycorrhizal (*Glomus deserticola*) colonized lettuce roots under drought condition (Ruiz-Lozano et al. 1996). Moreover, Banerjee et al. (2015) examined the increased activity of CAT enzyme under bacterial bioremediation (*Enterobacter cloacae* strain) under cadmium-toxic condition. The overexpression of SOD also is reported under bioremediation with other bacterial strains (*Streptococcus bovis* and *Selenomonas ruminantium*) upon exposure of mercury stress (Lenártová et al. 1998). Recently, it has been investigated that bioremediation with the fungal strain (*Trichoderma hamatum)* also resulted in more SOD contents, showed enhanced protection for oxidative injury (Russo et al. 2019b). Nevertheless, Russo et al. (2019a) noticed the enhanced SOD enzyme activity with other fungal strains (*P. simplicissimum*). Bioremediation with filamentous fungi *(Penicillium chrysogenum* XJ-1) also reported enhancing the SOD activities under toxic cadmium levels, indicating its vital role to mitigate the oxidative injury (Xu et al. 2015). Improved activeness of SOD enzyme was observed under the bioremediation of endophytic fungus (*Aspergillus tubingensis*) in toxic antimony conditions (Meghnous et al. 2019). Calderón-Delgado et al. (2019) also say that bioremediation with algae (*Chlorella vulgaris*) significantly increased SOD activity. Inoculation of *S. nigrum* L. with *M. circinelloides* also resulted in increased SOD activity in lead-contaminated soils (Sun et al. 2017).

4.1.2 Catalase

Bioremediation enhanced the generation of CAT that can reduce the effect of oxidative damage in plants. Bioremediation with AMF enhanced the activity of CAT under soil salinization conditions, and thus provide resistance in *Avena Nuda* (Huang et al. 2008). According to Porcel et al. (2003), more consecration of CAT is also noticed in *G. max* roots colonized through *G. mosseae*. The increased activities of CAT enzyme were also reported in *Scytosiphon lomentaria* under copper-toxic environment, indicating the protecting role of CAT under oxidative stress (Contreras et al. 2005). Moreover, increased activity of CAT was also observed under bacterial bioremediation (*Enterobacter cloacae* strain) under Cd conditions (Banerjee et al. 2015). Russo et al. (2019b) documented that bioremediation with the fungal strain (*Rhizopus arrhizus)* significantly enhanced the CAT contents, and showed advanced protection under oxidative stress. The increased activity of CAT was also reported under bioremediation with two fungal species (*T. harzianum* and *P. simplicissimum*) (Russo et al. 2019a). However, Xu et al. (2015) observed that antioxidant enzyme activities were investigated in the bioremediation with filamentous fungi (*Penicillium chrysogenum* XJ-1) under different cadmium level. These authors observed an increase in CAT activities in tested fungi bioremediation. Further, Calderón-Delgado et al. (2019) examined a similar trend, where CAT potential was increased under bioremediation with algae (*Chlorella vulgaris*). The increased activity of CAT was reported under bioremediation with Indian mustard (Ansari et al. 2009). These findings suggest that an increase in CAT activeness could result in mitigating the Hg-induced oxidative stress.

4.1.3 Ascorbate Peroxidase

Increased APX activity plays a crucial role in countering the ROS entities under a variety of environmental stresses (Pandey et al. 2017). Bioremediation technique has been recommended as an emerging approach to increase the accumulation of APX under several environmental stresses (Blilou et al. 2000; He et al. 2007). In tomato (*L. esculentum*) colonized with AMF, more APX contents were observed, indicating a defense response under high salt concentration (He et al. 2007). Another study conducted by Ansari et al. (2009) has noted the increased APX activity when the Indian mustard is used as bioremediation under mercury stress. According to Singh et al. (2016b), the bioremediation with bacterial strains (NBRIEAG-6, NBRIEAG-8, and NBRIEAG-9) has caused an increase in APX activity in *O. sativa* under As stress. The increased synthesis was observed in APX activity under bacterial bioremediation (*Providencia vermicola*) under copper-enriched conditions (Islam et al. 2016), and significantly contributed to the elimination of ROS.

4.1.4 Glutathione Reductase

Bioremediation strategies have been reported to increase the activities of GR in crops exposed to environmental contaminations. The enhanced synthesis was observed in GR activity under inoculation of lettuce with *Rhizobium* spp. (Han and Lee 2005). Similarly, Lenártová et al. (1998) reported the consequences of bioremediation with bacteria (*S. bovis* and *S. ruminantium*), and reported the increased activity of GR under $HgCl_2$ stress. Xu et al. (2015) revealed that bioremediation with filamentous fungi *(Penicillium chrysogenum* XJ-1) resulting in enhance the activities of GR under cadmium stress, indicating the protective role to mitigate the cadmium-induced oxidative damage. In another study, Ansari et al. (2009) have noted the enhanced activities of GR while evaluating the Indian mustard as a bioremediation technique under mercury stress. According to Chakraborty et al. (2014), fungal bioremediation (*Aspergillus foetidus*) also resulted in enhanced the activities of GR enzymes under Cd stress. Bioremediation with fungus (*Mucor hiemalis*) has been reported for their ability to enhance GR activity (Hoque 2003; Hoque et al. 2007). Extensive induced activities of GR by macrophyte (*Potamogeton pusillus*) bioremediation have also been reported by Monferrán et al. (2009).

4.1.5 Peroxidase

Pioneer studies on bioremediation had shown that bioremediation mediated activities of POD significantly decrease the effects of oxidative injury in crops (Kohler et al. 2009; Li et al. 2019). Higher POD activity in AM tomato significantly enhanced the plant growth under high concentration of salts (He et al. 2007). Kohler et al. (2009) documented the increased activity of POD under *Pseudomonas mendocina* Palleroni inoculation in *Lactuca sativa* L., and reported a positive effect on the development of crops under salinity. Increased activity of POD under various strains of plant growth-promoting rhizobacteria, e.g., *S. proteamaculans* and *R. leguminosarum* has also been reported (Maheshwari 2012). Recently, the enhanced POD activity has also been reported under bacterial bioremediation with *B. amyloliquefaciens* RWL-1, in rice seedling under copper-induced toxicity (Shahzad et al. 2019). However, as stated by Li et al. (2019), reduced activity of POD under bioremediation with rye-grass species was reported under As stress. Enhanced activities of POD under bioremediation with the fungal strain (*Rhizopus arrhizus* and *Trichoderma hamatum)* showed increased protection under oxidative stress (Russo et al. 2019b). Likewise, Meghnous et al. (2019) examined the increased activity of POD with bioremediation of endophytic fungus (*Aspergillus tubingensis*) exposed to antimony toxic conditions and protected the cells against oxidative stress.

4.1.6 Glutathione *S*-Transferases

Enhanced activities of GSTs in crops beneath environmental contaminants alleviate the oxidative stress (Dixon et al. 2010). The overexpression of GSTs has also been reported under bioremediation with the fungal strain (*Rhizopus arrhizus* and *Trichoderma hamatum)* (Russo et al. 2019a). These authors reported that more expression of GSTs significantly enhances the protection against oxidative stress. In another report, Russo et al. (2019a) reported the increased activity of GSTs enzyme under bioremediation with two fungal species (*T. harzianum* and *P. simplicissimum*). Fungus (*Mucor hiemalis*) bioremediation has also been reported for their ability to enhance the activity of GSTs enzymes (Hoque 2003; Hoque et al. 2007).

4.1.7 Glutathione Peroxidase

The activities of glutathione peroxidase (GPs) were reported to be increased under different bioremediation techniques (Jayanthy et al. 2014; Monferrán et al. 2009). Bacterial bioremediation (*Streptococcus bovis* and *Selenomonas ruminantium*) have been reported to enhance the activities of GPs under $HgCl_2$ stress (Lenártová et al. 1998), suggesting an important role to tackle with the oxidative damage under stress environments. Different macrophytes, such as *Potamogeton pusillus*, have been reported for their ability to enhance the activity of GPs (Monferrán et al. 2009). Increased activity of GPs in green alga (*S. quadricauda*) was reported under a high level of Se (Vítová et al. 2011). Pandey et al. (2013) also reported the increased activity of GPs in bacterial strains (Bacillus sp., PbSP6 and AsSP9) under the exposure to heavy metals. The enhanced activity of GPs under *L. leucocephala* remediation as reported in the dye contaminated soil (Jayanthy et al. 2014).

4.1.8 Monodehydroascorbate Reductase

Increased MDHAR activities facilitate AsA regeneration, which helps in the detoxification of ROS under contaminant environment (Hasanuzzaman et al. 2012; Nahar et al. 2016). The bioremediation techniques have also been reported to enhance MDHAR activities under environmental stresses. Therefore, Cuypers et al. (2000) reported increased MDHAR enzyme activity in *Phaseolus vulgaris*, when used as bioremediatory, under Cr-enriched soils. Huang et al. (2016) demonstrated that bioremediation with arbuscular mycorrhizal fungi (*G. versiforme*) could increase the activity of MDHAR significantly, in *Sorghum halepense* under Cs stress.

4.1.9 Dehydroascorbate Reductase

Under toxic metal conditions, decreased activities of dehydroascorbate reductase (DHAR) were reported in *Brassica napus* plants (Hasanuzzaman et al. 2017). While Dalton (1995) reported that DHAR is involved in detoxification of H_2O_2 in cellular compartments. Bioremediation had shown the increased activity of DHAR enzyme. For example, according to Huang et al. (2016), bioremediation with arbuscular mycorrhizal fungi (*G. mosseae* and *G. versiforme*) in Johnson grass resulted in an increase in DHAR activeness under Cs stress. Higher DHAR activity was reported in

Table 3 Sub-cellular localization of non-enzymatic antioxidants

Enzyme	Location
Ascorbate (ASA)	Mitochondria, chloroplasts, peroxisomes, vacuoles, apoplast, nucleoli, plastid, cytosol, and nuclear membrane
Glutathione (GSH)	Cytoplasm, mitochondria, and plasma membranes (peroxisomes, endoplasmic reticulum, and lysosomal membranes)
Carotenoids	Plastids and cellular membranes (photoautotrophic organisms)
Tocopherols	Nuclear and cellular membranes

mycorrhizal roots under drought stress conditions, which involved in shielding mechanisms under oxidative stress (Porcel et al. 2003).

4.2 Non-enzymatic Antioxidants

Non-enzymatic antioxidants, i.e., the ascorbate (ASA), GSH, carotenoids, and tocopherols; and the sub-cellular localization of these enzymes are presented in Table 3.

4.2.1 Ascorbate

Elevated production of ASA enzyme with different bioremediation techniques is well known (Islam et al. 2016). Increased activity of ASA in plant roots was observed on exposure to olive-mill residue bioremediation with *P. chrysogenum-10* (García-Sánchez et al. 2014). The raised activity of ASH enzyme was also reported with bacterial inoculation (*P. vermicola*), determine the potential of crops to survive under Cu-induced oxidative stress (Islam et al. 2016). Enhanced activity of ASH was also reported in phytoremediation plants (weeds plants) collected from metal-induced polluted soils (Singh et al. 2016a).

4.2.2 Glutathione

The reduced activity of total GSH was reported for bioremediation with bacterial strains (*Staphylococcus* sp., *Brevibacillus* sp., and *S. arlettae*) in rice and showed a bacteria-mediated reduction of oxidative stress under arsenic stress (Singh et al. 2016b). It has been examined by Liu et al. (2012b) that reduced activities of GSH in *Microbacterium* sp. also showed the ability to detoxify the environmental contaminants. Mala et al. (2015) reported that, in *B. methylotrophicus,* the GSH-dependent chromate reductase can detoxify chromium-induced oxidative stress. Decreased activity of GSH enzyme was also reported on exposure to olive-mill residue bioremediation with *P. chrysogenum-10* and *C. rigida* (García-Sánchez et al. 2014). Nevertheless, Singh et al. (2016a) reported the higher activity of GSH enzymes in phytoremediation plants (weeds plants) collected from metal-induced polluted soils. These authors reported that enhanced activity of GSH was found to detoxify heavy metals induced oxidative stress. Increased GSH as a result of microbial bioremediation was also reported (Xu et al. 2015). These researchers suggested that increased GSH activity significantly increased antioxidant capacity under heavy metal stress. The increased level of GSH was also reported in *Heliscus lugdunensis* by different researchers under heavy metals stress (Braha et al. 2007; Jaeckel et al. 2005). Mukherjee et al. (2010) also noted increased activity of GSH in *Aspergillus niger* under arsenate stress and reported their protective role under oxidative stress.

4.2.3 Carotenoids

The carotenoids synthesis has been reported in a different microorganism such as yeast, bacteria, and algae (Frengova and Beshkova 2009) when they are grown under contaminated environments (Breierová et al. 2008). According to Bhosale and Gadre (2001), the increased biosynthesis of carotenoids in yeast under metals toxicity was reported. Bioremediation of shrimp bio-waste (from natural probiotic) also resulted in increased the production of carotenoids (Prameela et al. 2010). However, decreased carotenoids contents were reported under the bioremediation of *Callitriche cophocarpa Sendtn* (aquatic macrophyte) under chromium stress (Augustynowicz et al. 2010). Increased production of carotenoids content was also reported for bacterial bioremediation (*S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*) (Radhika and Kannahi 2014). Bioremediation with photosynthetic bacteria also resulted in the recovery of carotenoids under contaminated environments (Idi et al. 2015; Pattanamanee et al. 2012). In another study, Azad et al. (2001), also reported the increased carotenoids contents under bioremediation techniques. Previously, Ponsano et al. (2003), showed that bioremediation with photosynthetic bacteria also generates biomass abundant in carotenoids, which can be employed as the feed of animals. Bioremediation with microalgae also can increase and/or the recovery of carotenoids contents (Raeesossadati et al. 2014). Bioremediation with manure vermicomposting showed high levels of carotenoids as compared with control (Ayyobi and Peyvast 2014). Besides, Amooaghaie and Golmohammadi (2017) reported the same results for the vermicomposting bioremediation on thyme (*Thymus vulgaris* L.) plants.

4.2.4 Tocopherols

Bioremediation strategies result in the production of non-enzymatic molecules like tocopherols, which triggered the production of ROS. Higher activities of tocopherol enzyme were reported in transgenic *Brassica* plants under abiotic stresses (Kumar et al. 2013). Higher tocopherols values were reported for bioremediation with pseudometallophytes (Epelde et al. 2010). Significant increases in tocopherols contents were also reported for bioremediation with microalgae under copper stressed conditions (Hamed et al. 2017). Increased synthesis of tocopherols was reported under bioremediation with rhizosphere associated bacteria (Salomon et al. 2016). The supplementation of 24-Epibrassinolide also increased the tocopherols contents in *R. sativus* seedlings under Cd and Hg stress (Kapoor et al. 2016).

5 Chelation of Metals and the Role of Antioxidants

In plants, the detoxification of heavy metals and their tolerance mechanisms could be separated into two unique grouped, i.e., the exterior-omission or internal-resistance. In response to the exterior detoxification, the organic acids are excreting out from plant roots, and formed a stable heavy metal-ligand bond with heavy metal ions, and thus significantly affecting their mobility and bioavailability. Thereby, in the process of internal metal-detoxification, the organic acids can chelate with heavy metals in the cytosol, and ions can be transferred into less or non-toxic forms (Clemens 2006; Hall 2002; Kushwaha et al. 2015). Below, we have briefly described the thiol and non-thiol compounds and metal chelation; while Fig. 3 shows the pivotal role and connection between these compounds and their systematization with other defense systems under metal-subjected crops.

5.1 Thiol-Compounds and Metal-Chelation

Different thiol compounds like GSH, phytochelatins (PCs), and metallothioneins (MTs) contain groups of sulfhydryl (-SH) are important for maintaining the cytosol concentration of free metal(loid) (Seth et al. 2012). In general, chelation is the inclusively spread intra-cellular process for the support of low amount and detoxification of freely available metal ions in the cytosol of plants, that could be carried out via thiol compounds like GSH, PCs, MTs, and amino acids and their derivatives (Anjum et al. 2012, 2015; Bjørklund et al. 2019; Jozefczak et al. 2012; Seth et al. 2012). Nevertheless, GSH, PCs, and MTs played a crucial part in the metal chelation in crops.

5.1.1 Glutathione-Induced Metal Chelation

GSH is widely known as a most vital metabolite associated with the defense responses under several abiotic and biotic factors; thus plants cannot live in the absence of GSH or its homologous (Kumar and Trivedi 2018; Nianiou-Obeidat et al. 2017; Noctor et al. 2012). GSH, a major -SH group present in compartments of plant cell (cytosol, endoplasmic reticulum, and mitochondria), plays an essential role in antioxidant defense mechanism and cellular redox homeostasis in plants; GSH is also associated with the detoxification and chelation of freely available metals/metalloids (Anjum et al. 2012; Nahar et al. 2015; Sabetta et al. 2017; Seth et al. 2012). Hence, Fig. 4a indicates the metabolic detoxification process of GSH under metal toxicity, and Fig. 4b shows the systematic layout for the key role of GSH in metal remediation, in which metal resistance is necessary preconditions affected by metal homeostasis. GSH plays a vital part in metal homeostasis, being a chelating agent/metabolite/substrate. However, under metal stress, ROS and antioxidant defense systems generate signaling, where GSH can affect the cellular pathways associated with the acclimation and repair process to tackle with oxidative damage caused by metal stress. Further, energy (ATP or NADPH) is required for equilibrium among antioxidant systems and ROS. Therefore, the maintenance of elevated GSH

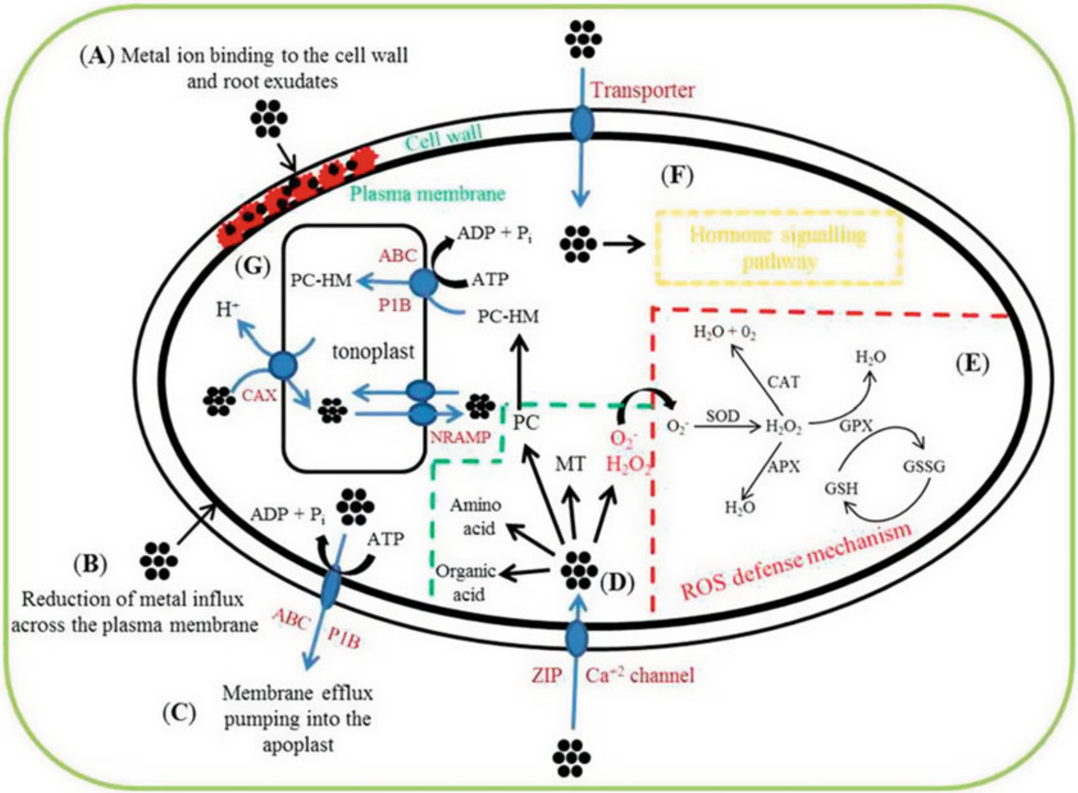

Fig. 3 Diagrammatic illustration of key role and connection between thiol and non-thiol compounds and their systematization with other defense systems under metal-subjected crops. Adapted from Anjum et al. (2015)

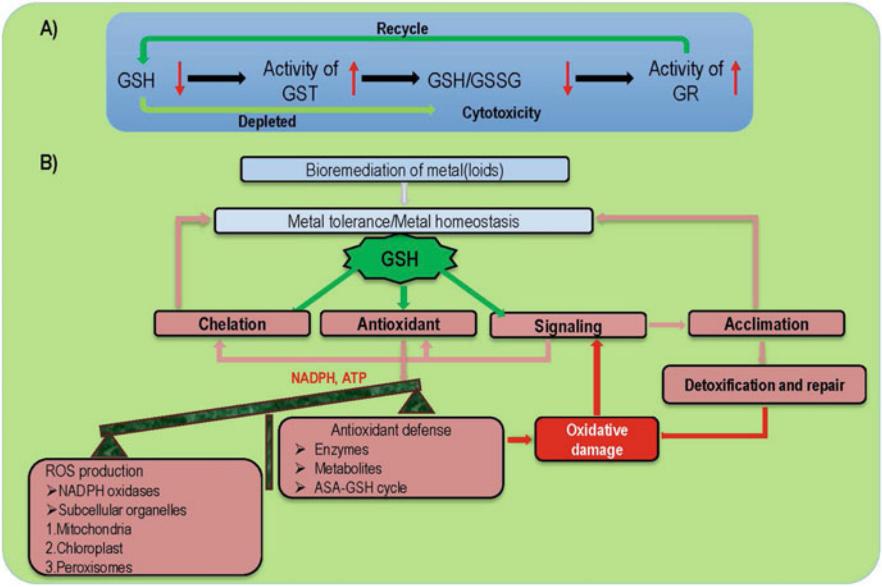

Fig. 4 Essentiality of GSH compound in the bioremediation process. (**a**) Metabolic detoxification process of GSH under metal toxicity; (**b**) Systematic layout for the key role of GSH in metals-remediation, in which metal-tolerance is a necessary precondition affected by metal homeostasis. See text for more detail

levels in plants is counted as an essential intrinsic feature, for reducing the heavy metals induced oxidative pressure in plants. The high levels of GSH in natural accruing plant species such as *T. goesingense*, *T. oxyceras*, and *H. lanatus* are well documented (Ernst et al. 2008).

Further, Hu et al. (2001) reported the higher levels of GSH in Cd-tolerant mutant of *C. reinhardtii* compared with wild-one. Therefore, Estrella-Gómez et al. (2012) described that *S. minima* plants showed glutathione synthase (GS) activity and increased GSH levels in response to Pb toxicity through a high expression level of SmGS gene. Hussain et al. (2016) documented that, the infectious disease of *A. thaliana* with a virulent pathogen *P. syringae* (*PstAvrB*) causes an improvement in the GSH level. Likewise, the increase in H_2O_2 was reported in cell subjected to stress; it also manages the GSH metabolism and protein action (Noctor et al. 2012). On the other hand, Balestrasse et al. (2001) noticed the deterioration in GSH concentration in soybean roots subjected to Cd stress; similar findings were also investigated by various researchers, e.g., in the roots of Scots pine (Schutzendubel et al. 2001), gray poplar roots (Schützendübel et al. 2002), and in *O. sativa* leaves (Hsu and Kao 2004). Piechalak et al. (2002) stated that Pb causes a reduction in the GSH level in legumes such as *V. faba*, and *P. vulgaris*. Additionally, Balestrasse et al. (2001) noticed the unchanged GSH concentration in the nodules of soybean plants under Cd stress.

5.1.2 Phytochelatins and Metal Chelation

The harmful impact of toxic metal(loids) ions in the cytoplasm can be removed with particular highly correlated ligands like PCs. Within the cytosol, firstly, PCs are generated and then moved to the vacuole in the form of a complex. However, in the existence of various metals, like Au, Zn, Mn, Mg, Se, Si, Cd, Pb, Hg, Cd, Ag, and Cu, etc. PCs can be quickly produced (Cobbett 2000; Cobbett and Goldsbrough 2002; Hasan et al. 2015). According to Volland et al. (2014), charophytic algae (*M. denticulata*) is the only one where the production of PCs has been discovered.

Hence, Zhang et al. (2010) examined the association among PCs biosynthesis and Cd amassing in *S. alfredii*, and reported that PCs are assumed as an inter-cellular Cd detoxification process in shoots instead of roots. Interestingly, Jambhulkar and Juwarkar (2009) reported that, *C. siamea* accumulated high concentrations of Pb, Ni, and Cr than other plant species cultivated on a fly ash dump having non-protein thiols; that are known for the synthesis of PCs and is accountable for the amassing of these metals. In contrast, Yadav et al. (2010) observed that, *J. curcas* accumulates an elevated concentration of Cr in roots; however, the concentration of freely available Cr ions in plant roots may last low, due to Cr ions sectionalize in the vacuole or making Cr-PCs complexes. On the other hand, knockdown of PCs genes that hold the production of PCs can enhance the susceptibility of *C. elegans* under Cd stress (Vatamaniuk et al. 2001).

An experiment performed by Mishra et al. (2006) deciphers the exposure of *B. monnieri* L. to Cd stress. The authors reported that the synthesis of PCs, along with the increased GSH levels and GR activities, plays a crucial part in the detoxification of metals and alleviation of metal-induced oxidative damage. Plants can store and resist a high level of Cd through an increased level of PCs and it is considered as a suitable antioxidant for phytoremediation. Moreover, Mukta et al. (2019) revealed that Cd-induced PCs help in reducing the Cr toxicity in rice by enhancing the GR activity and GSH level, which enhances the antioxidant defense mechanism to tackle with Cr toxicity. Under Cr stress, the enhanced PCs accumulation due to calcium treatments is also involved with vascular sequestration of Cr.

5.1.3 Metallothioneins

MTs possess a considerable affection for both vital and non-vital toxic metals, where MTs can furnish thiol compounds as a chelating agent in lessening form. Enormous affection of MTs for toxic metals such as Cd serve as a protective mechanism; on the other hand, it also plays a supporting for homeostasis of few crucial metals like Cu, Zn, Cd, etc. (Anjum et al. 2015). Previous studies show that MTs also induce or express in plants under various environmental stresses including wounding, senescence, salt, drought, high temperature, heavy metals, and cold; and these stresses can regulate MT genes expression in crops. Such as rgMT protein from rice regulated in *E. coli* and its expression were mediated by various abiotic stresses (Jin et al. 2006), *GhMT3a* from *G. hirsutum* enhanced resistance to few abiotic stresses in yeast and tobacco (Xue et al. 2008), ectopic expression of *OsMT1e-P* negotiates various abiotic stresses resistance in *Nicotiana tabacum* by ROS scavenging (Kumar et al.

2012), and functional characterization of *CsMT4* gene from cucumber increased resistance to high-salinity and osmotic stresses in *E. coli* (Zhou et al. 2019).

For heavy metals, Cu-induced H_2O_2 upregulation of *OsMT2c* gene from rice increased tolerance to Cu in *A. thaliana* (Liu et al. 2015), *CcMT1* gene from *C. cajan* regulated in *E. coli* and *Arabidopsis* improves resistance to Cd and Cu (Sekhar et al. 2011), *HbMT2a* from *H. brasiliensis* increases tolerance to Cu and Zn in *E. coli* (Li et al. 2015), *IlMT2b* from *I. lacteal* enhances tolerance to Cu in *Arabidopsis* (Gu et al. 2015), and a *PpMT2* gene from *P. patens* increased resistance to Cu and Cd in *A. thaliana* (Liu et al. 2019). In order to examine the phytoremediation availability of tobacco plants, Shestivska et al. (2011) demonstrated that the insertion of MTs in plants significantly enhanced the antioxidant properties than with non-transgenic plants. It is also reported that MTs-like proteins have also been present in *C. Vulgaris,* which can detoxify Cd and Zn toxicity (Huang et al. 2009).

5.2 Non-thiol-Compounds and Metal-Chelation

Numerous non-thiol compounds including amino acid and their derivatives (such as proline (Pro), cysteine (Cys), malate, and betaine), and organic acid (OAs; like citrate, malate, and oxalate) in isolation and coordination with different thiol compounds have been reported to be significantly contributed to the chelations of metals in plants (LaVoie et al. 2015; Pivato et al. 2014; Seth et al. 2012; Shaheen et al. 2017). Here, we discussed the recent studies on non-thiol compounds of metals in plants.

5.2.1 Organic Acids

Organic acids (OAs), low molecular weight compounds (LMWC), contain no less than one carboxyl group and are called as O_2-donor metal ligand. Among the various OA compounds, some compounds are elaborated in the oxidation of pyruvate when it existed in plant cells acting as a central point for the tricarboxylic acid cycle (TCA) cycle (Adeleke et al. 2017). OAs potentially perform various roles in the rhizospheres. OAs can carry the minus charge, therefore allowing movement of anions from soil origin and complexation of the metal cation in the solution (Jakkeral and Kajjidoni 2011). A major OA "citrate" is reported to belong to the crucial class of metabolites in plant cells. Large quantities of citrate have been reported in various plants such as in alfalfa (Lipton et al. 1987), tomato (Yilmaz et al. 2008), citrus (Sadka et al. 2001), tobacco (Lopez-Bucio et al. 2000), soybean (Yang et al. 2001), strawberry (Iannetta et al. 2004), and maize under light (Eprintsev et al. 2018).

In different cellular compartments, several metabolic pathways have been reported for the metabolism of citrate in plants. The involvement of citrate OA in carbon metabolism, and the abiotic and biotic stress resistance in plants have been reported in citrus under low temperature (Lin et al. 2016; Sheng et al. 2017), in rice under herbicide diclofop-methyl toxicity (Ding et al. 2014), soybean under Al stress (Zhou et al. 2018). Oxalate, an essential organic acid, is accumulated at a high level in plants, and plays an essential role in balancing the excess of inorganic cations over various anions (Lou et al. 2016; Ma et al. 2018; Osmolovskaya et al. 2018). The involvement oxalic acid in Al-resistance in *O. sativa* has been reported by Yokosho et al. (2011); Cu tolerance in *W. cocos* (Clausen et al. 2000); Cd elucidation in *B. nivea* (Li et al. 2014); Cu and Zn tolerance in fungi (Sazanova et al. 2015); Pb tolerance in *P. falcataria* (Setyaningsih et al. 2012), and phytoextraction of Pb, and Cd using *H. annuus* (Niu et al. 2010). Nevertheless, the role of dicarboxylic acid malate in photosynthesis process, respiration, oxidation of fatty acid, and biosynthesis of amino acids have been well documented (Casati et al. 1999; Fernie and Martinoia 2009; Musrati et al. 1998; Selinski and Scheibe 2019; Van Der Merwe et al. 2009; Zell et al. 2010).

5.2.2 Amino Acids and Their Derivatives

Under the high concentrations of metals, several amino acids like Pro, histidine, Cys, arginine, and the polyamines are synthesized in small amounts and showed their roles in stress resistance (Majumdar et al. 2019; Pivato et al. 2014). Nicotianamine is abundantly expressed in higher plants and plays a crucial part in metal chelation and homeostasis in plants (Banakar et al. 2019; Uraguchi et al. 2019). Arginine and Cys, diverse amino acids in living cells, play a significant part in the biosynthesis of other substances under higher concentrations of metals (Liu et al. 2018; Zhuang et al. 2017). The critical role of Arginine in metal chelation has been documented by various researchers (Futaki et al. 2004; Wojciechowska et al. 2015; Xu et al. 2018; Zhang et al. 2018). Polyamines are ubiquitous having differentiated properties such as acid neutralizing, antioxidant features, the stability of membrane and cell wall, and these characteristics make them effective protectants for abiotic stresses. The exogenous and endogenous applications of polyamines confer the tolerance against environmental stresses (Gill and Tuteja 2010a; Wen et al. 2008; Zhao and Yang 2008) including heavy metals, Cu tolerance in *N. peltatum* (Wang et al. 2007), Cd tolerance in wheat (Rady and Hemida 2015), Pb tolerance in wheat (Rady et al. 2016), and Cr tolerance in Kinnow mandarin (Shahid et al. 2018).

Another essential chelating agent, histidine is also present in some plants, especially in Ni-hyperaccumulators (Assunção et al. 2003). The significant roles of glycine betaine (GBs) as an osmoprotectant, ROS-scavenger, and as metal-chelators has been demonstrated in metal-subjected crops (Anjum et al. 2015; Asgher et al. 2013; Sharma and Dietz 2006; Theriappan et al. 2011). Proline plays a useful role in crops on exposure to different stress environments. Proline can act as an excellent osmolyte, and thus enhances the antioxidative defense mechanism and signaling molecules under stressed conditions. The involvement of flavonoids, phenylpropanoids, and phenolic acids in antioxidant defense mechanism is well understood (Ahmad et al. 2016; Gill and Tuteja 2010b; Rejeb et al. 2014).

6 Conclusion and Future Outlooks

Environmental pollution is enhancing gradually at a forbidding rate. Technological advancements, urbanization, and industrialization are the main contributors to pollution. Due to rapid industrializations, the increasing metals toxicity in the atmosphere is polluting the environment, not only air but also land and drinking water. Heavy metals, which are emitted from industries, are toxic, become persistent in the environment, and cause serious health problems. A sufficient concentration of heavy metal is needed for proper biological functions of animals and plants; hence, their elevated level causes metabolic interference. In the arrangement to tackle with the toxicity of heavy metals or to keep alive the level of some vital metals inside the range of physiological processes, the plant has evolved a wide range of multiplex mechanisms for metal tolerance. Plant and microorganisms possess various mechanisms for the metal-induced bioremediation of contaminated environments, including soils. Different microorganisms have been favorably employed to decrease the toxic effects of harmful metals.

Nevertheless, both enzymatic and non-enzymatic antioxidant defense systems thwart the impact of ROS. Thereby, these defense systems diffuse free radicals; hence, the oxidative stress risk becomes restricted. ROS also become inactivated at a cellular and molecular level. However, their low concentrations interrupt the radical chain reaction due to which the oxidative processes become delayed or inhibited. Antioxidants can easily chelate metal ions, which generate ROS. In coming years, it will be essential to comprehend that, how ROS modulate changes in crops function and development under the toxicity of heavy metals, consequently influence the crops, and boost their sustainable agricultural production.

Both antioxidant systems which are efficient enough play a vital role in achieving the detoxification or scavenging of excess ROS. Inside the cell, SOD, GPX, CAT, and enzymes from the cycle of AsA-GSH, such as MDHAR, APX, GR, and DHAR are included in enzymatic antioxidants, while GSH, AsA, phenolics, tocopherols, and carotenoids are non-enzymatic antioxidants. Many workers report that in plants, the enzymatic activities of defense systems of antioxidants increased in order to combat oxidative stresses, which are caused by various environmental factors. Increased antioxidant enzyme activities is linked with the scavenging of harmful ROS to enhance the high tolerance level of crops under metal stress. However, comprehensive research has been carried out on the production of ROS and response of defense systems in plants, thereby, more to the point works need to be done mainly in connection for the capabilities of antioxidant enzymes for the crop improvement under heavy metal stress.

Nevertheless, a good understanding is required on actual compounds that aid crops to manage the freely available metals in the cytoplasm. In this regard, thiol- and non-thiol compounds play a crucial part in the chelation of metals and control the low amount of freely available metals in the cytoplasm. Among these compounds, MTs and PCs are best-identified compounds due to their strong interaction with metals, metal chelation, reducing their level inside the cytosol, and ultimately reducing their toxic effect. Interestingly, GSH also plays an essential part in the bioremediation process as a chelating agent, due to its high kinship of metals, and it acts as a forerunner for PCs. Under metal stress, ROS and antioxidant defense systems generate signaling, where GSH can affect the cellular pathways associated with the acclimation and repair process to tackle with oxidative damage caused by metal toxicity. Besides this, GSH also acts as a crucial antioxidant defense agent, and redox signaling, including plant growth and development. Therefore, it is concluded that more studies are required to completely unrevealed the potential role of thiol- and non-thiol compounds for metal chelation. Transgenic plants can be introduced to boost the potential of metal bioremediation from toxic environments, and soils as well. Moreover, transgenic plants with modified GSH metabolisms have indicated encouraging outcomes; thereby, shortly, these plants should be implemented in field conditions on a large scale. Conjointly, the recommended investigations will help to generate the transgenic plants on a large scale for commercial usage, which can chelate various metals and prevent their toxicity in the environment.

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References

Abdal Dayem A et al (2017) The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. Int J Mol Sci 18:120

Adeleke R, Nwangburuka C, Oboirien B (2017) Origins, roles and fate of organic acids in soils: a review. S Afr J Bot 108:393–406

Ahmad P, Abdel Latef AA, Abd_Allah EF, Hashem A, Sarwat M, Anjum NA, Gucel S (2016) Calcium and potassium supplementation enhanced growth, osmolyte secondary metabolite production, and enzymatic antioxidant machinery in cadmium-exposed chickpea (*Cicer arietinum* L.). Front Plant Sci 7:513

Al Mahmud J, Hasanuzzaman M, Nahar K, Rahman A, Hossain MS, Fujita M (2017) Maleic acid assisted improvement of metal chelation and antioxidant metabolism confers chromium tolerance in *Brassica juncea* L. Ecotoxicol Environ Saf 144:216–226

Alqarawi AA, Abd Allah E, Hashem A (2014) Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. J Plant Interact 9:802–810

Amooaghaie R, Golmohammadi S (2017) Effect of vermicompost on growth, essential oil, and health of *Thymus vulgaris*. Compost Sci Util 25:166–177

Anjum NA et al (2012) Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids—a review. Environ Exp Bot 75:307–324

Anjum NA et al (2015) Jacks of metal/metalloid chelation trade in plants—an overview. Front Plant Sci 6:192

Ansari MKA, Ahmad A, Umar S, Iqbal M (2009) Mercury-induced changes in growth variables and antioxidative enzyme activities in Indian mustard. J Plant Interact 4:131–136

Asgher M, Khan MIR, Iqbal N, Masood A, Khan NA (2013) Cadmium tolerance in mustard cultivars: dependence on proline accumulation and nitrogen assimilation. J Funct Environ Bot 3:30–42

Assunção AG, Schat H, Aarts MG (2003) Thlaspi caerulescens, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351–360

Augustynowicz J, Grosicki M, Hanus-Fajerska E, Lekka M, Waloszek A, Kołoczek H (2010) Chromium (VI) bioremediation by aquatic macrophyte *Callitriche cophocarpa* Sendtn. Chemosphere 79:1077–1083

Ayyobi H, Peyvast G-A (2014) The effects of cow manure vermicompost and municipal solid waste compost on peppermint (*Mentha piperita* L.) in Torbat-e-Jam and Rasht regions of Iran. Int J Recycl Organic Waste Agric 3:147–153

Azad S, Vikineswary S, Ramachandran K, Chong V (2001) Growth and production of biomass of *Rhodovulum sulfidophilum* in sardine processing wastewater. Lett Appl Microbiol 33:264–268

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32:180

Balestrasse KB, Gardey L, Gallego SM, Tomaro ML (2001) Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. Funct Plant Biol 28:497–504

Banakar R, Fernandez AA, Zhu C, Abadia J, Capell T, Christou P (2019) The ratio of phytosiderophores nicotianamine to deoxymugenic acid controls metal homeostasis in rice. Planta 250:1339–1354

Banerjee G, Pandey S, Ray AK, Kumar R (2015) Bioremediation of heavy metals by a novel bacterial strain Enterobacter cloacae and its antioxidant enzyme activity, flocculant production, and protein expression in presence of lead, cadmium, and nickel. Water Air Soil Pollut 226:91

Bhosale P, Gadre R (2001) Production of β-carotene by a mutant of *Rhodotorula glutinis*. Appl Microbiol Biotechnol 55:423–427

Bjørklund G, Crisponi G, Nurchi VM, Cappai R, Buha Djordjevic A, Aaseth J (2019) A review on coordination properties of thiol-containing chelating agents towards mercury, cadmium, and lead. Molecules 24:3247

Blilou I, Bueno P, Ocampo JA, García-Garrido JM (2000) Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal Glomus mosseae. Mycol Res 104:722–725

Braha B, Tintemann H, Krauss G, Ehrman J, Bärlocher F, Krauss G-J (2007) Stress response in two strains of the aquatic hyphomycete *Heliscus lugdunensis* after exposure to cadmium and copper ions. Biometals 20:93

Breierová E, Gregor T, Marová I, Čertík M, Kogan G (2008) Enhanced antioxidant formula based on a selenium-supplemented carotenoid-producing yeast biomass. Chem Biodivers 5:440–446

Bulbovas P, Souza S, Esposito J, Moraes R, Alves E, Domingos M, Azevedo RA (2014) Assessment of the ozone tolerance of two soybean cultivars (*Glycine max* cv. Sambaíba and Tracajá) cultivated in Amazonian areas. Environ Sci Pollut Res 21:10514–10524

Calderón-Delgado IC, Mora-Solarte DA, Velasco-Santamaría YM (2019) Physiological and enzymatic responses of Chlorella vulgaris exposed to produced water and its potential for bioremediation. Environ Monit Assess 191:399

Casati P, Drincovich MF, Edwards GE, Andreo CS (1999) Malate metabolism by NADP-malic enzyme in plant defense. Photosynth Res 61:99–105

Chakraborty S, Mukherjee A, Khuda-Bukhsh AR, Das TK (2014) Cadmium-induced oxidative stress tolerance in cadmium resistant *Aspergillus foetidus*: its possible role in cadmium bioremediation. Ecotoxicol Environ Saf 106:46–53

Chaudhary K, Agarwal S, Khan S (2018) Role of phytochelatins (PCs), metallothioneins (MTs), and heavy metal ATPase (HMA) genes in heavy metal tolerance. In: Mycoremediation and environmental sustainability. Springer, Cham, pp 39–60

Cho Y-G, Rhee S-K, Lee S-T (2000) Influence of phenol on biodegradation of p-nitrophenol by freely suspended and immobilized *Nocardioides sp. NSP41*. Biodegradation 11:21–28

Clausen C, Green F III, Woodward B, Evans J, DeGroot R (2000) Correlation between oxalic acid production and copper tolerance in *Wolfiporia cocos*. Int Biodeterior Biodegradation 46:69–76

Clemens S (2006) Evolution and function of phytochelatin synthases. J Plant Physiol 163:319–332

Cobbett CS (2000) Phytochelatin biosynthesis and function in heavy-metal detoxification. Curr Opin Plant Biol 3:211–216

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Contreras L, Moenne A, Correa JA (2005) Antioxidant responses in *Scytosiphon lomentaria* (phaeophyceae) inhabiting copper-enriched coastal environments 1. J Phycol 41:1184–1195

Creus CM, Sueldo RJ, Barassi CA (1998) Water relations in Azospirillum-inoculated wheat seedlings under osmotic stress. Can J Bot 76:238–244

Cuypers A, Vangronsveld J, Clijsters H (2000) Biphasic effect of copper on the ascorbate-glutathione pathway in primary leaves of *Phaseolus vulgaris* seedlings during the early stages of metal assimilation. Physiol Plant 110:512–517

Dalton DA (1995) Antioxidant defenses of plants and fungi. In: Oxidative stress and antioxidant defenses in biology. Springer, Boston, pp 298–355

Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011:941810

Ding H et al (2014) Unraveling the toxicity mechanisms of the herbicide diclofop-methyl in rice: modulation of the activity of key enzymes involved in citrate metabolism and induction of cell membrane anion channels. J Agric Food Chem 62:10654–10660

Dixon DP, Skipsey M, Edwards R (2010) Roles for glutathione transferases in plant secondary metabolism. Phytochemistry 71:338–350

Doshi H, Ray A, Kothari I (2008) Bioremediation potential of Chlorella: spectroscopic, kinetics, and SEM studies. Int J Phytoremediation 10:264–277

Dwivedi S (2012) Bioremediation of heavy metal by algae: current and future perspective. J Adv Lab Res Biol 3:195–199

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015:756120

Epelde L, Becerril JM, Barrutia O, Gonzalez-Oreja JA, Garbisu C (2010) Interactions between plant and rhizosphere microbial communities in a metalliferous soil. Environ Pollut 158:1576–1583

Eprintsev AT, Fedorin DN, Dobychina MA, Igamberdiev AU (2018) Regulation of expression of the mitochondrial and peroxisomal forms of citrate synthase in maize during germination and in response to light. Plant Sci 272:157–163

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 1:529–539

Ernst WH, Krauss GJ, Verkleij JA, Wesenberg D (2008) Interaction of heavy metals with the sulphur metabolism in angiosperms from an ecological point of view. Plant Cell Environ 31:123–143

Estrella-Gómez NE, Sauri-Duch E, Zapata-Pérez O, Santamaría JM (2012) Glutathione plays a role in protecting leaves of *Salvinia minima* from Pb^{2+} damage associated with changes in the expression of *SmGS* genes and increased activity of GS. Environ Exp Bot 75:188–194

Fernie AR, Martinoia E (2009) Malate. Jack of all trades or master of a few? Phytochemistry 70:828–832

Flora SJ (2009) Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxidative Med Cell Longev 2:191–206

Flora SJ, Pachauri V (2010) Chelation in metal intoxication. Int J Environ Res Public Health 7:2745–2788

Frengova GI, Beshkova DM (2009) Carotenoids from Rhodotorula and Phaffia: yeasts of biotechnological importance. J Ind Microbiol Biotechnol 36:163

Fulthorpe RR, Rhodes AN, Tiedje JM (1996) Pristine soils mineralize 3-chlorobenzoate and 2, 4-dichlorophenoxyacetate via different microbial populations. Appl Environ Microbiol 62:1159–1166

Futaki S et al (2004) Arginine carrier peptide bearing Ni (II) chelator to promote cellular uptake of histidine-tagged proteins. Bioconjug Chem 15:475–481

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643

García-Sánchez M, Paradiso A, García-Romera I, Aranda E, De Pinto M (2014) Bioremediation of dry olive-mill residue removes inhibition of growth induced by this waste in tomato plants. Int J Environ Sci Technol 11:21–32

Ghelfi A, Gaziola S, Cia M, Chabregas S, Falco M, Kuser-Falcão P, Azevedo RA (2011) Cloning, expression, molecular modelling and docking analysis of glutathione transferase from *Saccharum officinarum*. Ann Appl Biol 159:267–280

Gill SS, Tuteja N (2010a) Polyamines and abiotic stress tolerance in plants. Plant Signal Behav 5:26–33

Gill SS, Tuteja N (2010b) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Gratão PL, Monteiro CC, Carvalho RF, Tezotto T, Piotto FA, Peres LE, Azevedo RA (2012) Biochemical dissection of diageotropica and never ripe tomato mutants to Cd-stressful conditions. Plant Physiol Biochem 56:79–96

Gu C-S, Liu L-Q, Deng Y-M, Zhu X-D, Huang S-Z, Lu X-Q (2015) The heterologous expression of the Iris lactea var. chinensis type 2 metallothionein *IlMT2b* gene enhances copper tolerance in *Arabidopsis thaliana*. Bull Environ Contam Toxicol 94:247–253

Hall J (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Hamed SM, Selim S, Klöck G, AbdElgawad H (2017) Sensitivity of two green microalgae to copper stress: growth, oxidative and antioxidants analyses. Ecotoxicol Environ Saf 144:19–25

Han H, Lee K (2005) Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. Res J Agric Biol Sci 1:210–215

Han L, Zhao D, Li C (2015) Isolation and 2, 4-D-degrading characteristics of *Cupriavidus campinensis BJ71*. Braz J Microbiol 46:433–441

Hasan M et al (2015) Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. Front Plant Sci 6:601

Hasanuzzaman M, Bhuyan MB, Raza A, Hawrylak-Nowak B, Matraszek-Gawron R, Al Mahmud J, Nahar K, Fujita M (2020a) Selenium in Plants: Boon or Bane?. Environ Exp Bot 29:104170

Hasanuzzaman M, Bhuyan MH, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V (2020b) Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants 9:681

Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M (2012) Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Crop stress and its management: perspectives and strategies. Springer, Dordrecht, pp 261–315

Hasanuzzaman M, Nahar K, Anee TI, Fujita M (2017) Exogenous silicon attenuates cadmium-induced oxidative stress in *Brassica napus* L. by modulating AsA-GSH pathway and glyoxalase system. Front Plant Sci 8:1061

Hassan TU, Bano A, Naz I (2017) Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field. Int J Phytoremediation 19:522–529

He Z, He C, Zhang Z, Zou Z, Wang H (2007) Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular mycorrhizae under NaCl stress. Colloid Surf B 59:128–133

He J et al (2011) Net cadmium flux and accumulation reveal tissue-specific oxidative stress and detoxification in Populus× canescens. Physiol Plant 143:50–63

Hoque E (2003) Verfahren zum Abbau von Xenobiotika durch Pilzarten mit Monooxygenase−/ Dioxygenase-Aktivität in Gegenwart von Pilzen mit Glutathion-S-Transferase-Aktivität. German Patent DE 101:365

Hoque E, Pflugmacher S, Fritscher J, Wolf M (2007) Induction of glutathione-transferase in biofilms and germinating spores of *Mucor hiemalis* strain EH5 from cold sulfidic spring waters. Appl Environ Microbiol 73:2697–2707

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 2012:872875

Hsu YT, Kao CH (2004) Cadmium toxicity is reduced by nitric oxide in rice leaves. Plant Growth Regul 42:227–238

Hu S, Lau KW, Wu M (2001) Cadmium sequestration in *Chlamydomonas reinhardtii*. Plant Sci 161:987–996

Huang L et al (2008) Antioxidant defenses of mycorrhizal fungus infection against SO 2-induced oxidative stress in *Avena nuda seedlings*. Bull Environ Contam Toxicol 81:440

Huang CF, Yamaji N, Mitani N, Yano M, Nagamura Y, Ma JF (2009) A bacterial-type ABC transporter is involved in aluminum tolerance in rice. Plant Cell 21:655–667

Huang R-H, Lu Y-M, Yang H-L, Huang W, Chen K (2016) Effects of arbuscular mycorrhizal fungi on caesium accumulation and the ascorbate-glutathione cycle of *Sorghum halepense*. Sci Asia 42:323–331

Hussain J et al (2016) Constitutive cyclic GMP accumulation in *Arabidopsis thaliana* compromises systemic acquired resistance induced by an avirulent pathogen by modulating local signals. Sci Rep 6:36423

Iannetta PP, Escobar NM, Ross HA, Souleyre EJ, Hancock RD, Witte CP, Davies HV (2004) Identification, cloning and expression analysis of strawberry (Fragaria× ananassa) mitochondrial citrate synthase and mitochondrial malate dehydrogenase. Physiol Plant 121:15–26

Idi A, Nor MHM, Wahab MFA, Ibrahim Z (2015) Photosynthetic bacteria: an eco-friendly and cheap tool for bioremediation. Rev Environ Sci Biotechnol 14:271–285

Islam F et al (2016) Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. Environ Sci Pollut Res 23:220–233

Jadoon S, Malik A (2018) A review of formation, toxicity of reactive oxygen species by heavy metals and tolerance in plants. Int J Biochem Res Rev 21:1–12

Jaeckel P, Krauss G, Menge S, Schierhorn A, Rücknagel P, Krauss G-J (2005) Cadmium induces a novel metallothionein and phytochelatin 2 in an aquatic fungus. Biochem Biophys Res Commun 333:150–155

Jain S, Arnepalli D (2019) Biominerlisation as a remediation technique: a critical review. In: Geotechnical characterisation and geoenvironmental engineering. Springer, Singapore, pp 155–162

Jakkeral SA, Kajjidoni S (2011) Root exudation of organic acids in selected genotypes under phosphorus deficient condition in blackgram (*Vigna mungo* L. Hepper). Karnataka J Agric Sci 24:316–319

Jambhulkar HP, Juwarkar AA (2009) Assessment of bioaccumulation of heavy metals by different plant species grown on fly ash dump. Ecotoxicol Environ Saf 72:1122–1128

Jan AT, Azam M, Ali A, Haq QMR (2014) Prospects for exploiting bacteria for bioremediation of metal pollution. Crit Rev Environ Sci Technol 44:519–560

Jayanthy V, Geetha R, Rajendran R, Prabhavathi P, Sundaram SK, Kumar SD, Santhanam P (2014) Phytoremediation of dye contaminated soil by *Leucaena leucocephala* (subabul) seed and growth assessment of *Vigna radiata* in the remediated soil. Saudi J Biol Sci 21:324–333

Ji G, Silver S (1995) Bacterial resistance mechanisms for heavy metals of environmental concern. J Ind Microbiol 14:61–75

Jin S, Cheng Y, Guan Q, Liu D, Takano T, Liu S (2006) A metallothionein-like protein of rice (rgMT) functions in E. coli and its gene expression is induced by abiotic stresses. Biotechnol Lett 28:1749–1753

Jozefczak M, Remans T, Vangronsveld J, Cuypers A (2012) Glutathione is a key player in metal-induced oxidative stress defenses. Int J Mol Sci 13:3145–3175

Kapoor D, Rattan A, Gautam V, Bhardwaj R (2016) Alleviation of cadmium and mercury stress by supplementation of steroid hormone to Raphanus sativus seedlings. Proc Natl Acad Sci India Sect B Biol Sci 86:661–666

Kohler J, Hernández JA, Caravaca F, Roldán A (2009) Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. Environ Exp Bot 65:245–252

Krumova E, Kostadinova N, Miteva-Staleva J, Gryshko V, Angelova M (2016) Cellular response to Cu-and Zn-induced oxidative stress in *Aspergillus fumigatus* isolated from polluted soils in Bulgaria. Clean Soil Air Water 44:657–666

Kumar S, Trivedi PK (2018) Glutathione S-transferases: role in combating abiotic stresses including arsenic detoxification in plants. Front Plant Sci 9:751

Kumar G et al (2012) Clustered metallothionein genes are co-regulated in rice and ectopic expression of *OsMT1e-P* confers multiple abiotic stress tolerance in tobacco via ROS scavenging. BMC Plant Biol 12:107

Kumar D, Yusuf MA, Singh P, Sardar M, Sarin NB (2013) Modulation of antioxidant machinery in α-tocopherol-enriched transgenic *Brassica juncea* plants tolerant to abiotic stress conditions. Protoplasma 250:1079–1089

Kushwaha A, Rani R, Kumar S, Gautam A (2015) Heavy metal detoxification and tolerance mechanisms in plants: implications for phytoremediation. Environ Rev 24:39–51

LaVoie SP et al (2015) Organic and inorganic mercurials have distinct effects on cellular thiols, metal homeostasis, and Fe-binding proteins in *Escherichia coli*. J Biol Inorg Chem 20:1239–1251

Lenártová V, Holovská K, Javorský P (1998) The influence of mercury on the antioxidant enzyme activity of rumen bacteria *Streptococcus bovis* and *Selenomonas ruminantium*. FEMS Microbiol Ecol 27:319–325

Li H et al (2014) Enhanced efficiency of cadmium removal by *Boehmeria nivea* (L.) gaud. In the presence of exogenous citric and oxalic acids. J Environ Sci 26:2508–2516

Li Y, Chen YY, Yang SG, Tian WM (2015) Cloning and characterization of *HbMT2a*, a metallothionein gene from *Hevea brasiliensis Muell.* Arg differently responds to abiotic stress and heavy metals. Biochem Biophys Res Commun 461:95–101

Li J, Zhao Q, Xue B, Wu H, Song G, Zhang X (2019) Arsenic and nutrient absorption characteristics and antioxidant response in different leaves of two ryegrass (*Lolium perenne*) species under arsenic stress. PLoS One 14:e0225373

Lin Q et al (2016) Low temperature induced changes in citrate metabolism in ponkan (*Citrus reticulata Blanco* cv. *Ponkan*) fruit during maturation. PLoS One 11:e0156703

Lipton DS, Blanchar RW, Blevins DG (1987) Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol 85:315–317

Liu S, Yang C, Xie W, Xia C, Fan P (2012a) The effects of cadmium on germination and seedling growth of *Suaeda salsa*. Proc Environ Sci 16:293–298

Liu Z, Wu Y, Lei C, Liu P, Gao M (2012b) Chromate reduction by a chromate-resistant bacterium, *Microbacterium sp*. World J Microbiol Biotechnol 28:1585–1592

Liu J, Shi X, Qian M, Zheng L, Lian C, Xia Y, Shen Z (2015) Copper-induced hydrogen peroxide upregulation of a metallothionein gene, *OsMT2c*, from *Oryza sativa* L. confers copper tolerance in Arabidopsis thaliana. J Hazard Mater 294:99–108

Liu M, Li N, He Y, Ge Y, Song G (2018) Dually emitting gold-silver nanoclusters as viable ratiometric fluorescent probes for cysteine and arginine. Microchim Acta 185:147

Liu Y et al (2019) Heterologous expression of the metallothionein *PpMT2* gene from Physcomitrella patens confers enhanced tolerance to heavy metal stress on transgenic Arabidopsis plants. Plant Growth Regul:1–10

Lopez-Bucio J, de la Vega OM, Guevara-Garcia A, Herrera-Estrella L (2000) Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nat Biotechnol 18:450

Lou HQ et al (2016) An oxalyl-CoA synthetase is involved in oxalate degradation and aluminum tolerance. Plant Physiol 172:1679–1690

Ma XL, Ren J, Dai WR, Yang W, Bi YF (2018) Effects of aluminium on the root activity, organic acids and free proline accumulation of alfalfa grown in nutrient solution. N Z J Agric Res:1–12

Maheshwari DK (2012) Bacteria in agrobiology: plant probiotics. Springer, Berlin

Mai P, Jacobsen OS, Aamand J (2001) Mineralization and co-metabolic degradation of phenoxyalkanoic acid herbicides by a pure bacterial culture isolated from an aquifer. Appl Microbiol Biotechnol 56:486–490

Majumdar R et al (2019) Contribution of maize polyamine and amino acid metabolism towards resistance against *Aspergillus flavus* infection and aflatoxin production. Front Plant Sci 10:692

Mala JGS, Sujatha D, Rose C (2015) Inducible chromate reductase exhibiting extracellular activity in *Bacillus methylotrophicus* for chromium bioremediation. Microbiol Res 170:235–241

Martins MNC, de Souza VV, da Silva Souza T (2016) Cytotoxic, genotoxic and mutagenic effects of sewage sludge on *Allium cepa*. Chemosphere 148:481–486

Megharaj M, Singh N, Kookana RS, Naidu R, Sethunathan N (2003) Hydrolysis of fenamiphos and its oxidation products by a soil bacterium in pure culture, soil and water. Appl Microbiol Biotechnol 61:252–256

Meghnous O, Dehimat L, Doumas P, Kassa-Laouar M, Mosbah F, Rached O (2019) Oxidative and antioxidative responses to antimony stress by endophytic fungus *Aspergillus tubingensis* isolated from antimony accumulator Hedysarum pallidum Desf. Biologia 74:1711–1720

Mishra S, Srivastava S, Tripathi R, Govindarajan R, Kuriakose S, Prasad M (2006) Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol Biochem 44:25–37

Mishra S, Jha A, Dubey R (2011) Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. Protoplasma 248:565–577

Monferrán MV, Agudo JAS, Pignata ML, Wunderlin DA (2009) Copper-induced response of physiological parameters and antioxidant enzymes in the aquatic macrophyte *Potamogeton pusillus*. Environ Pollut 157:2570–2576

Mukherjee A, Das D, Mondal SK, Biswas R, Das TK, Boujedaini N, Khuda-Bukhsh AR (2010) Tolerance of arsenate-induced stress in *Aspergillus niger*, a possible candidate for bioremediation. Ecotoxicol Environ Saf 73:172–182

Mukta RH, Khatun MR, Nazmul Huda A (2019) Calcium induces phytochelatin accumulation to cope with chromium toxicity in rice (*Oryza sativa* L.). J Plant Interact 14:295–302

Mulligan C, Yong R, Gibbs B (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207

Musrati R, Kollarova M, Mernik N, Mikulasova D (1998) Malate dehydrogenase: distribution, function and properties. Gen Physiol Biophys 17:193–210

Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015) Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methylglyoxal detoxification system. Environ Exp Bot 112:44–54

Nahar K, Hasanuzzaman M, Alam MM, Rahman A, Suzuki T, Fujita M (2016) Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems. Ecotoxicol Environ Saf 126:245–255

Nawaz K et al (2011) Eco-friendly role of biodegradation against agricultural pesticides hazards. Afr J Microbiol Res 5:177–183

Nematian MA, Kazemeini F (2013) Accumulation of Pb, Zn, Cu and Fe in plants and hyperaccumulator choice in Galali iron mine area, Iran. Int J Agric Crop Sci 5:426

Nianiou-Obeidat I, Madesis P, Kissoudis C, Voulgari G, Chronopoulou E, Tsaftaris A, Labrou NE (2017) Plant glutathione transferase-mediated stress tolerance: functions and biotechnological applications. Plant Cell Rep 36:791–805

Niu Z, Sun L, Sun T (2010) Relationships between changes of three organic acids (oxalic acid, citric acid and tartaric acid) and phytoextration by Sunflower (*Helianthus annuus* L.) in sand cultures contaminated with cadmium and lead. In: International conference on digital manufacturing & automation. IEEE, pp 149–153

Noctor G et al (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35:454–484

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res 14:1504

Osmolovskaya N, Dung VV, Kuchaeva L (2018) The role of organic acids in heavy metal tolerance in plants. Biol Commun 63:9–16

Pandey S, Barai PK, Maiti TK (2013) Influence of heavy metals on the activity of antioxidant enzymes in the metal resistant strains of *Ochrobactrum* and *Bacillus sp*. J Environ Biol 34:1033

Pandey S et al (2017) Abiotic stress tolerance in plants: myriad roles of ascorbate peroxidase. Front Plant Sci 8:581

Pattanamanee W, Choorit W, Deesan C, Sirisansaneeyakul S, Chisti Y (2012) Photofermentive production of biohydrogen from oil palm waste hydrolysate. Int J Hydrogen Energy 37:4077–4087

Piechalak A, Tomaszewska B, Baralkiewicz D, Malecka A (2002) Accumulation and detoxification of lead ions in legumes. Phytochemistry 60:153–162

Pierart A, Shahid M, Sejalon-Delmas N, Dumat C (2015) Antimony bioavailability: knowledge and research perspectives for sustainable agricultures. J Hazard Mater 289:219–234

Pivato M, Fabrega-Prats M, Masi A (2014) Low-molecular-weight thiols in plants: functional and analytical implications. Arch Biochem Biophys 560:83–99

Ponsano EHG, Lacava PM, Pinto MF (2003) Chemical composition of *Rhodocyclus gelatinosus* biomass produced in poultry slaughterhouse wastewater. Arch Biochem Biophys 46:143–147

Porcel R, Barea JM, Ruiz-Lozano JM (2003) Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytol 157:135–143

Prameela K, Murali Mohan C, Smitha P, Hemalatha K (2010) Bioremediation of shrimp biowaste by using natural probiotic for chitin and carotenoid production an alternative method to hazardous chemical method. Int J Appl Biol Pharma Technol 1:903–910

Radhika M, Kannahi M (2014) Bioremediation of pesticide (Cypermethrin) using bacterial species in contaminated soil. Int J Curr Microbiol Appl Sci 3:427–435

Rady MM, Hemida KA (2015) Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. Ecotoxicol Environ Saf 119:178–185

Rady MM, El-Yazal MAS, Taie HA, Ahmed SM (2016) Response of wheat growth and productivity to exogenous polyamines under lead stress. J Crop Sci Biotechnol 19:363–371

Raeesossadati M, Ahmadzadeh H, McHenry M, Moheimani N (2014) CO_2 bioremediation by microalgae in photobioreactors: impacts of biomass and CO_2 concentrations, light, and temperature. Algal Res 6:78–85

Rahman A, Nahar K, Hasanuzzaman M, Fujita M (2016) Manganese-induced cadmium stress tolerance in rice seedlings: coordinated action of antioxidant defense, glyoxalase system and nutrient homeostasis. C R Biol 339:462–474

Raza A (2020) Eco-physiological and biochemical responses of rapeseed (*Brassica napus* L.) to abiotic stresses: consequences and mitigation strategies. J Plant Growth Regul. https://doi.org/10.1007/s00344-020-10231-z

Raza A, Habib M, Kakavand SN, Zahid Z, Zahra N, Sharif R, Hasanuzzaman M (2020a) Phytoremediation of cadmium: physiological, biochemical, and molecular mechanisms. Biology 9:177

Raza A, Ashraf F, Zou X, Zhang X, Tosif H (2020b) Plant adaptation and tolerance to environmental stresses: mechanisms and perspectives. In: Plant ecophysiology and adaptation under climate change: mechanisms and perspectives I. Springer, Singapore, pp 117–145

Raza A, Charagh S, Sadaqat N, Jin W (2020c) *Arabidopsis thaliana*: Model plant for the study of abiotic stress responses. In: The plant family brassicaceae. Springer, Singapore, pp 129–180

Raza A, Mehmood SS, Tabassum J, Batool R (2019a) Targeting plant hormones to develop abiotic stress resistance in wheat. In: Wheat production in changing environments. Springer, Singapore, pp 557–577

Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, Xu J (2019b) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. Plants 8:34

Rehman A, Anjum MS (2011) Multiple metal tolerance and biosorption of cadmium by Candida tropicalis isolated from industrial effluents: glutathione as detoxifying agent. Environ Monit Assess 174:585–595

Rejeb KB, Abdelly C, Savouré A (2014) How reactive oxygen species and proline face stress together. Plant Physiol Biochem 80:278–284

Ruiz-Lozano J, Azcón R, Palma J (1996) Superoxide dismutase activity in arbuscular mycorrhizal *Lactuca sativa* plants subjected to drought stress. New Phytol 134:327–333

Russo F et al (2019a) Understanding fungal potential in the mitigation of contaminated areas in the Czech Republic: tolerance, biotransformation of hexachlorocyclohexane (HCH) and oxidative stress analysis. Environ Sci Pollut Res 26:24445–24461

Russo F et al (2019b) Bioremediation of DDT-contaminated agricultural soils: the potential of two autochthonous saprotrophic fungal strains. Appl Environ Microbiol 85:01720–01719

Sabetta W, Paradiso A, Paciolla C, de Pinto MC (2017) Chemistry, biosynthesis, and antioxidative function of glutathione in plants. In: Glutathione in plant growth, development, and stress tolerance. Springer, Cham, pp 1–27

Sadka A, Dahan E, Or E, Roose ML, Marsh KB, Cohen L (2001) Comparative analysis of mitochondrial citrate synthase gene structure, transcript level and enzymatic activity in acidless and acid-containing Citrus varieties. Funct Plant Biol 28:383–390

Salim N, Raza A (2020) Nutrient use efficiency (NUE) for sustainable wheat production: a review. J Plant Nutr 43:297–315

Salomon MV, Purpora R, Bottini R, Piccoli P (2016) Rhizosphere associated bacteria trigger accumulation of terpenes in leaves of *Vitis vinifera* L. cv. Malbec that protect cells against reactive oxygen species. Plant Physiol Biochem 106:295–304

Sazanova K, Osmolovskaya N, Schiparev S, Yakkonen K, Kuchaeva L, Vlasov D (2015) Organic acids induce tolerance to zinc-and copper-exposed fungi under various growth conditions. Curr Microbiol 70:520–527

Scarano G, Morelli E (2002) Characterization of cadmium-and lead-phytochelatin complexes formed in a marine microalga in response to metal exposure. Biometals 15:145–151

Schutzendubel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL, Polle A (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. Plant Physiol 127:887–898

Schützendübel A, Nikolova P, Rudolf C, Polle A (2002) Cadmium and H2O2-induced oxidative stress in Populus× canescens roots. Plant Physiol Biochem 40:577–584

Sekhar K, Priyanka B, Reddy V, Rao K (2011) Metallothionein 1 (*CcMT1*) of pigeonpea (*Cajanus cajan*, L.) confers enhanced tolerance to copper and cadmium in *Escherichia coli* and *Arabidopsis thaliana*. Environ Exp Bot 72:131–139

Selinski J, Scheibe R (2019) Malate valves: old shuttles with new perspectives. Plant Biol 21:21–30

Seth CS et al (2012) Phytoextraction of toxic metals: a central role for glutathione. Plant Cell Environ 35:334–346

Setyaningsih L, Setiadi Y, Sopandie D, Budi SW (2012) Organic acid characteristics and tolerance of Sengon (*Paraserianthes falcataria* L Nielsen) to lead. J Man Hut Trop 18:177–183

Shaheen S, Mahmood Q, Asif M, Ahmad R (2017) Genetic control of metal sequestration in hyper-accumulator plants. In: Phytoremediation. Springer, Cham, pp 343–368

Shahid M et al (2015) Heavy metal stress and crop productivity. In: Crop production and global environmental issues. Springer, Cham, pp 1–25

Shahid MA et al (2018) Polyamines provide new insights into the biochemical basis of Cr-tolerance in Kinnow mandarin grafted on diploid and double-diploid rootstocks. Environ Exp Bot 156:248–260

Shahzad R et al (2019) Amelioration of heavy metal stress by endophytic *Bacillus amyloliquefaciens RWL-1* in rice by regulating metabolic changes: potential for bacterial bioremediation. Biochem J 476:3385–3400

Sharma SS, Dietz K-J (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726

Sheng L et al (2017) Exogenous γ-aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit. Food Chem 216:138–145

Shestivska V et al (2011) Investigation of the antioxidant properties of metallothionein in transgenic tobacco plants using voltammetry at a carbon paste electrode. Int J Electrochem Sci 6:2869–2883

Silva P, Matos M (2016) Assessment of the impact of aluminum on germination, early growth and free proline content in *Lactuca sativa* L. Ecotoxicol Environ Saf 131:151–156

Singh JS, Abhilash P, Singh H, Singh RP, Singh D (2011) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480:1–9

Singh A, Prasad SM, Singh S, Singh M (2016a) Phytoremediation potential of weed plants' oxidative biomarker and antioxidant responses. Chem Ecol 32:684–706

Singh N, Srivastava S, Rathaur S, Singh N (2016b) Assessing the bioremediation potential of arsenic tolerant bacterial strains in rice rhizosphere interface. J Environ Sci 48:112–119

Srivastava S, Dubey R (2011) Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings. Plant Growth Regul 64:1–16

Sun L, Cao X, Li M, Zhang X, Li X, Cui Z (2017) Enhanced bioremediation of lead-contaminated soil by *Solanum nigrum* L. with Mucor circinelloides. Environ Sci Pollut Res 24:9681–9689

Tak HI, Ahmad F, Babalola OO (2013) Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. In: Reviews of environmental contamination and toxicology, vol 223. Springer, New York, pp 33–52

Tandon PK, Singh SB (2016) Redox processes in water remediation. Environ Chem Lett 14:15–25

Theriappan P, Gupta AK, Dhasarrathan P (2011) Accumulation of proline under salinity and heavy metal stress in cauliflower seedlings. J Appl Sci Environ Manag 15

Tsuji N, Hirayanagi N, Okada M, Miyasaka H, Hirata K, Zenk MH, Miyamoto K (2002) Enhancement of tolerance to heavy metals and oxidative stress in *Dunaliella tertiolecta* by Zn-induced phytochelatin synthesis. Biochem Biophys Res Commun 293:653–659

Uraguchi S, Weber M, Clemens S (2019) Elevated root nicotianamine concentrations are critical for Zn hyperaccumulation across diverse edaphic environments. Plant Cell Environ 42:2003–2014

Van Der Merwe MJ, Osorio S, Moritz T, Nunes-Nesi A, Fernie AR (2009) Decreased mitochondrial activities of malate dehydrogenase and fumarase in tomato lead to altered root growth and architecture via diverse mechanisms. Plant Physiol 149:653–669

Vatamaniuk OK, Bucher EA, Ward JT, Rea PA (2001) A new pathway for heavy metal detoxification in animals phytochelatin synthase is required for cadmium tolerance in *Caenorhabditis elegans*. J Biol Chem 276:20817–20820

Verma S, Kuila A (2019) Bioremediation of heavy metals by microbial process. Environ Technol Inn 14:100369

Vítová M, Bišová K, Hlavová M, Zachleder V, Rucki M, Čížková M (2011) Glutathione peroxidase activity in the selenium-treated alga Scenedesmus quadricauda. Aquat Toxicol 102:87–94

Volland S, Bayer E, Baumgartner V, Andosch A, Lütz C, Sima E, Lütz-Meindl U (2014) Rescue of heavy metal effects on cell physiology of the algal model system Micrasterias by divalent ions. J Plant Physiol 171:154–163

Wang X, Shi G, Xu Q, Hu J (2007) Exogenous polyamines enhance copper tolerance of Nymphoides peltatum. J Plant Physiol 164:1062–1070

Wasi S, Jeelani G, Ahmad M (2008) Biochemical characterization of a multiple heavy metal, pesticides and phenol resistant Pseudomonas fluorescens strain. Chemosphere 71:1348–1355

Wasi S, Tabrez S, Ahmad M (2011) Suitability of immobilized Pseudomonas fluorescens *SM1* strain for remediation of phenols, heavy metals, and pesticides from water. Water Air Soil Pollut 220:89–99

Wasi S, Tabrez S, Ahmad M (2013) Use of *Pseudomonas spp.* for the bioremediation of environmental pollutants: a review. Environ Monit Assess 185:8147–8155

Wen X-P et al (2008) Over-expression of the *apple spermidine synthase* gene in pear confers multiple abiotic stress tolerance by altering polyamine titers. Transgenic Res 17:251–263

Wojciechowska A, Gągor A, Zierkiewicz W, Jarząb A, Dylong A, Duczmal M (2015) Metal–organic framework in an l-arginine copper (ii) ion polymer: structure, properties, theoretical studies and microbiological activity. RSC Adv 5:36295–36306

Wu QS, Zou YN, Liu W, Ye X, Zai H, Zhao L (2010) Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: changes in leaf antioxidant defense systems. Plant Soil Environ 56:470–475

Xu X, Xia L, Zhu W, Zhang Z, Huang Q, Chen W (2015) Role of Penicillium chrysogenum *XJ-1* in the detoxification and bioremediation of cadmium. Front Microbiol 6:1422

Xu P, Zheng Y, Zhu X, Li S, Zhou C (2018) L-lysine and L-arginine inhibit the oxidation of lipids and proteins of emulsion sausage by chelating iron ion and scavenging radical. Asian Australas J Anim 31:905

Xue T, Li X, Zhu W, Wu C, Yang G, Zheng C (2008) Cotton metallothionein *GhMT3a*, a reactive oxygen species scavenger, increased tolerance against abiotic stress in transgenic tobacco and yeast. J Exp Bot 60:339–349

Yadav SK, Dhote M, Kumar P, Sharma J, Chakrabarti T, Juwarkar AA (2010) Differential antioxidative enzyme responses of *Jatropha curcas* L. to chromium stress. J Hazard Mater 180:609–615

Yamanaka K, Hoshino M, Okamoto M, Sawamura R, Hasegawa A, Okada S (1990) Induction of DNA damage by dimethylarsine, a metabolite of inorganic arsenics, is for the major part likely due to its peroxyl radical. Biochem Biophys Res Commun 168:58–64

Yang ZM, Nian H, Sivaguru M, Tanakamaru S, Matsumoto H (2001) Characterization of aluminium-induced citrate secretion in aluminium-tolerant soybean (*Glycine max*) plants. Physiol Plant 113:64–71

Yap L, Lee Y, Poh C (1999) Mechanism for phenol tolerance in phenol-degrading Comamonas testosteroni strain. Appl Microbiol Biotechnol 51:833–840

Yilmaz E, Batislam E, Basar M, Tuglu D, Erguder I (2008) Citrate levels in fresh tomato juice: a possible dietary alternative to traditional citrate supplementation in stone-forming patients. Urology 71:379–383

Yokosho K, Yamaji N, Ma JF (2011) An Al-inducible *MATE* gene is involved in external detoxification of Al in rice. Plant J 68:1061–1069

Zell MB et al (2010) Analysis of Arabidopsis with highly reduced levels of malate and fumarate sheds light on the role of these organic acids as storage carbon molecules. Plant Physiol 152:1251–1262

Zhang ZC, Chen BX, Qiu BS (2010) Phytochelatin synthesis plays a similar role in shoots of the cadmium hyperaccumulator Sedum alfredii as in non-resistant plants. Plant Cell Environ 33:1248–1255

Zhang Y, Zhang H, Liu Y, Zhang Z, Ding C (2018) Chelating ability and microbial stability of an L-arginine-modified chitosan-based environmental remediation material. J Polym Environ 26:885–894

Zhao H, Yang H (2008) Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in *Malus hupehensis* Rehd. Sci Hortic 116:442–447

Zhou Y et al (2018) Soybean NADP-malic enzyme functions in malate and citrate metabolism and contributes to their efflux under Al stress. Front Plant Sci 8:2246

Zhou Y, Liu J, Liu S, Jiang L, Hu L (2019) Identification of the metallothionein gene family from cucumber and functional characterization of *CsMT4* in *Escherichia coli* under salinity and osmotic stress. 3 Biotech 9:394

Zhuang Q, Sun L, Ni Y (2017) One-step synthesis of graphitic carbon nitride nanosheets with the help of melamine and its application for fluorescence detection of mercuric ions. Talanta 164:458–462

Current Research on the Role of Plant Primary and Secondary Metabolites in Response to Cadmium Stress

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Contents

Abstract

Cadmium (Cd) is known to be among the most toxic heavy metals to plants, posing severe risks to human health. Understanding Cd response mechanisms can provide insights into obtaining plants to be used for phytoremediation of Cd-contaminated soils and selection of plants with different degrees of Cd tolerance. Metabolites are the end products of cellular regulatory processes and

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define the cellular phenotype induced by stresses such as Cd toxicity. This chapter aims to summarize the current panorama of research concerning the role of plant metabolites in response to cadmium stress through metabolomic profiling studies as well as studies of preselected compounds in plants exposed to Cd stress.

Keywords

Abiotic stress · Cadmium tolerance in plants · Metabolomics · Plant responses to cadmium · Phytoremediation

1 Introduction

The intensification of industrial activities in recent decades has introduced alarming amounts of heavy metals (HMs) into the environment. In general, HMs are resistant to biodegradation and can persist in the environment for hundreds of years, making them a chronic source of contamination that poses risks to human health and the entire ecosystem (Nagajyoti et al. 2010; Filimonova et al. 2016). Once deposited in the environment, HMs are able to undergo bioaccumulation and biomagnification processes along the trophic chain and may cause changes in the genetic material of various organisms (Leme and Marin-Morales 2009; Liman et al. 2010; Pizzaia et al. 2019).

Cadmium (Cd) is known to be among the most toxic HMs to humans and plants. With the exception of the diatom *Thalassiosira weissflogii* (Thalassiosiraceae), which has a carbonic anhydrase capable of using Cd^{2+} as an enzymatic cofactor (Lane et al. 2005), Cd is a nonessential and extremely toxic HM, justifying the need for monitoring their concentration in biological systems. In land plants, Cd phytotoxicity is related to its high mobility in the soil–plant system (Benavides et al. 2005). Bioaccumulation can be understood as the ability of any plant species to absorb and accumulate HMs, along with associated effects on plant development and metabolism, and ultimately on the plant's ability to complete its life cycle (Kumar et al. 2013).

Cd is released into the environment as Cd^{2+} primarily by anthropogenic activities, including industrial and agricultural waste disposal, waste incineration, and mining activities (Loganathan et al. 2012; Cuypers et al. 2016; Devesa and Vélez 2016). In addition, oil refining, processing and use of other fossil fuels, and atmospheric deposition have contributed to increased Cd levels in the environment over recent decades (Nagajyoti et al. 2010). Perhaps most critically, because phosphate fertilizers derived from geologic deposits of rock phosphate often naturally contain high levels of Cd, the use of phosphate fertilizers contributes to a large fraction of soil Cd contamination (Gilbert 2018). Increased soil contamination by excessive use of phosphate fertilizers contributes to an elevated concentration of Cd and other various toxic compounds in the soil, leading to greater retention in plant tissues (Satarug et al. 2003). Ingesting either plants or products derived from contaminated plants is one route for human and animal Cd exposure (Kubo et al. 2016). There has been an effort to gradually replace high-Cd fertilizers with less toxic alternatives given growing recognition that concentrations of Cd and other toxic metals have been increasing in the environment, and especially accumulating in plants where such elements may enter food webs (Gallego et al. 2012).

Cd can cause severe morphophysiological damage to plants, in addition to bioaccumulating in various parts of plants such as edible vegetable leaves (Baldantoni et al. 2016). This often results in reduced agricultural productivity and ultimately health risks to the human population through bioaccumulation and biomagnification (Gratão et al. 2015; Peng et al. 2017). Plant Cd uptake can affect the homeostasis of various essential metals in the plant organism, including Zn, Ca, Fe, Mg, and Cu. In cellular metabolism, Cd toxicity is related to high sulfur-binding affinity and strong interactions with sulfhydryl groups of many proteins. Cd phytotoxicity is also related to physiological and biochemical modifications, such as alteration of photosynthetic processes, and metabolic and enzymatic activities (DalCorso et al. 2008; Gallego et al. 2012; Choppala et al. 2014) that lead to growth inhibition and chlorosis (Gill and Tuteja 2011).

At the cellular level, Cd interferes with the formation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals. When the amount of oxidizing species produced is greater than that of the plants are able to eliminate, this leads to oxidative stress (Yi et al. 2015). Increased concentrations of oxidizing species are extremely reactive and cytotoxic to virtually all organisms due to protein oxidation, DNA damage, and membrane lipid peroxidation, the latter of which is considered the most drastic effect of HMs on cells (Quan et al. 2008; Arruda and Azevedo 2009; Gill and Tuteja 2010). In addition, cytogenetic studies have demonstrated substantial effects of HMs on the cell cycle, resulting in chromosomal changes and even apoptosis (Leme and Marin-Morales 2009; Moraes et al. 2015, 2018; Carvalho et al. 2018; Pizzaia et al. 2019).

Metal tolerance and hyperaccumulation should be considered distinct continuous traits based on their distinct physiological mechanisms and separate genetic basis (Goolsby and Mason 2015). In this context, understanding Cd tolerance and response mechanisms can contribute to research on selection of Cd-tolerant plants, Cd hyperaccumulators, and/or Cd low accumulators. Also, understanding such mechanisms can provide insights into the engineering of plants for phytoremediation, biofortification of food crops, and as biomarkers for the identification of environmental pollutants.

Plant responses to Cd include modulation of synthesis and activity of metabolites, since they are the end products of cellular regulatory processes and define the cellular phenotype induced by stresses such as Cd toxicity (Fiehn et al. 2007). When plants are exposed to stress conditions, an energy shift from normal plant growth is required. In this context, plant survival involves a complex network of various regulatory pathways, including metabolite adjustment, leading to physiological and morphological acclimatization (Muscolo et al. 2015).

Plants produce myriad primary and secondary metabolites, and a wide variety of classes of plant metabolites have received attention in various subfields since the introduction of gas chromatography (GC) in the early 1950s with the analysis of plant essential oils (Rochfort 2005; Ryan and Robards 2006; Verpoorte et al. 2008;

Wink et al. 2015). The complete set of metabolites used or formed by an organism in association with its metabolism is referred to as the "metabolome" (Nielsen and Oliver 2005). Metabolomics, in turn, is the measurement of all metabolites in a given system (cell, tissue, or organism) under a given set of conditions (Goodacre 2004; Kopka et al. 2004; Rochfort 2005).

Metabolomics is currently applied in a variety of research fields, including several applications in the plant sciences (Taylor et al. 2002; Pinu 2015; Rizhsky et al. 2016). Within metabolomics, there are two principal approaches employed to provide comprehensive knowledge of plant metabolism and the response to environmental conditions: targeted metabolomics, which is focused on analyzing selected classes of metabolites, and untargeted metabolomics, which aims to screen the entire metabolite content of biological samples (Navarro-Reig et al. 2017). In addition to metabolomic approaches, less comprehensive studies focused on understanding the response of individual metabolites, and small groups of preselected compounds to Cd are also important for understanding plant Cd response and tolerance mechanisms.

This chapter aims to present information on plant primary and secondary metabolites related to the plant response to Cd as obtained through metabolomic profiling studies as well as studies of preselected compounds in plants exposed to Cd stress.

2 Cadmium Response-Related Metabolites

A number of studies have analyzed the specific metabolic responses of plants exposed to Cd, as can be seen in Table 1. In addition, metabolomic analysis also has been used for investigating Cd response in plants and requires specific techniques, such as those listed in Table 2.

The most widely used techniques for plant metabolomic research are gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) spectroscopy, and capillary electrophoresis-mass spectrometry (CE-MS). These techniques all have specific strengths and weaknesses and can be used in combination according to the type of metabolite to be quantified (Tomás-Barberán 1995; Obata and Fernie 2012; Jorge et al. 2016). Other methods may be employed to quantify metabolites including high-performance liquid chromatography with photodiode array detection (HPLC-DAD) and thin-layer chromatography (TLC). Also, metabolite detection by Fourier transform ion cyclotron resonance mass analyzers (FT-ICR MS) has been described (Hagel and Facchini 2008; Moritz and Johansson 2008; Flis et al. 2016).

Indeed, given the great diversity and complexity of plant metabolites, metabolomics is an important tool to provide a comprehensive and simultaneous perspective of metabolic interactions and has been widely used in research involving plant stress by HMs such as Cd in recent years. When compared to transcriptomics and proteomics, metabolomics is often considered to provide a more complete view of the mechanisms of biological regulation (Osorio et al. 2012; Dos Santos et al.

Table 1 Examples of specific metabolites analyzed in studies on plants exposed to cadmium stress

Plant species	Plant materials (age of the plants when treated with Cd)	Technologies for metabolite identification	Metabolites	[Cd]; Exposure time	References
Amaranthus hypochondriacus	Leaves (5 months)	Liquid chromatography-tandem mass spectrometry, high-performance liquid chromatography	Phytochelatins, thiol compounds	Cd added in the form of a $CdCl_2 \cdot 2.5H_2O$ solution at a concentration of 100 mg kg^{-1} (corresponding to an actual concentration of 126.34 mg kg^{-1} in soil); 2 months	Xie et al. (2019)
Brassica juncea	Leaves (55 days)	Colorimetric methods	Chlorophyll, proline, ascorbic acid, α-tocopherol	$CdSO_4 \cdot 8H_2O$ treatments (100 and 200 mg L^{-1} in plastic pots containing sand plus vermicompost); 45 days	Ahmad et al. (2016)
Brassica rapa	Roots (seeds)	Colorimetric methods	Alkaloids, proline, phenolic compounds	$CdCl_2$ concentrations: 25, 50, 75, and 100 mg kg^{-1} in soil; 30 days (the early vegetative stage), 65 days (late vegetative stage), and 155 days (flowering stage)	Abo-Hamad et al. (2013)
Drimia elata	Shoots and bulbs (154 days)	Inductively coupled plasma optical emission spectroscopy	Chlorophyll, proline, total phenolic, flavonoids	2, 5, and 10 mg L^{-1} $cd(NO_3)_2$ in pots containing acid washed quartz sand; 42 days	Okem et al. (2015)
Matricaria chamomilla	Shoots and roots (5 weeks)	High-performance liquid chromatography and detection by a mass selective HP MSD quadrupole detector	Phenolic acids	60 μM of Cd in solution; 7 days	Kovácik et al. (2011)

(continued)

Table 1 (continued)

Plant species	Plant materials (age of the plants when treated with Cd)	Technologies for metabolite identification	Metabolites	[Cd]; Exposure time	References
Hypericum perforatum	Shoots and roots (42 days)	Liquid chromatography with mass detection	Reduced and oxidized glutathione, ascorbic acid, phenolic acids, flavonoids	10 µM Cd (added as $CdCl_2 \cdot 2½H_2O$) in solution; 14 days	Babula et al. (2015)
Robinia pseudoacacia	Leaves and stems (age was not communicated)	Inductively coupled plasma mass spectrometry	Saponins, phenolic compounds, flavonoids, alkaloids, condensed tannins	0.23, 1.23, and 5.23 mg Cd kg^{-1} dry soil; 30 days	Zhao et al. (2016)

Table 2 Examples of metabolomics-based studies on plants exposed to cadmium stress

Plant species	Plant materials (age of the plants when treated with Cd)	Technologies for metabolite identification	[Cd]; exposition time	References
Amaranthus hypochondriacus	Leaves (5 months)	Liquid chromatography-tandem mass spectrometry, high-performance liquid chromatography	Cd added in the form of a $CdCl_2 \cdot 2.5H_2O$ solution at a concentration of 100 mg kg^{-1} (corresponding to an actual concentrations of 126.34 mg kg^{-1} in soil); 2 months	Xie et al. (2019)
Cynodon dactylon	Roots and shoots (4 months)	Inductively coupled plasma mass spectroscopy	1.5 mM $CdSO_4$ in $solution_4$; 14 days	Xie et al. (2014)
Drimia elata	Shoots and bulbs (154 days)	Inductively coupled plasma optical emission spectroscopy	2, 5, and 10 mg L^{-1} of $Cd(NO_3)_2$ in pots containing acid washed quartz sand; 42 days	Okem et al. (2015)
Hypericum perforatum	Shoot and roots (42 days)	Liquid chromatography with mass detection	10 μM of Cd (added as $CdCl_2 \cdot 2½H_2O$) in solution; 14 days	Babula et al. (2015)
Raphanus sativus	Roots (17 days)	Chromatography-mass spectrometry	400 mg L^{-1} $CdCl_2 \cdot 2.5H_2O$ in culture pots filled with modified half-strength Hoagland nutrient solution; 72 h	Wang et al. (2015a)
Solanum lycopersicum	Leaves (116 days)	Proton nuclear magnetic resonance spectroscopy, high-pressure liquid chromatography with photo diode array detection, colorimetric methods	20 and 100 μM $CdCl_2$ in solution; 90 days	Hédiji et al. (2010)

2017), especially for biological processes mediated directly by metabolites, such as metal chelation.

In some studies metabolomic results have been correlated with synthesis of phytochelatins (PCs). For example, Xie et al. (2019) performed a full-scale metabolomic analysis of metabolic responses of *Amaranthus hypochondriacus* (Amaranthaceae) under Cd stress and found that 12 metabolites were highly linearly correlated with PCs involved in three pathways (Val, Leu, and Ile biosynthesis; Ala, Asp, and Glu metabolism; and Arg and Pro metabolism). Cd^{2+}–PC complexes (formed in the cytoplasm) are transported to the vacuoles, contributing to modulation of Cd tolerance in plants. In fact, several studies have associated PC levels with differential Cd tolerance in some species (Wang et al. 2012; Brunetti et al. 2015).

In studies using omics approaches other than metabolomics, such as proteomics (Borges et al. 2019), transcriptomics (Chen et al. 2019a), and phenomics (Piotto et al. 2018), it is possible to observe the correlation of Cd-induced toxicity with oxidative stress, interference with photosynthetic processes, and related aspects of metabolism. Some studies also indicate that signaling molecules such as plant hormones may contribute to the initiation of a response cascade. Hereafter, the current state of knowledge is presented on classes of metabolites that have been demonstrated to be potentially related to Cd response, as well as a discussion of the technologies that allow the quantification of these compounds. Subsequently, we present evidence on several aspects of plant physiology that are related to the metabolic response of plants to Cd, including those related to hormones and photosynthesis.

2.1 Introducing Metabolites in Plants and Their Classification

Plants are sessile multicellular organisms, and the evolution of their metabolism has moved toward fantastic complexity, which has led to plant survival under highly diverse environmental conditions (Knudsen et al. 2018). Plant metabolism can be divided into two categories: primary metabolism and secondary metabolism. Plant primary metabolism encompasses metabolites, reactions, and pathways, which are essential for plant growth and development, and thus survival to reproduction (Pott et al. 2019). Thus, primary metabolites are those with universal distribution among plants and produced in larger quantities in plant tissue, such as amino acids, proteins, nucleotides, carbohydrates, lipids, and pigments such as chlorophyll (Jorge et al. 2016). Plant secondary metabolism encompasses all other functions, including plant abiotic stress resistance, plant defense against herbivore and disease, mutualisms with symbionts and pollinators, and many other functions not strictly essential for completion of the life cycle under idealized conditions (Wolfender et al. 2015). Of particular relevance here, secondary metabolism plays an important role in combatting HM toxicity. Phytotoxicity caused by exposure to Cd strongly interferes with plant growth and development, since it compromises essential physiological mechanisms related to the composition of primary metabolites and also involves interactions with secondary metabolites through the activation of stress response pathways.

Several primary metabolism products often serve as precursors for the synthesis of the tens of thousands of secondary metabolites. However, compared to primary metabolism (which is generally highly conserved), a much greater diversity is observed in secondary (or specialized) metabolism pathways at the level of species, organs, tissues, developmental stages, and cell types. The high level of catalytic promiscuity of the enzymes, which manufacture many secondary metabolites, is presumably necessary to their high diversity (Pott et al. 2019). It is estimated that the plant kingdom contains between 200,000 and 1,000,000 metabolites, of which 7000–15,000 are found in each plant species, and of this total, about 3000–5000 are present in leaves (Davies et al. 2012; Matsuda et al. 2015). This number of metabolites may vary within the same plant species or genotype, both in composition and quantity, depending on natural variations in plant development and environmental conditions such as temperature, rainfall, soil composition, etc. (Yang et al. 2018).

Many secondary metabolites not only have advantages for the plants that produce them but they are also of economic, industrial, and pharmaceutical interest to humanity. The main classes of secondary metabolites with proven action on plant metabolism to date and some examples of studies on Cd response are presented below.

2.1.1 Polysaccharides

Polysaccharides are not only energetic and structural components of plants but also have a variety of other biological functions, some of which are associated with secondary metabolism (Zong et al. 2012). These are monosaccharide polymers connected by glycosidic bonds, which usually have straight or branched side chains in their molecular structures (Chen et al. 2019b). Plant polysaccharides can be divided into homogeneous or homoglycans when resulting from the condensation of a large number of molecules of the same sugar (e.g., starch and cellulose), and heterogeneous or heteroglycans when formed by the condensation of different types of sugars (e.g., gums, mucilages, and pectins). The characteristics of the different polysaccharides are determined by their primary structure, type, and sequence of monosaccharides and also by their degree of polymerization and their conformation. These compounds can also be classified by their solubility in water, being either soluble (e.g., gum, mucilage, and pectin) or insoluble (e.g., cellulose and hemicellulose) (Zhang et al. 2019).

Chromatography is currently the most common method for identifying polysaccharides and their homogeneity. Gel permeation chromatography and high-performance liquid chromatography (HPLC) combined with refractive index (IR) detector are examples of chromatographic methods used for polysaccharide identification (Lv et al. 2013). Polysaccharide content in plant samples can also be measured by using the phenol-sulfuric acid method (Wang et al. 2015b). Other techniques such as cleavage are not only used to determine the molecular weight of polysaccharides but also to identify structural fragments (Guo et al. 2012). In the measurement process, the matrix and sample concentration are selected according to the structure of the targeted polysaccharide to achieve the desired result (Ren et al. 2019).

In plants exposed to Cd, changes in cellulosic cell wall structure and homogalacturonan methylation pattern have been observed (Gutsch et al. 2019). It is noteworthy that polysaccharides such as pectins are of great importance in the binding and accumulation of metals in the plant cell wall and may influence the ability of the cell wall to accumulate and immobilize metals (Krzesłowska 2011).

2.1.2 Phenolic Acids

Plant phenolic acids include derivatives of benzoic (Widhalm and Dudareva 2015) and cinnamic acid (Tinikul et al. 2018). Major benzoic acid derivatives include *p*-hydroxybenzoic, salicylic, protocatechuic, vanillic, gentisic, gallic, and syringic acids. Several of these can be obtained by acid hydrolysis of gymnosperm and angiosperm leaves, indicating a close relationship with lignin biosynthesis. Most benzoic acid derivatives are widespread across land plants, and salicylic acid also has a major conserved function as a plant hormone involved in pathogen defense and several other functions (Li et al. 2003, 2019; Khan et al. 2015; Tanaka et al. 2019). Major cinnamic acid derivatives include *p*-coumaric, caffeic, ferulic, and synapic acids, among many others, and this class of compounds is present in virtually all plant tissues. All cinnamic acid derivatives have a double bond in their chemical structures and can exist in two isomeric forms (*Z* and *E*), *E*-isomers being the most common and also the most stable (Sova 2012). The third class, esters, contain a wide variety of compounds formed by the esterification of a carboxylic acid (e.g., a cinnamic acid derivative or benzoic acid derivative) with another carboxylic acid or other organic acid containing an alcohol group. These include compounds like the chlorogenic acids (esters of cinnamic acid derivatives and quinic acid), chicoric acid (ester of caffeic acid and tartaric acid), and rosmarinic acid (ester of caffeic acid and a lactic acid derivative).

Most phenolic acids are very soluble in water and polar organic solvents. Analyzing these compounds can be performed by using TLC on cellulose, silica, or polyamide gel. This technique is very useful for the separation and identification of various phenolic acids, especially when associated with fluorescence staining under UV light, staining with chemical reagents (ferric chloride, phosphomolybdic acid salts, vanillin, and other aromatic aldehydes in medium acid) (Rastogi et al. 2008). Both HPLC and CE can also be used to isolate phenolic acids, as can GC after derivatization (Tomás-Barberán 1995; Restivo et al. 2014).

The protective effect of some phenolic metabolites against Cd toxicity is due to their ability to chelate Cd. Some phenolic acids are capable of eliminating oxidizing species and forming metal chelates, making Cd unavailable in plant tissues (Kovácik et al. 2009). Cd can be retained by polymerized phenols in aquatic plants, demonstrating a correlation not with only increased phenolic metabolite synthesis but also with the activity of polyphenol oxidase and peroxidase in these plants (Lavid et al. 2001a, b).

Plants that have an ability to increase the synthesis of these metabolites may be able to efficiently withstand Cd stress. For example, the importance of different phenolic metabolites for Cd plant tolerance was verified in chamomile (*Matricaria chamomilla*, Asteraceae) (Kovácik et al. 2009). In addition to the direct importance described above, it has been suggested that environmental monitoring of Cd may be possible through the analysis of some natural phenolic acids synthesized by plants under metal exposure (Dresler et al. 2016, 2017).

2.1.3 Flavonoids

Flavonoids are an important class of polyphenolic metabolites biosynthesized from the phenylpropanoid pathway. Flavonoids in various structural forms can be identified, but most representatives of this class have 15 carbon atoms in their nucleus, consisting of two phenyl groups linked by a three-carbon chain, forming a skeleton called C6–C3–C6 (Pietta 2000).

Around 4200 structurally different plant flavonoids are known, belonging to several subclasses, such as flavones (Heilmann and Merfort 1998; Hostetler et al. 2017), flavonols (Leo and Woodman 2015), flavanones (Barreca et al. 2017), flavanonols (Sunil and Xu 2019), catechins (Fan et al. 2017), chalcones (Karthikeyan et al. 2015), aurones (Haudecoeur and Boumendjel 2012), anthocyans (Wallace and Giusti 2015), leucoanthocyanidins (Franklin et al. 2018), proanthocyanidins (Cos et al. 2004), isoflavonoids (Douglas et al. 2013), neoflavonoids (Olmedo et al. 2017), biflavonoids (Gontijo et al. 2017), and others.

Chromatic assays are important for plant flavonoid measurement. The colors obtained vary according to the chemical structure of the substance, specifically the number and arrangement of hydroxyl groups in the fundamental nucleus. Examples of these assays include Shinoda's test and TLC (by using silica gel or polyamide as stationary phases and various chromogenic or physical agents to identify separate compounds) (Glavnik et al. 2017). Quantitative methods like HPLC and CE are commonly used for flavonoid quantification (Tomás-Barberán 1995; Motta et al. 2017). Infrared and mass spectrometry are important for determining the chemical structure of flavonoids and its molecular weight and, when associated with techniques such as HPLC, are also highly useful during the screening process (Villiers et al. 2016).

Regarding Cd response, it has been shown that 23 transcripts were differentially expressed and mapped for flavonoid biosynthesis, of which ten were upregulated in Cd-treated cell suspensions of cape periwinkle *Catharanthus roseus* (Apocynaceae) (Thakur et al. 2019). Depending on their different molecular structure, flavonoids can act as chelating agents of metal ions such as Cd, reducing the presence of free radicals and protecting against oxidative stress (Mierziak et al. 2014). In addition, flavonoids can be oxidized by peroxidase and act in the H_2O_2-scavenging, phenolic/ASC/POX system upon HM treatment (Michalak 2006). Mongkhonsin et al. (2016) in turn verified that the accumulation of Zn and Cd in the cell wall of *Gynura pseudochina* (Asteraceae) involves flavonoid compounds. Li et al. (2015a) pointed out that chelation of flavonoids makes much critical difference to their anti-oxidation, and the anti-lipid peroxidation capability of flavonoids is the cause of the activity of scavenging free radical and metal chelation.

2.1.4 Tannins

Tannins are compounds responsible for the astringency of many fruits and plant products. The biological activities of tannins are due to three general characteristics: ability to complex with macromolecules such as proteins and polysaccharides, complexation with metal ions, and antioxidant activity (Olejar et al. 2016). Tannins are classified according to their chemical structure into two main groups:

hydrolyzable tannins and condensed tannins (Salminen and Karonen 2011). Hydrolyzable tannins are characterized by a central sugar whose hydroxyls are esterified with phenolic acids and thus two subtypes can be obtained, the gallotannins (formed from gallic acid units) or ellagitannins (formed from hexahydroxydiphenyl units). Condensed tannins in turn are oligomers and polymers formed by the polycondensation of two or more flavan-3-ol or flavan-3,4-diol units (Cai et al. 2017).

HPLC analysis of tannins is also possible, but only in reverse phase, and restricted to smaller molecules from monomers to tetramers (Ma et al. 2018). In fact, in principle all hydrolyzable tannins can be quantified using liquid chromatography coupled with mass spectrometry, while polymeric condensed tannins with many isomeric forms of many species are so complex that they cannot be separated from each other even by modern chromatographic techniques (Salminen and Karonen 2011).

Regarding the role of tannins in plant Cd response, significant increases in the concentrations of polyphenolics including condensed tannins have been observed in response to Cd in leaves and roots of the river mangrove *Aegiceras corniculatum* (Primulaceae) (Jiang et al. 2017). The presence of tannins and other polyphenols within the symplast and apoplast can act against the accumulation of $O_2^{\bullet -}$ to protect the membrane structures, such as the chloroplast and mitochondrial membranes within plant cells (Jiang et al. 2017). In addition, tannins can chelate metal ions with hydroxyl and carboxyl functional groups (Lavid et al. 2001a, b). Cd microlocation within the cell is associated with regions of high production of tannins, which provide cell wall-binding sites for Cd (Sanita di Toppi and Gabbrielli 1999; Vollenweider and Günthardt-Goerg 2005).

Plant detoxification of metals like Cd generates tannin accumulation in many plants, which can be considered a self-protecting function (Vollenweider and Günthardt-Goerg 2005). However, increasing tannin concentration may result in increased Cd accumulation, as observed in the mangrove *Kandelia candel* (Rhizophoraceae) (Qin et al. 2006). For this species, the accumulation of Cd generated by the increase in tannin levels was possibly counterbalanced by the increase in the amounts of soluble sugar and proline, which allowed cellular osmotic control, attenuating the metal toxicity (Qin et al. 2006).

2.1.5 Coumarins and Xanthones

Coumarins are secondary metabolites widely distributed in plants and, according to their chemical structure, are considered *o*-hydroxycinnamic acid lactones. Most of the plant-derived coumarins are oxygenated at C-7, and the hydroxyl group may be methylated or glycosylated. Prenylation at various positions of the coumarin backbone can also be present, resulting in pyranocoumarins or furanocoumarins. In addition, coumarins can dimerize to form dimeric coumarins (Kaur et al. 2015; Sarker and Nahar 2017). More than 1000 coumarins have been isolated from natural sources, and their biological properties depend on their substitution patterns (Stringlis et al. 2019). Xanthones, in turn, are secondary metabolites derived from dibenzo-γ-pyrone. In higher plants, xanthones are biosynthesized from the shikimic acid and acetate pathways (Yang et al. 2017).

The presence of a lactonic ring facilitates the extraction and detection of coumarins and xanthones, since in an alkaline medium this ring is opened, converting these compounds into water-soluble salts extractable in aqueous solvents. Relactonization occurs by acidification of the aqueous solvent, permitting the recovery of these compounds by subsequent extraction with organic solvent (Kim 2017). The development of diverse stationary phases for TLC and column chromatography, such as chemically bound phase silica gels, contributed to the capacity to achieve separation of mixtures of these compounds in plant samples (Sarker and Nahar 2017). In addition, techniques such as HPLC now enable separation in less time and with higher resolutions than conventional open column methods (Wei et al. 2016; Lima et al. 2017). In fact, coumarins are identified by m/z ratio on HPLC or using the UV-DAD fingerprint (characteristic UV spectrum). Coumarins and xanthones have characteristic UV spectra, which are strongly influenced by the nature and position of the substituent groups. Spots of TLC chromatograms of extracts rich in these compounds under UV light appear in different colors, such as green, blue, yellow, and purple, which can be enhanced after exposure to ammonia vapor (Kovač-Bešović and Durić 2003).

Coumarins have already been related to electrochemical determination of Cd in pomegranate (*Punica granatum*, Lythraceae) and cork oak (*Quercus suber*, Fagaceae) (Zazoua et al. 2018). Regarding photosynthetic physiology, in one study Cd-treated liverworts, *Jungermannia exsertifolia* (Jungermanniaceae), showed significantly lower concentrations of chlorophyll and carotenoids than control plants. The response of these aspects of photosynthetic physiology was inversely proportional to concentrations of xanthophylls, antioxidants, ferulic acid, phosphoric acid, and coumarins, which all showed an increase in the presence of Cd, highlighting the relevance of these metabolites in the stress caused by metal (Otero et al. 2006).

2.1.6 Lignoids

Lignoid is a generic name that characterizes polymeric molecules whose molecular scaffold is formed exclusively by the phenylpropanic group $(C6–C3)_n$ (Monte Neto et al. 2007). These compounds can be classified according to chemical structures into lignins, lignans, neolignans, and oligolignans.

Lignins are substances that deposit on the walls of plant cells, giving them some rigidity. These compounds form from 15% to 35% of the dry matter of gymnosperm and angiosperm trunks and are constituents of the cell wall of stems, leaves, and roots of all vascular plants, including herbaceous species. Lignins have several phenylpropanic units, being considered polymers (Vinardell and Mitjans 2017). Lignans are dimers formed by the oxidative coupling of cinnamic alcohols with each other or with cinnamic acids. Structurally, the two phenylpropanic residues have the oxygenated gamma carbon (C9) (Solyomváry et al. 2017). Neolignans are oxidative dimers of allylphenols and propenylphenols, interconnected or cross-linked, which lack an oxygenated C9 (Teponno et al. 2016). Finally, oligolignans are lignoid oligomers, that is, the products resulting from the condensation of 3 to 5 phenylpropanoid units. Lignins can be further subdivided into sesquilignans and neolignans, in analogy to terpenoid nomenclature (Smeds et al. 2016).

Regarding the main techniques for the detection of lignoids, in relation to their polarity, neolignans are soluble in nonpolar solvents such as petroleum ether, especially if the aromatic ring moiety is methoxylated. Lignins are of intermediate polarity given their oxygenated gamma carbon. On the contrary, glycosylated lignans and oligolignoids are largely nonpolar and effectively extracted with alcohols such as methanol, ethanol, and butanol (Cheng et al. 2015). For these molecules, high-performance liquid chromatography coupled with quadrupole tandem time-of-flight mass (HPLC-QTOF-MS) and ultraviolet spectrometry (HPLC-UV) are suitable for the qualitative and quantitative analyses of some plant lignans (Liu et al. 2015).

Lignins are extremely important when it comes to coping with Cd toxicity, since cell wall lignification helps prevent Cd from entering the plant cell. Indeed, increasing cell wall lignification to prevent Cd entry and transport by the apoplastic pathway is one of the defense mechanisms used by some higher plants against Cd toxicity (Loix et al. 2017).

2.1.7 Terpenoids

Terpenoids are a wide variety of plant substances whose biosynthetic origin derives from isoprene units. Terpene skeletons are formed by the condensation of isoprene units, being monoterpenes (10 °C) and sesquiterpenes (15 °C) the main representatives found in essential oils. Compared with other living organisms, green plants exhibit an unusually high number of terpenoids, both per species and in total (Pichersky and Raguso 2018). Terpenes may be acyclic, monocyclic, bicyclic, unsaturated, alcohols, aldehydes, ketones, lactones, or tropolones. In each of these subgroups, numerous substances with more than 8000 different skeleton types are classified (Groot and Schmidt 2016). Currently, the use of TLC, GC-MS, and NMR has been adopted for terpenoid quantification (Tomi et al. 2016).

According to the structure of terpenoids, they may act as antioxidant molecules in different in vitro/in vivo analyses, including in response to HMs (Arif et al. 2016). When it comes to the stress generated by HMs such as Cd and other HMs, Hojati et al. (2017) observed that high Cd supply enhanced the relative proportion of monoterpene hydrocarbons. Changes in contents of menthol (a well-known terpenoid) in essential oils were observed in Cd-exposed peppermint plants, *Mentha piperita* (Lamiaceae) (Zheljazkov et al. 2006).

2.1.8 Saponins

Saponins are chemically described as steroid or polycyclic triterpene heterosides. This type of structure, which has a lipophilic part (triterpene or steroid) and a hydrophilic part (sugars), drives the characteristic saponin property of reducing the surface tension of water and thus acting as detergents and emulsifiers (Güçlü-Üstündağ and Mazza 2007). These metabolites are high molecular weight substances and generally occur in complex mixtures, so that the sugar chain can be straight or branched and the carbons of the interheterosidic bonds are variable. Recently these compounds have been studied more intensely, in parallel with the knowledge about their biological properties and the evolution of chromatographic and spectroscopic techniques (He et al. 2019). Saponins can be acidic, basic, or neutral. As for the number of aglycone-linked sugar chains, they may be monodesmosidic (one chain) or bidesmosidic (two chains) (Singh et al. 2017a).

The chromatographic profile of saponins, established by TLC or HPLC, can be used as a method of identifying plant extracts rich in these compounds. With the same aim, the coupling of these techniques to mass spectrometry has greatly helped the structural elucidation of saponins (Mikołajczyk-Bator et al. 2016). Correlation between saponins and Cd has been verified, since the saponin production quantified by diosgenin in longevity spinach, *Gynura procumbens* (Asteraceae), was altered when exposed to Cd treatments and reduced in combined Cd and Cu treatments (Ibrahim et al. 2017).

2.1.9 Alkaloids

Alkaloids are a group of nitrogen-containing plant secondary metabolites. Alkaloids have large structural diversity but are typically heterocycles with at least one nitrogen atom in a negatively oxidized state (Kabera et al. 2014). Alkaloids derived from amino acids that contain a nitrogen atom in a heterocyclic ring are called true alkaloids and classified according to the ring system present. On the contrary, alkaloids derived from amino acids that contain a nitrogen atom not within the heterocycle are called protoalkaloids. Alkaloids that are not derived from amino acids are called pseudoalkaloids (Rashid et al. 2019). These metabolites can be found in all plant organs, but mainly in four types of tissues or cells: actively growing tissues, epidermal and hypodermic cells, vascular sheaths, or lactating vessels (Ge and Wang 2018).

There are several general reagents for alkaloid detection through precipitation, including Mayer, Bouchardat, Dragendorff, and Bertrand reagents (Mascato et al. 2015). In addition, alkaloid characterization can be done by TLC using the same precipitation reagents (Sreevidya and Mehrotra 2003). These substances are normally extracted from the plant by acid–base extraction due to the presence of the nitrogen atom with mismatched electron pairs, which give these substances a typically basic nature. Quantitative analysis is usually done by HPLC coupled other techniques such as MS and NMR (Khatib et al. 2016).

In the opium poppy, *Papaver somniferum* (Papaveraceae), alkaloids are known to play an important role in plant defense as well as mechanisms against various types of abiotic stresses (Szabó et al. 2003). Lachman et al. (2006) subjected poppy to different concentrations of Cd and Zn and found a relationship between alkaloid concentration and stress caused by metals. In addition, these authors detected the complexation of metals in the hydroxyl groups of some molecules. Chen et al. (2018) in turn verified that cape periwinkle, *Catharanthus roseus* (Apocynaceae), has potential application in phytoremediation of Cd due to relatively substantial accumulation of biomass, as well as terpenoid indole alkaloids.

2.2 Physiological Context of the Plant Metabolic Response to Cd Stress

Photosynthesis, the central process in which light energy is used to fix carbon and produce carbohydrates and other organic compounds in plants, can be directly influenced by plant exposure to Cd (Cheng and Kaplan 2003). One of the first readily visible symptoms of the presence of Cd in plants is chlorosis, which is the manifestation of damage to multiple components of the photosynthetic machinery that results in reduced photosynthetic rate and ultimately causes a decrease in growth rate (DalCorso et al. 2008). Indeed, chlorosis is a predominant symptom in plants contaminated with Cd, resulting from competition between Cd and iron ions for plasma membrane uptake sites. When plants have chlorosis, other symptoms can also be detected such as leaf dryness and leaf epinastia (Gill and Tuteja 2011). Changes in photosynthetic rates of plants exposed to Cd are generally studied and evaluated together in a logical sequence of metabolic events, and each researcher generally makes observations according to pre-established criteria. Thus, the identification of the specific aspect of photosynthesis that is being inhibited by Cd exposure (e.g., light harvesting complex, electron transport chain, Calvin cycle) is not often assessed in the scope of many studies of Cd dynamics in plants. However, several studies have indicated that exposure to Cd affects carbon assimilation through disturbance of chlorophyll synthesis, thylakoid structure, the efficiency of photosystem I (PSI) and photosystem II (PSII), the oxygen evolving complex, the electron transport chain, Rubisco activity, and even stomatal conductance (Otero et al. 2006; Choppala et al. 2014).

In the liverwort *Jungermannia exsertifolia* (Jungermanniaceae), Cd exposure caused a decrease in chlorophyll concentrations, along with a compensatory increase in nonphotochemical quenching via the xanthophyll cycle to dissipate excess light energy (Otero et al. 2006). In safflower, *Carthamus tinctorius* (Asteraceae), Cd was also found to reduce chlorophyll synthesis and the efficiency of PSII (Moradi and Ehsanzadeh 2015). In winter rape, *Brassica napus* (Brassicaceae), Cd exposure did not alter chlorophyll content but was found to reduce the efficiency of PSII as well as the rate of electron transport, while plants experienced increased dissipation of light energy as heat (Janeczko et al. 2005). From multiple studies in distantly related plants, an upregulation of nonphotochemical quenching appears to be a physiological mechanism of protection of the photosynthetic apparatus against high light stress induced by Cd through reduction in light-harvesting capacity via either chlorophyll degradation or photosystem damage.

Changes in PSI and PSII are also highlighted in multiple studies as among the first detectable symptoms in plants exposed to Cd and can be considered one of the main drivers of Cd-induced phytotoxicity. In general, the influence of Cd on photosystems is more severe in PSII than in PSI (Mallick and Mohn 2003), as PSI is more tolerant to metal ions than PSII (Romanowska et al. 2006). In addition, Keren and Krieger-Liszkay (2011) found that plants controlled the number of PSII reaction centers through the PSII repair cycle, which could effectively prevent permanent damage to the PSI. In another study, Sigfridsson et al. (2004) observed that Cd^{2+} has multiple effects on both the donor side (Cd inhibits oxygen evolution in a high-affinity site by competition with Ca^{2+}) and the acceptor side of PSII.

Reductions in chlorophyll concentrations in response to Cd are often due to disturbances in the structure of the thylakoid membrane itself, which may be minimized in some cases with an increase in the amount of alpha-tocopherol. This subunit of vitamin E exhibits antioxidant activity under metal stress and has been shown to be associated with increased stability of the thylakoid membrane, preventing damage to photosystem II (Havaux et al. 2005). In this context, several studies evaluating the metabolome of Cd-exposed plants have observed an increase in the amount of alpha-tocopherol in Cd-exposed plants, and this increase is observed under both increased Cd concentration and increased exposure time (Villiers et al. 2011; Sun et al. 2013; Hédiji et al. 2015).

Beyond light capture physiology, the light-independent reactions of the Calvin cycle are also adversely affected by Cd exposure. In *Elsholtzia argyi*, exposure to Cd has been demonstrated to inhibit enzymatic activity, including carboxylation by Rubisco (Li et al. 2015b). Furthermore, Cd was found to prevent NADPH from participating in the Calvin cycle, which was suggested to lead to excessive electron accumulation in the electron transport chain and reduced performance of PSI (Li et al. 2015b). A similar response has been observed in lettuce, *Lactuca sativa* (Asteraceae), where Cd exposure reduces the carboxylation activity of Rubisco (Dias et al. 2013).

While photoprotective mechanisms are key to dealing with excess light levels when Cd exposure has reduced plant photosynthetic capacity, this response often also includes antioxidant molecules to prevent Cd-induced oxidative stress, especially in PSII. Cd causes changes in the plant antioxidant system, and plants with different tolerance levels also differ in levels and activity of antioxidant enzymes. Major Cd-induced stress-related antioxidant enzymes include: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), and glutathione peroxidase (GPX). Modifications in nonenzymatic antioxidants have also been demonstrated (Borges et al. 2018; Marques et al. 2019).

Studies on endogenous antioxidants have greatly helped to understand some mechanisms of plant tolerance to Cd (Borges et al. 2018; Marques et al. 2019). Many of these antioxidant substances have already been described in this chapter, as in the case of flavonoids, among so many other antioxidant metabolites (Marques et al. 2019).

Plant hormones, or phytohormones, are compounds produced by plants that act to regulate development and growth. Phytohormones act as chemical messengers between cells, tissues, and organs of higher plants and can be directly affected by stress induced by HMs (Wani et al. 2016). These substances, even in small amounts, are involved in signaling cascade of response to different biotic and abiotic stresses, including metabolic responses to Cd. In this context, hormones can act on plant tolerance to adverse environmental conditions such as the stress induced by Cd toxicity (DalCorso et al. 2008; Popova et al. 2009). Among the major classes of plant hormones, auxins, cytokinins, salicylates, jasmonates, ethylene, and abscisic acid can be highlighted. These are the main phytohormones capable of inducing the expression of many stress-related genes through different metabolic pathways (Gao et al. 2011; Singh et al. 2017b). It is important to mention that most studies that assess plant hormonal response to stress by Cd focus on quantifying certain classes of phytohormones over others, predetermined by the researcher in view of the limitations and interest of each study. Although this type of selective study for certain classes of phytohormones is important, it highlights the potential utility of metabolomics studies focused on the simultaneous characterization of the whole set of molecules involved in the plant stress response by Cd. In fact, changes in the content of several hormones, such as abscisic acid (Zhao et al. 2014), cytokinin (Hashem 2013), and auxin (Bücker-Neto et al. 2017) have been reported in plants treated with toxic metals including Cd.

Abscisic acid is involved in increasing the expression of several stress-related genes (Sah et al. 2016). Jasmonic acid (JA) is another hormone that is part of the plant stress cascade caused by Cd. JA is involved in numerous physiological processes in plants and plays important roles in regulating the expression of plant defense genes (Sharma et al. 2019) and Cd response (Lei et al. 2019). Maksymiec et al. (2007) observed that JA (as a signaling factor) led to reduced growth of various plant parts in response to stress with Cd, and not only to changes in photosynthesis and related metabolites. In another study, Noriega et al. (2012) observed that, depending on concentration of JA, this hormone could improve plant antioxidant responses in response to Cd in soybean plants. These authors also observed an increase in the activities of antioxidant enzymes, such as CAT and SOD, showing that JA plays an important role in plant tolerance to Cd.

Kavulych et al. (2019) found that SA is an important hormone for regulating synthesis of phenolic compounds in buckwheat, *Fagopyrum esculentum* (Polygonaceae). This regulation is made by the action of stressors, mainly with a combined effect between salicylic acid and Cd. The use of external SA may serve as an important regulator of the formation of phenolic compounds by the action of stressors, especially with two-factor combined effect (salicylic acid + Cd) (Kavulych et al. 2019). Several other studies demonstrate that the application of salicylic acid helps several plant species to tolerate Cd toxicity (Shakirova et al. 2016; Guo et al. 2019).

In addition to stress hormones altered by HMs such as Cd, polyamines are also important metabolites for plant response to Cd. Several studies have been using different polyamine treatments and their interaction with compounds such as glutathione and nitric oxide among others (Nahar et al. 2016; Rady et al. 2016; Tajti et al. 2018; Alzahrani and Rady 2019; Seifi and Shelp 2019). Studies on interaction of putrescine and nitric oxide have shown that the addition of both putrescine alone and it together with nitric oxide led to increased Cd tolerance in mung bean, *Vigna radiata* (Fabaceae), and higher plant Cd tolerance was observed in nitric oxide and putrescine-treated treatments. These treatments also increased the amount of polyamines, highlighting their importance in coping with Cd stress (Nahar et al. 2016). Benavides et al. (2018) working with wheat (*Triticum aestivum*, Poaceae) and sunflower (*Helianthus annuus*, Asteraceae) observed an increase in tolerance to Cd when plants were exposed to Cd in conjunction with putrescine, spermidine, or spermine, and deleterious effect induced by Cd could be only partially prevented by plant pretreatment with the polyamines.

3 Conclusion

Current research strongly suggests that plant metabolites act in response to Cd toxicity and have important functions for plant tolerance to this heavy metal. Understanding the role of plant metabolites can contribute to plant selection focused on phytoremediation of soils contaminated with HMs. Recent advances in plant metabolome research and studies of hormone signaling networks have shown that while there are overarching patterns in the plant metabolic response to Cd, there is still much to discover, especially considering the vast number of plant primary and secondary metabolites and the nuance inherent in species-specific responses.

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References

Abo-Hamad SAEH, Saad-Allah KMG, Abo-Kassem EEDM (2013) Effect of gamma irradiation or potassium on some primary and secondary metabolites of *Brassica rapa* (L.) root under cadmium stress. Int Res J Agric Sci Soil Sci 3:408–415

Ahmad P, Allah EA, Hashem A, Sarwat M, Gucel S (2016) Exogenous application of selenium mitigates cadmium toxicity in *Brassica juncea* L. (Czern & Cross) by up–regulating antioxidative system and secondary metabolites. J Plant Growth Regul 35:936–950

Alzahrani Y, Rady MM (2019) Compared to antioxidants and polyamines, the role of maize grain-derived organic biostimulants in improving cadmium tolerance in wheat plants. Ecotoxicol Environ Saf 182:109378

Arif N, Yadav V, Singh S, Kushwaha BK, Singh S, Tripathi DK, Vishwakarma K, Sharma S, Dubey NK, Chauhan DK (2016) Assessment of antioxidant potential of plants in response to heavy metals. In: Singh A, Prasad S, Singh R (eds) Plant responses to xenobiotics. Springer, Singapore

Arruda MAZ, Azevedo RA (2009) Metallomics and chemical speciation: towards a better understanding of metal-induced stress in plants. Ann Appl Biol 155:301–307

Babula P, Klejdus B, Kovacik J, Hedbavny J, Hlavna M (2015) Lanthanum rather than cadmium induces oxidative stress and metabolite changes in *Hypericum perforatum*. J Hazard Mater 286:334–342

Baldantoni D, Morra L, Zaccardelli M, Alfani A (2016) Cadmium accumulation in leaves of leafy vegetables. Ecotoxicol Environ Saf 123:89–94

Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, Laganà G, Daglia M, Meneghini S, Nabavi SM (2017) Flavanones: citrus phytochemical with health-promoting properties. Biofactors 43:495–506

Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34

Benavides MP, Groppa MD, Recalde L, Verstraeten SV (2018) Effects of polyamines on cadmium- and copper-mediated alterations in wheat (*Triticum aestivum* L) and sunflower (*Helianthus annuus* L) seedling membrane fluidity. Arch Biochem Biophys 654:27–39

Borges KLR, Salvato F, Alcântara BK, Nalin RS, Piotto FA, Azevedo RA (2018) Temporal dynamic responses of roots in contrasting tomato genotypes to cadmium tolerance. Ecotoxicology 27:245–258

Borges KLR, Salvato F, Loziuk PL, Muddiman DC, Azevedo RA (2019) Quantitative proteomic analysis of tomato genotypes with differential cadmium tolerance. Environ Sci Pollut Res 26:26039–26051

Brunetti P, Zanella L, De Paolis A, Di Litta D, Cecchetti V, Falasca G, Barbieri M, Altamura MM, Costantino P, Cardarelli M (2015) Cadmium-inducible expression of the ABC-type transporter AtABCC3 increases phytochelatin-mediated cadmium tolerance in Arabidopsis. J Exp Bot 66:3815–3829

Bücker-Neto L, Paiva ALS, Machado RD, Arenhart RA, Margis-Pinheiro M (2017) Interactions between plant hormones and heavy metals responses. Genet Mol Biol 40:373–386

Cai Y, Zhang J, Chen NG, Shi Z, Qiu J, He C, Chen M (2017) Recent advances in anticancer activities and drug delivery systems of tannins. Med Res Rev 37:665–701

Carvalho MEA, Piotto FA, Nogueira ML, Gomes-Junior FG, Chamma HMCP, Pizzaia D, Azevedo RA (2018) Cadmium exposure triggers genotype-dependent changes in seed vigor and germination of tomato offspring. Protoplasma 255:989–999

Chen Q, Lu X, Guo X, Pan Y, Yu B, Tang Z, Guo Q (2018) Differential responses to Cd stress induced by exogenous application of Cu, Zn or Ca in the medicinal plant *Catharanthus roseus*. Ecotoxicol Environ Saf 157:266–275

Chen C, Cao Q, Jiang Q, Li J, Yu R, Shi G (2019a) Comparative transcriptome analysis reveals gene network regulating cadmium uptake and translocation in peanut roots under iron deficiency. BMC Plant Biol 19:35

Chen L, Ge MD, Zhu YJ, Song Y, Cheung PC, Zhang BB, Liu LM (2019b) Structure, bioactivity and applications of natural hyperbranched polysaccharides. Carbohydr Polym 223:115076

Cheng X, Kaplan LA (2003) Simultaneous analyses of neutral carbohydrates and amino sugars in freshwaters with HPLC-PAD. J Chromatogr Sci 41:434–438

Cheng Z, Song H, Yang Y, Zhou H, Liu Y, Liu Z (2015) Smashing tissue extraction of five lignans from the fruit of *Schisandra chinensis*. J Chromatogr Sci 54:246–256

Choppala G, Saifullah BN, Bibi S, Iqbal M, Rengel Z, Kunhikrishnan A, Ashwath N, Ok YS (2014) Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. Crit Rev Plant Sci 33:374–391

Cos P, Bruyne TD, Hermans N, Apers S, Berghe DV, Vlietinck AJ (2004) Proanthocyanidins in health care: current and new trends. Curr Med Chem 11:1345–1359

Cuypers A, Hendrix S, Reis RA, Smet S, Deckers J, Gielen H, Jozefczak M, Loix C, Vercampt H, Vangronsveld J, Keunen E (2016) Hydrogen peroxide, signaling in disguise during metal phytotoxicity. Front Plant Sci 7:470

DalCorso G, Farinati S, Maistri S, Furini A (2008) How plants cope with cadmium: staking all on metabolism and gene expression. J Integr Plant Biol 50:1268–1280

Davies O, Mendes P, Smallbone K, Malys N (2012) Characterisation of multiple substrate-specific (d) ITP/(d) XTPase and modelling of deaminated purine nucleotide metabolism. BMB Rep 45:259–264

Devesa V, Vélez D (2016) Cadmium: properties and determination. In: Caballero B, Finglas PM, Tolbrá F (eds) Encyclopedia of food and health. Academic Press, Oxford, pp 543–549

Dias MC, Monteiro C, Pereira JMM, Correia C, Gonçalves B, Santos C (2013) Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiol Plant 35:1281–1289

Dos Santos VS, Macedo FA, Do Vale JS, Silva DB, Carollo CA (2017) Metabolomics as a tool for understanding the evolution of *Tabebuia sensu lato*. Metabolomics 13:72

Douglas CC, Johnson SA, Arjmandi BH (2013) Soy and its isoflavones: the truth behind the science in breast cancer. Anti Cancer Agents Med Chem 13:1178–1187

Dresler S, Kubrak T, Rutkowska E, Gagoś M, Bogucka-Kocka A, Świeboda R, Wójcik M (2016) Comparison of analytical methods in chemometric fingerprinting of metallicolous and non-metallicolous populations of *Echium vulgare* L. Phytochem Anal 27:239–248

Dresler S, Rutkowska E, Bednarek W, Stanisławski G, Kubrak T, Bogucka-Kocka A, Wójcik M (2017) Selected secondary metabolites in *Echium vulgare* L. populations from nonmetalliferous and metalliferous áreas. Phytochemistry 133:4–14

Fan FY, Sang LX, Jiang M (2017) Catechins and their therapeutic benefits to inflammatory bowel disease. Molecules 22:484

Fiehn O, Sumner LW, Ward J, Dickerson J, Lange MB, Lane G, Roessner U, Last R, Rhee SY, Nikolau B (2007) Minimum reporting standards for plant biology context information in metabolomic studies. Metabolomics 3:195–201

Filimonova V, Gonçalves F, Marques JC, De Troch M, Gonçalves AM (2016) Fatty acid profiling as bioindicator of chemical stress in marine organisms: a review. Ecol Indic 67:657–672

Flis P, Ouerdane L, Grillet L, Curie C, Mari S, Lobinski R (2016) Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection. New Phytol 211:1129–1141

Franklin R, Bispo RFM, Sousa-Rodrigues CF, Pires LAS, Fonseca A Jr, Babinski MA (2018) Grape leucoanthocyanidin protects liver tissue in albino rabbits with nonalcoholic hepatic steatosis. Cells Tissues Organs 205:129–136

Gallego SM, Pena LB, Barcia RA, Azpilicueta CE, Iannone MF, Rosales EP, Zawoznika MS, Groppaa MD, Benavides MP (2012) Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environ Exp Bot 83:33–46

Gao QM, Venugopal S, Navarre D, Kachroo A (2011) Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. Plant Physiol 155:464–476

Ge YC, Wang KW (2018) New analogues of aporphine alkaloids. Mini Rev Med Chem 18:1590–1602

Gilbert N (2018) European Union debates controversial plans to limit cadmium in fertilizer. Science. https://doi.org/10.1126/science.aat8448

Gill SS, Tuteja N (2010) Reactive oxygen species and oxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Gill SS, Tuteja N (2011) Cadmium stress tolerance in crop plants: probing the role of sulfur. Plant Signal Behav 6:215–222

Glavnik V, Vovk I, Albreht A (2017) High performance thin-layer chromatography-mass spectrometry of Japanese knotweed flavin-3-ols and proanthocyanidins on silica gel plates. J Chromatogr A 1482:97–108

Gontijo VS, dos Santos MH, Viegas J (2017) Biological and chemical aspects of natural biflavonoids from plants: a brief review. Mini Rev Med Chem 17:834–862

Goodacre R (2004) Making sense of the metabolome using evolutionary computation: seeing the wood with the trees. J Exp Bot 56:245–254

Goolsby EW, Mason CM (2015) Toward a more physiologically and evolutionarily relevant definition of metal hyperaccumulation in plants. Front Plant Sci 6:33. https://doi.org/10.3389/fpls.2015.00033

Gratão PL, Monteiro CC, Tezotto T, Carvalho RF, Alves LR, Peters LP, Azevedo RA (2015) Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. Biometals 28:803–816

Groot AC, Schmidt E (2016) Essential oils, part I: Introduction. Dermatitis 27:39–42

Güçlü-Üstündağ Ö, Mazza G (2007) Saponins: properties, applications and processing. Crit Rev Food Sci Nutr 47:231–258

Guo Q, Cui SW, Wang Q, Hu X, Kang J, Yada RY (2012) Structural characterization of a low-molecular-weight heteropolysaccharide (glucomannan) isolated from Artemisiasphaerocephala Krasch. Carbohydr Res 350:31–39

Guo B, Liu C, Liang Y, Li N, Fu Q (2019) Salicylic acid signals plant defense against cadmium toxicity. Int J Mol Sci 20(12):2960

Gutsch A, Sergeant K, Keunen E, Prinsen E, Guerriero G, Renaut J, Hausman JF, Cuypers A (2019) Does long-term cadmium exposure influence the composition of pectic polysaccharides in the cell wall of *Medicago sativa* stems? BMC Plant Biol 19:271

Hagel JM, Facchini PJ (2008) Plant metabolomics: analytical platforms and integration with functional genomics. Phytochem Rev 7:479–497

Hashem HA (2013) Cadmium toxicity induces lipid peroxidation and alters cytokinin content and antioxidant enzyme activities in soybean. Botany 92:1–7

Haudecoeur R, Boumendjel A (2012) Recent advances in the medicinal chemistry of aurones. Curr Med Chem 19:2861–2875

Havaux M, Eymery F, Porfirova S, Rey P, Dörmann P (2005) Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. Plant Cell 17:3451–4369

He Y, Hu Z, Li A, Zhu Z, Yang N, Ying Z, He J, Wang C, Yin S, Cheng S (2019) Recent advances in biotransformation of saponins. Molecules 24:2365

Hédiji H, Djebali W, Cabasson C, Maucourt M, Baldet P, Bertrand A, Boulila Zoghlami L, Deborde C, Moing A, Brouquisse R, Chaïbi W, Gallusci P (2010) Effects of long-term cadmium exposure on growth and metabolomic profile of tomato plants. Ecotoxicol Environ Saf 73 (8):1965–1974

Hédiji H, Djebali W, Belkadhi A, Cabasson C, Moing A, Rolin D, Brouquisse R, Gallusci P, Chaïbi W (2015) Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: consequences on fruit production. S Afr J Bot 97:176–181

Heilmann J, Merfort I (1998) Aktueller kenntnisstand zum metabolismus von flavonoiden II. Resorption und metabolismus von flavonen, flavanonen, flavanen, proanthocyanidinen und isoflavonoiden. Pharm Unserer Zeit 27:173–183

Hojati M, Modarres-Sanavy SAM, Enferadi ST, Majdi M, Ghanati F, Farzadfar S, Pazoki A (2017) Cadmium and copper induced changes in growth, oxidative metabolism and terpenoids of *Tanacetum parthenium*. Environ Sci Pollut Res Int 24(13):12261–12272

Hostetler GL, Ralston RA, Schwartz SJ (2017) Flavones: food sources, bioavailability, metabolism, and bioactivity. Adv Nutr 8:423–435

Ibrahim M, Chee Kong Y, Mohd Zain N (2017) Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant *Sambung Nyawa* (*Gynura procumbens* (Lour.) Merr). Molecules 22:1623

Janeczko A, Koscielniak J, Pilipowicz M, Szarek–Lukaszewska G, Skoczowski A (2005) Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 43:293–298

Jiang S, Weng B, Liu T, Su Y, Liu J, Lu H, Yan C (2017) Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) Blanco (Ac). Ecotoxicol Environ Saf 141:290–297

Jorge TF, Rodrigues JA, Caldana C, Schmidt R, van Dongen JT, Thomas-Oates J, António C (2016) Mass spectrometry-based plant metabolomics: metabolite responses to abiotic stress. Mass Spectrom Rev 35:620–649

Kabera JN, Semana E, Mussa AR, He X (2014) Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. J Pharm Pharmacol 2:377–392

Karthikeyan C, Narayana SHNM, Ramasamy S, Vanam U, Manivannan E, Karunagaran D, Trivedi P (2015) Advances in chalcones with anticancer activities. Recent Pat Anticancer Drug Discov 10:97–115

Kaur M, Kohli S, Sandhu S, Bansal Y, Bansal G (2015) Coumarin: a promising scaffold for anticancer agents. Anti Cancer Agents Med Chem 15:1032–1048

Kavulych Y, Kobyletska M, Terek O (2019) Investigation of salicylic acid-induced change on flavonoids production under cadmium toxicity in buckwheat (*Fagopyrum esculentum* Moench) plants. Eureka Life Sci 5:13–18

Keren N, Krieger-Liszkay A (2011) Photoinhibition: molecular mechanisms and physiological significance. Physiol Plant 142:1–5

Khan AK, Rashid R, Fatima N, Mahmood S, Mir S, Khan S, Jabeen N, Murtaza G (2015) Pharmacological activities of protocatechuic acid. Acta Pol Pharm 72:643–650

Khatib M, Pieraccini G, Innocenti M, Melani F, Mulinacci N (2016) An insight on the alkaloid content of *Capparis spinosa* L. root by HPLC–DAD–MS, MS/MS and 1H qNMR. J Pharm Biomed Anal 123:53–62

Kim JH (2017) Extraction time and temperature affect the extraction efficiencies of coumarin and phenylpropanoids from *Cinnamomum cassia* bark using a microwave-assisted extraction method. J Chromatogr B 1063:196–203

Knudsen C, Gallage NJ, Hansen CC, Møller BL, Laursen T (2018) Dynamic metabolic solutions to the sessile life style of plants. Nat Prod Rep 35:1140–1155

Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmüller E, Dörmann P, Weckwerth W, Gibon Y, Stitt M, Willmitzer L, Fernie AR, Steinhauser D (2004) GMD@CSB. DB: the Golm metabolome database. Bioinformatics 21:1635–1638

Kovač-Bešović EE, Durić K (2003) Thin layer chromatography-application in qualitative analysis on presence of coumarins and flavonoids in plant material. Bosnian J Basic Med 3:19–26

Kovácik J, Klejdus B, Hedbavny J, Stork F, Backor M (2009) Comparison of cadmium and copper effect on phenolic metabolism, mineral nutrients and stress-related parameters in *Matricaria chamomilla* plants. Plant Soil 320:231

Kovácik J, Klejdus B, Hedbavny J, Zoń J (2011) Significance of phenols in cadmium and nickel uptake. J Plant Physiol 168(6):576–584

Krzesłowska M (2011) The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta Physiol Plant 33:35–51

Kubo K, Kobayashi H, Fujitac M, Otaa T, Minamiyamad Y, Watanabee Y, Nakajimaf T, Shinanoa T (2016) Varietal differences in the absorption and partitioning of cadmium in common wheat (*Triticum aestivum* L). Environ Exp Bot 124:79–88

Kumar S, Asif MH, Chakrabarty D, Tripathi RD, Dubey RS, Trivedi PK (2013) Expression of a rice lambda class of glutathione S-transferase, *OsGSTL2*, in *Arabidopsis* provides tolerance to heavy metal and other abiotic stresses. J Hazard Mater 249:228–237

Lachman J, Hejtmankova A, Miholova D, Kolihová D, Tluka P (2006) Relations among alkaloids, cadmium and zinc contents in opium poppy (*Papaver somniferum* L.). Plant Soil Environ 52:282

Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FMM (2005) Biochemistry: a cadmium enzyme from a marine diatom. Nature 435:42

Lavid N, Schwartz A, Lewinsohn E, Tel-Or E (2001a) Phenols and phenol oxidases are involved in cadmium accumulation in the water plants *Nymphoides peltata* (Menyanthaceae) and Nymphaeae (Nymphaeaceae). Planta 214:189–195

Lavid N, Schwartz A, Yarden O, Tel-Or E (2001b) The involvement of polyphenols and peroxidase activities in heavy-metal accumulation by epidermal glands of the waterlily (Nymphaeaceae). Planta 212(3):323–331

Lei GJ, Sun L, Sun Y, Zhu XF, Li GX, Zheng SJ (2019) Jasmonic acid alleviates cadmium toxicity in Arabidopsis via suppression of cadmium uptake and translocation. J Integr Plant Biol 62:218–227

Leme DM, Marin-Morales MA (2009) *Allium cepa* test in environmental monitoring: a review on its application. Mutat Res 682:71–81

Leo CH, Woodman OL (2015) Flavonols in the prevention of diabetes-induced vascular dysfunction. J Cardiovasc Pharmacol 65:532

Li W, Sun Y, Joseph J, Fitzloff JF, Fong HH, van Breemen RB (2003) p-Hydroxybenzoic acid alkyl esters in *Andrographis paniculata* herbs, commercial extracts, and formulated products. J Agric Food Chem 51:524–529

Li J, Lu H, Liu J, Hong H, Yan C (2015a) The influence of flavonoid amendment on the absorption of cadmium in *Avicennia marina* roots. Ecotoxicol Environ Saf 120:1–6

Li S, Yang W, Yang T, Chen Y, Ni W (2015b) Effects of cadmium stress on leaf chlorophyll fluorescence and photosynthesis of *Elsholtzia argyi*—a cadmium accumulating plant. Int J Phytoremediation 17(1–6):85–92

Li N, Han X, Feng D, Yuan D, Huang LJ (2019) Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: do we understand what they are whispering? Int J Mol Sci 20:671

Lima RDCL, Gramsbergen SM, Van Staden J, Jäger AK, Kongstad KT, Staerk D (2017) Advancing HPLC–PDA–HRMS–SPE–NMR analysis of coumarins in Coleonema album by use of orthogonal reversed-phase C18 and pentafluorophenyl separations. J Nat Prod 80:1020–1027

Liman R, Akyıl D, Eren Y, Konuk M (2010) Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/Salmonella and Allium test. Chemosphere 80:1056–1061

Liu XT, Wang XG, Xu R, Meng FH, Yu NJ, Zhao YM (2015) Qualitative and quantitative analysis of lignan constituents in *Caulis trachelospermi* by HPLC–QTOF–MS and HPLC–UV. Molecules 20:8107–8124

Loganathan P, Vigneswaran S, Kandasamy J, Naidu R (2012) Cadmium sorption and desorption in soils: a review. Crit Rev Environ Sci Technol 42:489–533

Loix C, Huybrechts M, Vangronsveld J, Gielen M, Keunen E, Cuypers A (2017) Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. Front Plant Sci 8:1867

Lv X, Wang C, Cheng Y, Huang L, Wang Z (2013) Isolation and structural characterization of a polysaccharide LRP4-A from *Lycium ruthenicum* Murr. Carbohydr Res 365:20–25

Ma W, Waffo-Téguo P, Paissoni MA, Jourdes M, Teissedre PL (2018) New insight into the unresolved HPLC broad peak of Cabernet Sauvignon grape seed polymeric tannins by combining CPC and Q-ToF approaches. Food Chem 249:168–175

Maksymiec W, Wójcik M, Krupa Z (2007) Variation in oxidative stress and photochemical activity in *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence or absence of jasmonate and ascorbate. Chemosphere 66(3):421–427

Mallick N, Mohn FH (2003) Use of chlorophyll fluorescence in metal-stress research: a case study with the green microalga *Scenedesmus*. Ecotoxicol Environ Saf 55:64–69

Marques DN, Carvalho MEA, Piotto FA, Batagin-Piotto KD, Nogueira ML, Gaziola SA, Azevedo RA (2019) Antioxidant defense response in plants to cadmium stress. In: Hasanuzzaman M, Prasad MNV, Nahar K (eds) Cadmium tolerance in plants: agronomic, molecular, signaling, and omic approaches. Elsevier, Cambridge, pp 423–461. https://doi.org/10.1016/B978-0-12-815794-7.00016-3

Mascato DRLH, Monteiro JB, Passarinho MM, Galeno DML, Cruz RJ, Ortiz C, Morales L, Lima ES, Carvalho RP (2015) Evaluation of antioxidant capacity of *Solanum sessiliflorum* (Cubiu) extract: an in vitro assay. J Nutr Metab:364185

Matsuda F, Nakabayashi R, Yang Z, Okazaki Y, Yonemaru JI, Ebana K, Yano M, Saito K (2015) Metabolome-genome-wide association study dissects genetic architecture for generating natural variation in rice secondary metabolism. Plant J 81:13–23

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol J Environ Stud 15:523–530

Mierziak J, Kostyn K, Kulma A (2014) Flavonoids as important molecules of plant interactions with the environment. Molecules 19:16240–16265

Mikołajczyk-Bator K, Błaszczyk A, Czyżniejewski M, Kachlicki P (2016) Identification of saponins from sugar beet (*Beta vulgaris*) by low and high-resolution HPLC-MS/MS. J Chromatogr B 1029:36–47

Mongkhonsin B, Nakbanpote W, Hokura A, Nuengchamnong N, Maneechai S (2016) Phenolic compounds responding to zinc and/or cadmium treatments in *Gynura pseudochina* (L.) DC. extracts and biomass. Plant Physiol Biochem 109:549–560

Monte Neto RL, Barbosa Filho JM, Sousa LM, Athayde Filho PF, Dias CS, Oliveira MR (2007) Crude ethanolic extract, lignoid fraction and yangambin from *Ocotea duckei* (Lauraceae) show antileishmanial activity. Z Naturforsch 62:348–352

Moradi L, Ehsanzadeh P (2015) Effects of Cd on photosynthesis and growth of safflower (*Carthamus tinctorius* L.) genotypes. Photosynthetica 53:506–518

Moraes RM, Santos Filho PR, Carvalho M, Nogueira ML, Barbosa S (2015) Effects of copper on physiological and cytological aspects in *Lactuca sativa* L. Rev Bras Bio 13:115–121

Moraes RM, Carvalho M, Nery FC, Santos Filho PR, Nogueira ML, Barbosa S (2018) Effects of hydrogen peroxide on initial growth and enzymatic antioxidant system of *Lactuca sativa* L. (Asteraceae). Pak J Bot 50:1769–1774

Moritz T, Johansson AI (2008) Plant metabolomics. In: Griffiths WJ (ed) Metabolomics, metabonomics and metabolite profiling. The Royal Society of Chemistry, Cambridge, pp 254–272

Motta EVS, Costa JC, Bastos JK (2017) A validated HPLC–UV method for the analysis of galloylquinic acid derivatives and flavonoids in *Copaifera langsdorffii* leaves. J Chromatogr B 1061:240–247

Muscolo A, Junker A, Klukas C, Weigelt-Fischer K, Riewe D, Altmann T (2015) Phenotypic and metabolic responses to drought and salinity of four contrasting lentil accessions. J Exp Bot 66:5467–5480

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8(3):199–216

Nahar K, Hasanuzzaman M, Alam MM, Rahman A, Suzuki T, Fujita M (2016) Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems. Ecotoxicol Environ Saf 126:245–255

Navarro-Reig M, Jaumot J, Piña B, Moyano E, Galceran MT, Tauler R (2017) Metabolomic analysis of the effects of cadmium and copper treatment in *Oryza sativa* L. using untargeted liquid chromatography coupled to high resolution mass spectrometry and all-ion fragmentation. Metallomics 9(6):660–675

Nielsen J, Oliver S (2005) The next wave in metabolome analysis. Trends Biotechnol 23:544–546

Noriega G, Santa Cruz D, Batlle A, Tomaro M, Balestrasse K (2012) Heme oxygenase is involved in the protection exerted by jasmonic acid against cadmium stress in soybean roots. J Plant Growth Regul 31:79–89

Obata T, Fernie AR (2012) The use of metabolomics to dissect plant responses to abiotic stresses. Cell Mol Life Sci 69(19):3225–3243

Okem A, Southway C, Stirk WA, Street RA, Finnie JF, Staden JV (2015) Effect of cadmium and aluminum on growth, metabolite content and biological activity in *Drimia elata* (Jacq.) Hyacinthaceae. S Afr J Bot 98:142–147

Olejar KJ, Ray S, Kilmartin PA (2016) Enhanced antioxidant activity of polyolefin films integrated with grape tannins. J Sci Food Agric 96:2825–2831

Olmedo DA, López-Pérez JL, Del Olmo E, Bedoya LM, Sancho R, Alcamí J, Muñoz E, San Feliciano A, Gupta MP (2017) Neoflavonoids as inhibitors of HIV-1 replication by targeting the tat and NF-κB pathways. Molecules 22:321

Osorio S, Do PT, Fernie AR (2012) Profiling primary metabolites of tomato fruit with gas chromatography/mass spectrometry. In: Hardy NW, Hall RD (eds) Plant metabolomics: methods and protocols. Springer/Humana Press, pp 101–109

Otero S, Núñez-Olivera E, Martínez-Abaigar J, Tomás R, Arróniz-Crespo M, Beaucourt N (2006) Effects of cadmium and enhanced UV radiation on the physiology and the concentration of UV-absorbing compounds of the aquatic liverwort *Jungermannia exsertifolia* subsp. cordifolia. Photochem Photobiol Sci 5:760–769

Peng Q, Bakulski KM, Nan B, Park SK (2017) Cadmium and Alzheimer's disease mortality in US adults: updated evidence with a urinary biomarker and extended follow-up time. Environ Res 157:44–51

Pichersky E, Raguso RA (2018) Why do plants produce so many terpenoid compounds? New Phytol 220(3):692–702

Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042

Pinu FR (2015) Metabolomics—the new frontier in food safety and quality research. Food Res Int 72:80–81

Piotto FA, Carvalho MEA, Souza LA, Rabêlo FHS, Franco MR, Batagin-Piotto KD, Azevedo RA (2018) Estimating tomato tolerance to heavy metal toxicity: cadmium as study case. Environ Sci Pollut Res 25:27535–27544

Pizzaia D, Nogueira ML, Mondin M, Carvalho MEA, Piotto FA, Rosario MF, Azevedo RA (2019) Cadmium toxicity and its relationship with disturbances in the cytoskeleton, cell cycle and chromosome stability. Ecotoxicology 28:1046–1055

Popova LP, Maslenkova LT, Yordanova RY, Ivanova AP, Krantev AP, Szalai G, Janda T (2009) Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. Plant Physiol Biochem 47:224–231

Pott DM, Osorio S, Vallarino JG (2019) From central to specialized metabolism: an overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit. Front Plant Sci 10:835

Qin GQ, Yan CL, Wei LL (2006) Effect of cadmium stress on the contents of tannin, soluble sugar and proline in *Kandelia candel* (L.) Druce seedlings. Acta Ecol Sin 10

Quan ZX, Rhee SK, Zuo JE, Yang Y, Bae JW, Park JR, Sung-Taik L, Park YH (2008) Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. Environ Microbiol 10:3130–3139

Rady MM, El-Yazal MAS, Taie HAA, Ahmed SMAM (2016) Response of *Triticum aestivum* (L.) plants grown under cadmium stress to polyamines pretreatments. Plant 4:29–36

Rashid MU, Alamzeb M, Ali S, Ullah Z, Shah ZA, Naz I, Khan MR (2019) The chemistry and pharmacology of alkaloids and allied nitrogen compounds from *Artemisia* species: a review. Phytother Res 33:2661–2684

Rastogi S, Pandey MM, Rawat AKS (2008) High-performance thin-layer chromatography densitometric method for the simultaneous determination of three phenolic acids in *Syzygium aromaticum* (L.) Merr. & Perry. J AOAC Int 91:1169–1173

Ren Y, Bai Y, Zhang Z, Cai W, Del Rio Flores A (2019) The preparation and structure analysis methods of natural polysaccharides of plants and fungi: a review of recent development. Molecules 24:3122

Restivo A, Degano I, Ribechini E, Colombini MP (2014) Development and optimisation of an HPLC-DAD-ESI-Q-TOF method for the determination of phenolic acids and derivatives. PLoS One 9:e88762

Rizhsky L, Jin H, Shepard MR, Scott HW, Teitgen AM, Perera MA, Mhaske V, Jose A, Zheng X, Crispin M, Wurtele ES, Jones D, Hur M, Góngora-Castillo E, Robin Buell C, Minto RE, Nikolau BJ (2016) Integrating metabolomics and transcriptomics data to discover a biocatalyst that can generate the amine precursors for alkamide biosynthesis. Plant J 88:775–793

Rochfort S (2005) Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. J Nat Prod 68:1813–1820

Romanowska E, Wróblewska B, Drozak A, Siedlecka M (2006) High light intensity protects photosynthetic apparatus of pea plants against exposure to lead. Plant Physiol Biochem 44:387–394

Ryan D, Robards K (2006) Metabolomics: the greatest omics of them all? Anal Chem 78:7954–7958

Sah SK, Reddy KR, Li J (2016) Abscisic acid and abiotic stress tolerance in crop plants. Front Plant Sci 7(571)

Salminen J-P, Karonen M (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. Funct Ecol 25:325–338

Sanita di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Sarker SD, Nahar L (2017) Progress in the chemistry of naturally occurring coumarins. In: Kinghorn A, Falk H, Gibbons S, Kobayashi J (eds) Progress in the chemistry of organic natural products. Progress in the chemistry of organic natural products, vol 106, pp 241–304

Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PEB, Williams DJ, Moore MR (2003) A global perspective on cadmium pollution and toxicity in non occupationally exposed population. Toxicol Lett 137:65–83

Seifi HS, Shelp BJ (2019) Spermine differentially refines plant defense responses against biotic and abiotic stresses. Front Plant Sci 10:117

Shakirova FM, Allagulova CR, Maslennikova DR, Klyuchnikova EO, Avalbaev AM, Bezrukova MV (2016) Salicylic acid-induced protection against cadmium toxicity in wheat plants. Environ Exp Bot 122:19–28

Sharma P, Jha AB, Dubey RS (2019) Oxidative stress and antioxidative defense system in plants growing under abiotic stresses. In: Pessarakli M (ed) Handbook of plant and crop stress. CRC Press, pp 93–136

Sigfridsson KGV, Bernat G, Mamedoy F, Styring S (2004) Molecular interference of Cd^{2+} with photosystem II. Biochim Biophys Acta 1659:19–31

Singh B, Singh JP, Singh N, Kaur A (2017a) Saponins in pulses and their health promoting activities: a review. Food Chem 233:540–549

Singh VP, Prasad SM, Munné-Bosch S, Müller M (2017b) Phytohormones and the regulation of stress tolerance in plants: current status and future directions. Front Plant Sci 8:1871

Smeds AI, Eklund PC, Willför SM (2016) Chemical characterization of high-molar-mass fractions in a Norway spruce knotwood ethanol extract. Phytochemistry 130:207–217

Solyomváry A, Beni S, Boldizsar I (2017) Dibenzylbutyrolactone lignans—a review of their structural diversity, biosynthesis, occurrence, identification and importance. Mini Rev Med Chem 17:1053–1074

Sova M (2012) Antioxidant and antimicrobial activities of cinnamic acid derivatives. Mini Rev Med Chem 12:749–767

Sreevidya N, Mehrotra S (2003) Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J AOAC Int 86:1124–1127

Stringlis IA, De Jonge RR, Pieterse CMJ (2019) The age of coumarins in plant-microbe interactions. Plant Cell Physiol 60:1405–1419

Sun Y, Sun G, Xu Y, Wang L, Liang X, Lin D (2013) Assessment of sepiolite for immobilization of cadmium-contaminated soils. Geoderma 193:149–155

Sunil C, Xu B (2019) An insight into the health-promoting effects of taxifolin (dihydroquercetin). Phytochemistry 166:112066

Szabó B, Tyihák E, Szabó G, Botz L (2003) Mycotoxin and drought stress induced change of alkaloid content of *Papaver somniferum* plantlets. Acta Bot Hung 45:409–417

Tajti J, Janda T, Majláth I, Szalai G, Pál M (2018) Comparative study on the effects of putrescine and spermidine pre-treatment on cadmium stress in wheat. Ecotoxicol Environ Saf 148:546–554

Tanaka T, Onuma H, Shigihara T, Kimura E, Fukuta Y, Shirasaka N, Moriyama T, Homma Y (2019) Anti-osteoporotic effects of syringic acid and vanilic acid in the extracts of waste beds after mushroom cultivation. J Biosci Bioeng 128:622–629

Taylor J, King RD, Altmann T, Fiehn O (2002) Application of metabolomics to plant genotype discrimination using statistics and machine learning. Bioinformatics 18:241–248

Teponno RB, Kusari S, Spiteller M (2016) Recent advances in research on lignans and neolignans. Nat Prod Rep 33:1044–1092

Thakur S, Choudhary S, Bhardwaj P (2019) Comparative transcriptome profiling under cadmium stress reveals the uptake and tolerance mechanism in *Brassica juncea*. J Plant Growth Regul 38:1141–1152

Tinikul R, Chenprakhon P, Maenpuen S, Chaiyen P (2018) Biotransformation of plant-derived phenolic acids. Biotechnol J 13(6):e1700632

Tomás-Barberán FA (1995) Capillary electrophoresis: a new technique in the analysis of plant secondary metabolites. Phytochem Anal 6(4):177–192

Tomi K, Kitao M, Konishi N, Murakami H, Matsumura Y, Hayashi T (2016) Enantioselective GC-MS analysis of volatile components from rosemary (*Rosmarinus officinalis* L.) essential oils and hydrosols. Biosci Biotechnol Biochem 80:840–847

Verpoorte R, Choi YH, Mustafa NR, Kim HK (2008) Metabolomics: back to basics. Phytochem Rev 7:525–537

Villiers F, Ducruix C, Hugouvieux V, Jarno N, Ezan E, Garin J, Junot C, Bourguignon J (2011) Investigating the plant response to cadmium exposure by proteomic and metabolomic approaches. Proteomics 11:1650–1663

Villiers A, Venter P, Pasch H (2016) Recent advances and trends in the liquid-chromatography-mass spectrometry analysis of flavonoids. J Chromatogr A 1430:16–78

Vinardell M, Mitjans M (2017) Lignins and their derivatives with beneficial effects on human health. Int J Mol Sci 18:1219

Vollenweider P, Günthardt-Goerg MS (2005) Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. Environ Pollut 137:455–465

Wallace TC, Giusti MM (2015) Anthocyanins. Adv Nutr 6:620–622

Wang F, Wang Z, Zhu C (2012) Heteroexpression of the wheat phytochelatin synthase gene (*TaPCS1*) in rice enhances cadmium sensitivity. Acta Biochim Biophys Sin 44(10):886–893

Wang Y, Xu L, Shen H, Wang J, Liu W, Zhu X, Wang R, Sun X, Liu L (2015a) Metabolomic analysis with GC-MS to reveal potential metabolites and biological pathways involved in Pb & Cd stress response of radish roots. Sci Rep 5:18296

Wang CZ, Zhang HY, Li WJ, Ye JZ (2015b) Chemical constituents and structural characterization of polysaccharides from four typical bamboo species leaves. Molecules 20:4162–4179

Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176

Wei L, Wang X, Zhang P, Sun Y, Jia L, Zhao J, Dong S, Sun L (2016) An UPLC-MS/MS method for simultaneous quantitation of two coumarins and two flavonoids in rat plasma and its application to a pharmacokinetic study of *Wikstroemia indica* extract. J Chromatogr B 1008:139–145

Widhalm JR, Dudareva N (2015) A familiar ring to it: biosynthesis of plant benzoic acids. Mol Plant 8(1):83–97

Wink CS, Meyer GM, Zapp J, Maurer HH (2015) Lefetamine, a controlled drug and pharmaceutical lead of new designer drugs: synthesis, metabolism, and detectability in urine and human liver preparations using GC-MS, LC-MS(n), and LC-high resolution-MS/MS. Anal Bioanal Chem 407:1545–1557

Wolfender JL, Marti G, Thomas A, Bertrand S (2015) Current approaches and challenges for the metabolite profiling of complex natural extracts. J Chromatogr A 1382:136–164

Xie Y, Hu L, Du Z, Sun X, Amombo E, Fan J, Fu J (2014) Effects of cadmium exposure on growth and metabolic profile of bermudagrass [*Cynodon dactylon* (L.) Pers.]. PLoS One 9(12):e115279

Xie M, Chen W, Lai X, Dai H, Sun H, Zhou X, Chen T (2019) Metabolic responses and their correlations with phytochelatins in *Amaranthus hypochondriacus* under cadmium stress. Environ Pollut 252(Pt B):1791–1800

Yang R, Li P, Li N, Zhang Q, Bai X, Wang L, Xiao Y, Sun L, Yang Q, Yan J (2017) Xanthones from the pericarp of *Garcinia mangostana*. Molecules 22:683

Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q (2018) Response of plant secondary metabolites to environmental factors. Molecules 23:762

Yi J, Lam TI, Yokoyama W, Cheng LW, Zhong F (2015) Beta-carotene encapsulated in food protein nanoparticles reduces peroxyl radical oxidation in Caco-2 cells. Food Hydrocoll 43:31–40

Zazoua A, Bouraoui S, Halim R, Khedimallah N, Jaffrezic-Renault N (2018) Polyphenolic natural products for the electrochemical determination of cadmium. Anal Lett 51:359–370

Zhang J, Wen C, Zhang H, Duan Y (2019) Review of isolation, structural properties, chain conformation, and bioactivities of psyllium polysaccharides. Int J Biol Macromol 139:409–420

Zhao FY, Wang K, Zhang SY, Ren J, Liu T, Wang X (2014) Crosstalk between ABA, auxin, MAPK signaling, and the cell cycle in cadmium-stressed rice seedlings. Acta Physiol Plant 36:1879–1892

Zhao YH, Jia X, Wang WK, Liu T, Huang SP, Yang MY (2016) Growth under elevated air temperature alters secondary metabolites in *Robinia pseudoacacia* L. seedlings in Cd- and Pb-contaminated soils. Sci Total Environ 565:586–594

Zheljazkov VD, Craker LE, Xing B (2006) Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. Environ Exp Bot 58(1):9–16

Zong A, Cao H, Wang F (2012) Anticancer polysaccharides from natural resources: a review of recent research. Carbohydr Polym 90:1395–1410

The Current Scenario and Prospects of Immobilization Remediation Technique for the Management of Heavy Metals Contaminated Soils

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Abstract

Approximately, more than 200 million hectares are contaminated with heavy metals (HMs) having very high concentrations greater than the standard values worldwide. Thus, the urgent remediation of HMs contaminated soils is the need of the hour. In situ immobilization of HMs through organic, inorganic, and other stabilizing additives seems to be the most promising remediation technique in managing HMs pollution. The efficiency of different stabilizing agents has been previously tested for the rehabilitation of HMs contaminated soils with the immediate estimation of their leaching and availability from them. Among tested amendments, biochar and iron base amendments have shown their high efficiency in removing multi-HMs polluted soils. Thus, the immobilization technique seems to be a preferable alternative over other traditional remediation methods owing to its vast applicability, easy availability of raw materials, and wide acceptability. However, weathering activities may increase the risk of HMs remobilization due to the breakdown of organic amendments. Thus continuous monitoring of HMs soils is recommended.

Keywords

Heavy metals · Immobilization · Inorganic amendments · Organic amendments · Remediation

1 Introduction

The pollution of arable soils with heavy metals (HMs) is an emergent environmental issue worldwide. The term “HMs” refers to “the elements (metals and metalloids) with potential capability to harm living organisms (plants, animals, humans) in a very small amount.” These HMs consist of cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) (Singh et al. 2011).

Both natural such as weathering and geological processes while in parallel, the additions of artificial fertilizers into agricultural lands, the use of organic and agricultural residues, tannery industry, improper dumping of Pb–acid as well as Ni–Cd batteries, electronic wastes, and military firing ranges are the most common anthropogenic source of HMs pollution (Tauqeer et al. 2019).

When the concentrations of HMs increased in the soil from their threshold limits, noteworthy toxic symptoms observed on soil quality, which severely affect the performance of major soil functions. Further, the additions of HMs in arable lands increase the risk of their accumulation in plants grown on them, which leads to dietary exposure through biomagnification and thus causing metal poisoning in animals and humans. Likewise, the presence of HMs in such soils also resulted in poor seed germination, which adversely affects the yield and nutritional quality of crops grown on them. Additionally, leaching and surface water runoff is the additional output pathways of HMs migration into other surrounding ecosystems (Kumar et al. 2019; Hembrom et al. 2020).

Keeping in mind the above-narrated issues, the remediation of HMs contaminated lands via adopting efficient remediation methods is necessary. Several conventional remediation methods such as soil replacement, soil washing, and thermal desorption have been reported in managing HMs pollution. However, these reclamation methods are difficult in operation, required high cost, and is destructive. Therefore, these remediation methods are not preferred for the remediation of HMs contaminated soils (Yao et al. 2012).

Recently, the (ex situ) immobilization remediation method for HMs contaminated soils through organic and inorganic amendments has attained the attention of the scientific community across the globe due to its several advantages over traditional methods such as (1) less site destructive, (2) easy to handle due to its simplicity, (3) required comparatively cheap and easily available raw materials for amendments, (4) improved soil quality and soil health through the provision of essential nutrients in degraded soils, and (5) controlled the dispersal risk of contamination (Peng et al. 2009; Wuana and Okieimen 2011).

The addition of stabilizing agents in contaminated soils reduced the bioavailability of HMs by forming precipitates, HMs complexes, and adsorption. Furthermore, the addition of organic amendments not only immobilize HMs but also have additional benefits such as (1) increase the soil biological activities and provide them habitat, (2) improve physicochemical properties of degraded soil by enhancing organic content, (3) enhance water-holding capacity of the soil, (4) support root proliferation and elongation, (5) provide essential nutrients to the plants, and (6) enhance cation exchange capacity (CEC) of the soil.

The objective of this chapter is to collect the latest information about modern trends in the immobilization technique deployed for the management of HMs contaminated soils especially focusing on the interaction of amendments with HMs in soil and their influence on soil quality.

2 Classification of HMs Contaminated Soils

Several anthropogenic sources of HMs have already been cited in the literature, but here we discuss major sources that contributed to high levels of HMs pollution.

2.1 Contamination of Agricultural Soils from Agrochemicals and Wastewater

A dramatic increase in population, food insecurity, and other environmental factors (drought, etc.) puts huge burdens on agricultural resources worldwide. Recently, the farmers having low socio−economic status from developing countries, the excessive use of agrochemicals (fertilizers, pesticides, herbicides, etc.) and irrigation with wastewater are still being a common practice to fulfill the needs of huge population. These practices resulted in HMs accumulation and their transfer from soil to crops, which pose a serious threat to humans due to their direct or indirect bioaccumulation and biomagnification through soil–plant and soil–plant–animal pathways (Turan et al. 2018).

The applications of phosphorus fertilizers are another source of HMs especially Cd pollution in agricultural soils. For example, Cd is the major constituent of phosphorus fertilizers, which contains Cd from smaller amounts up to 300 mg kg^{-1} (Selim 2018). Except for Cd, the concentrations of other HMs such as Pb and Cr in phosphorus fertilizers have also previously been reported (Atafar et al. 2010; Cheraghi et al. 2012; Nacke et al. 2013). The applications of phosphorus additives in agricultural soils resulted in HMs deposition, which negatively influences the soil quality and crop production (Cheraghi et al. 2012; Nacke et al. 2013).

The overexploitation and excessive use of groundwater for irrigation depleted fresh and groundwater resources worldwide, especially in developing countries. Unfortunately, the farmers from economically poor status countries are compelled to rely on municipal (MWW) and industrial wastewater (IWW) to irrigate their croplands for crop and fodder production. The continuous applications of wastewater may result in HMs pollution of the arable lands (Turan et al. 2018). For example, irrigation with MWW in an arable field has resulted in the contamination of Cd, Ni, Cr, and Pb (Turan et al. 2018). Likewise, the irrigational use of effluents discharged from Pb−acid batteries and electroplating industries resulted in the serious contamination of surrounding agricultural soils with Pb (Khan et al. 2020) and Ni (Shahbaz et al. 2018a, b, 2019), respectively. The continuous irrigation of arable lands with MWW in Marrakech, Morocco, increased the levels of Cd, Cu, Pb, and Zn loading in agricultural soils and crops grown on them (Chaoua et al. 2019).

2.2 Aerially Deposited HMs from Mining, Smelting, and Fuel Burning

The areas with long-term industrial activities and their nearby ecosystems in Europe, South Asia, Western, and South Africa were supposed to be highly contaminated with HMs. Mining and smelting actions were previously performed to extract metals from their natural ores and caused significant damage to the ecosystem. The mining of Ni from ferronickel mines resulted in Ni accumulation in the agricultural soils of the Sinú River in northwestern Colombia (Marrugo-Negrete et al. 2017). The production of acid mine drainage (AMD) is another challenging task associated with mining actions due to the presence of HMs in excessive amounts. When water is released from abandoned mines and tailings, it dissolves a significant amount of HMs, which contaminate freshwater resources, nearby agricultural lands through rainwater runoff, and groundwater via leaching (Naidu et al. 2019). The aerial depositions of HMs from the burning of Pb−based gasoline and dust from mining and metal smelters contributed to the HMs contamination of their surrounding environment (Walraven et al. 2014; Žibret et al. 2013; Huang et al. 2018). For instance, the aerial depositions from Pb–Zn smelters in Hungary, Czech Republic, Austria, and Slovakia (Iqbal et al. 2012; Muhammad et al. 2012) and across Europe have resulted in the accumulation of Pb, Cd, and Zn in surrounding soils (Ettler 2016). Likewise, significantly the higher concentrations of As, Sb, Hg, Zn, Cd, and Pb have been reported in the surrounding soil of a 60-year-old Pb–Zn smelter in the town near Yunnan province, China (Li et al. 2015). The HMs solubility and bioavailability increased under acid rain conditions, which probably increased the risk of human exposure through runoff in urban areas (Žibret et al. 2013). The intense industrial and agricultural practices and high traffic activities caused remarkable HMs accumulation in agricultural soils (Cai et al. 2019). The dissolution of smelter wastes also resulted in the release of Pb (up to 200 mg kg^{-1}), Cu (4000 mg kg^{-1}), As (300 mg kg^{-1}), and Zn (1500 mg kg^{-1}) (Kierczak et al. 2013).

2.3 Firing Range Soils

Military firing or sports shooting ranges also play a key role in the deterioration of the environment via the release of HMs from the bullets and their fragments. The extent of firing ranges depends upon several factors including range type, the type and composition of ammunition, frequency of shooting, soil chemistry, and geochemistry of pollutants. Fayiga (2019) and references therein reported very high concentrations of Zn, Sb, Pb, Cu, and other HMs (Cd, Cr, Ni, Co, etc.) in military range soils from Canada, South Korea, the USA, Spain, Pakistan, and the Czech Republic. The continuous dissolution of bullets and their fragments due to redox reactions in the soil increased the labile fractions of HMs in firing range soils. The excessive release of HMs from highly polluted firing ranges significantly caused the contamination of groundwater, nearby water bodies as well as agricultural lands through leaching and rainwater runoff. In Norway, the surface runoff from a small-arms shooting range resulted in the contamination of Stitjønn and Kyrtjønn lakes. This contamination increased up to 161 mg Pb kg^{-1}DW in *Salmo trutta* and up to 2700 mg Pb kg^{-1} DW in bottom sediments of the respective lakes (Mariussen et al. 2017). Similarly, the contamination of agricultural land through runoff from 30-year-old firing range soil in the Czech Republic has previously been described (Chrastný et al. 2010). Reportedly, the decommissioned ranges were also often used as pastures for livestock in Switzerland (Tandy et al. 2017), which may increase the accumulation risk of HMs in animals grazing on them (Johnsen et al. 2019). On the contrary, the contamination of sports shooting ranges due to Pb deposition from Pb pellets has been reported worldwide causing the significant deaths of more than million birds including endangered species because of Pb poisoning (Pain et al. 2019).

2.4 Fishponds

Aquaculture regarded as the backbone for economic development in African and Asian countries, which increases the GDP through fish export (Muddassir et al. 2019). Generally, the fish ponds are prepared in specific areas with distinctive characteristics such as (1) flat or depression lands, (2) waterlogged soils, (3) degraded agricultural lands due to salinity, (4) perennial source of sufficient water supply, i.e., rivers, canals, streams, etc., and (5) high water retention rate due to high clay content. However, the conversion of fertile agricultural lands into fishponds is also a common practice being observed in various areas of Pakistan. Fish ponds established in saline soils are mostly provided with brackish groundwater, which creates a new challenging task for sustainable fish forming (Shaheen et al. 2020). Likewise, fertilizer additions to such ponds are a common practice to increase the growth of aquatic animals and plants (Muddassir et al. 2019). The contamination of ponds could occur due to irrigation with brackish water and applications of different fertilizers (Shaheen et al. 2020; Muddassir et al. 2019). The contamination of ponds with HMs has been reported (Shaheen et al. 2020; Adeyeye 1994).

3 Factors Influencing the Efficiency of Remediation Strategies

The stabilization of HMs in soil primarily depends upon chemical speciation and binding potential of HMs, which are controlled by physicochemical characteristics of the soil as well as several environmental factors. Geochemical processes occurring in the soil such as precipitation, adsorption, complexation, redox reactions, organic matter, and interaction of Al, Fe, and Mn with HMs play a key role in HMs mobility (Komárek et al. 2013). Several soil factors such as soil texture, particle size, bulk density, pH, electrical conductivity (EC), CEC, water-holding capacity organic matter, redox potential, and the presence of clay contents as well as oxides and hydroxides of Fe, Al, and Mn influenced the geochemical reactions such as precipitation, adsorption, complexation, and redox reactions, thereby increasing or decreasing the bioavailability of HMs (Beiyuan et al. 2017).

3.1 Soil pH

Soil pH not only influenced the bioavailability of HMs but also affect cation exchange, surface–complexation interactions of cations, and other several binding mechanisms. Generally, a rise in pH increased the concentrations of OH^-, which promotes strong and preferential adsorption of HMs through forming carbonate, precipitate, and hydroxides, thereby reducing their bioavailability (Wu et al. 2016).

3.2 Cation Exchange Capacity (CEC)

The CEC refers to the ion binding ability of the soil, which also plays a key role in governing the mobility of HMs. The presence of organic matter, metal oxides, and clay minerals increased CEC and furnished large surfaces for the adsorption of HMs, which reduced their mobility in soil solution (Kelebemang et al. 2017; Finzgar et al. 2007).

3.3 Organic Matter

The breakdown of plants and animal residues through soil microbial communities increased the concentrations of carboxyl, phenols, amino, and carboxylate functional groups, which restricts HMs mobility in the soil. These functional groups provide binding sites to HMs, which form stable metal complexes with organic matter through adsorption, ion exchange, and complexation mechanisms resultantly reducing their bioavailability in the soil at higher pH levels (Zeng et al. 2011; Quenea et al. 2009).

3.4 Abundance of Oxides and Hydroxides

The oxides and hydroxides of Fe, Mn, and Al are considered as the key components for the stabilization of HMs in soil due to their majestic features such as specific affinity for metal ions, strong binding ability, inner surface complexation, and the formation of minerals via precipitation mechanism (Zeng et al. 2017). Additionally, the oxide concentrations found to be higher under oxidized conditions, which promote HMs immobilization by metal oxides (Komárek et al. 2013).

3.5 The Contents and Types of Clay Minerals

The presence of clay minerals such as kaolinite, chlorite, smectite, illite, vermiculite, bentonite, and zeolite plays a vital role in the geochemistry of HMs in the soil. Owing to partial and stable negative charges on clay minerals, the soils having high clay contents showed higher adsorption tendency, thereby reducing HMs bioavailability (Rieuwerts 2007).

3.6 Soil Biota

The presence or absence of key soil (micro)organisms may (im)mobilize HMs in the soil. Large quantities of HMs may alter the functioning and activities of essential soil (micro)organisms. These (micro)organisms tend to tolerate and resist HMs stress, which influences the efficiency of a remediation strategy (Rajkumar et al. 2012). The *Azotobacter* spp., arbuscular mycorrhizal fungi (AMF), and *Cellulosimicrobium cellulans* are known to reduce HMs bioavailability in the soil through complexation, sorption, and reduction mechanisms. These microorganisms released several extracellular polymeric substances such as, glomalin, and insoluble glycoprotein, which strongly bound HMs and reduced their mobility in the soil. These HMs were sorbed by melanin and chitin in fungal cell wall as well as accumulated in the vacuoles of mycorrhizal fungi, which restrict their mobility (González-Guerrero et al. 2008; Rajkumar et al. 2012; Khan et al. 2020).

4 Traditional Remediation Methods

This section covers different remediation approaches deployed for the management of HMs contaminated soils.

4.1 Soil Excavation and Replacement

Soil excavation or replacement is effectively insulating the soil and environment for minimizing the effects of HMs in the environment (Nejad et al. 2018). This technique works with the usage of clean soil to partially or completely replace the contaminated soil to dilute the concentrations of toxic pollutants (Yao et al. 2012). This technique effectively controls the mobility of HMs but not permanently removes them from the environment. This method comprises of three steps: In the first step, contaminated soil is replaced with clean soil by removing its contaminated surface. The second and third steps are soil spading and soil importing, associated with the digging of contaminated soil at higher depths and stored for further treatment (Yao et al. 2012; Nejad et al. 2018).

4.2 Soil Washing and Flushing

The soil washing is an easy, reliable method used for the removal of HMs present in the soil (Peng et al. 2018; Ou-Yang et al. 2010; Nejad et al. 2018). In this method, several ingredients such as organic and inorganic acids, chelating substances, and surfactants are used to dissolve HMs from the solid phase to soil solution (Akcil et al. 2015). Some treatments effectually leached down the HMs from the soil, such as EDTA for the leaching of Cd (Peng et al. 2018), sulfuric, oxalic, and citric acid for the removal of various HMs through washing from the soil (Beolchini et al. 2013). Interestingly, phosphoric acid also showed the highest extraction efficiency of HMs removal from the soil with a positive influence on soil and plant health (Nejad et al. 2018). Above all treatments, diluted sulfuric acid is best for soil washing as it has fewer negative impacts on the environment. Likewise, some washing agents may not effectively work when remediating contaminated calcareous soils, for the reason that calcite and carbonates neutralize the protons (Fonti et al. 2013; Akcil et al. 2015).

4.3 Vitrifying Methods

Vitrification enforced intense energy source to melt contaminated soil at 1600–2000 °C temperature (Nejad et al. 2018). The steam generated by high temperature was collected by the off-gas treatment system (Yao et al. 2012). Interestingly, this method immobilizes and fixes the HMs into a divine glass pattern at molecular levels. This powerful in situ technique is often known as joule-heating vitrification, used to remediate tons of HMs contaminated soils by melting and heating, thereby subsequently immobilizing them in the soil (Nejad et al. 2018; Li and Zhang 2013; Wuana and Okieimen 2011). Extremely the highest power produced glass stone binds the HMs and decreases their leaching, thus preventing groundwater contamination (Nejad et al. 2018).

4.4 Electro-Kinetic Methods

The new technique based on the principle of vitrification method is “Electro-kinetic/Electrochemical” in which electric field (AC/DC flow) is produced at both sides of contaminated soil (Akcil et al. 2015; Peng et al. 2018; Nejad et al. 2018). Several researchers investigated the successful immobilization of HMs in the contaminated sites via electro-kinetic technology (Juris et al. 2015; Ottosen et al. 2012; Nejad et al. 2018) via the movement of water, ions, and charged particles between both electrodes. This method is suitable for fine particles because of the higher adsorption of HMs due to the relatively higher flow of electric current (Pedersen et al. 2015; Peng et al. 2018). Likewise, HMs mobilization associated with oxides, carbonates, nitrate, and hydroxides can also be enhanced via the electro-kinetic method. Electromigration, electroosmosis, electrolysis, and electrophoresis are associated mechanisms involved in the removal of HMs from the contaminated soils (Peng et al. 2018). Co-application of several surfactants and desorbing agents with this technique can also be used to enhance the HMs removal efficiency (Peng et al. 2018).

5 Negative Consequences of Traditional Remediation Strategies

The speciation, labile–immobile fractions of HMs and pollution levels strongly affect the efficiency of the remediation method. Therefore, the prior knowledge about the selected site such as characteristics of the contaminated soil, level, and distribution of HMs and climatic conditions must be acquired to design the most precise remedial alternative.

The main features that affect the applicability and choice of remediation strategies are (1) alleviate HMs toxicity, (2) efficient in reducing HMs bioavailability, (3) effective in managing multicontaminated soils (such as shooting range, E-waste, mining as well as industrially contaminated soils), (4) applicable to highly HMs contaminated soils, (5) support plant establishment and growth, (6) environment-friendly, economically feasible, and commercially available, (7) widely acceptable by the community as well as stakeholders, and (8) long-lasting effects. Although traditional or harsh remediation strategies are efficient in removing HMs from the soil, they severely damage and alter soil properties, which demands additional improvement. This section discusses the major limitations of the above-mentioned harsh remediation methods.

Soil washing required (1) enough space, (2) sufficient amount of water for washing, (3) washing chemicals (such as redox as well as chelating agents, surfactants, acids–bases, and salts), and (4) off-site dumping of residual solids and associated risk of HMs leaching from them, which increased the overall cost. Additionally, this remediation method is not recommended for the soils having high clay as well as organic matter contents (Nejad et al. 2018; Sharma et al. 2018). The drawbacks of soil replacement and excavation remediation process increased the costs due to the requirements of (1) heavy machinery for transportation of both contaminated and uncontaminated soils, (2) skilled labor and technically sound experts, (3) dilution with the import of new excessive uncontaminated soils, (4) deep digging, (5) large working volume, (6) recommended for upper layer soils, and (7) severely destroy soil structure by the compaction through the movement of heavy machinery (Nejad et al. 2018; Yao et al. 2012; Zhou et al. 2004). Usually, prior information about soil properties, dissolution, and transport of HMs, stirring rate and time, cell set-up, current density are the key factors influencing the efficiency of electrochemical remediation method (Alshawabkeh 2009; Nejad et al. 2018; Pedersen et al. 2015; Yeung and Gu 2011). Additionally, other demerits of this remediation technique, which increased the overall cost and longer treatment times, are (1) the formation of stable precipitates due to higher contents of OH^- around the anode, (2) need of desorbing substances such as surfactants and acidification, (3) only applicable for low permeable soils with relatively higher concentrations of

HMs, (4) efficient in removing HMs when sufficient pore fluid is available in soil pores, which facilitates the transport of pollutants, (5) as well as electric current, (6) modifications in soil microstructure as well as geochemistry due to acidification at anodes, and (7) higher energy requirements (Nejad et al. 2018; Peng et al. 2018; Kuppusamy et al. 2016; Pedersen et al. 2015; Alshawabkeh 2009). Likewise, the key limitations of vitrification remediation approach are (1) only effective for a certain depth (approximately 2–7 m below) of soil, (2) unfit for soils with low clay contents, (3) dewatering is necessary to remove HMs from permeable aquifers, (4) required dynamic compaction to decrease large voids, (5) need of soil, sand, and clay as well as electric current to acquire certain features of vitrified material, (6) should be avoided for soils with higher alkaline contents (K_2O, $Na_2O = 1.4$ wt%) due to less conductance of current by the molten soil, (7) inefficient in removing HMs from the deeper layers, which increased energy requirements, (8) regular monitoring is required to ensure the stabilization of HMs, and (9) required skilled labor and technical expertise, which increased the overall cost (Nejad et al. 2018; Peng et al. 2018; Kuppusamy et al. 2016; Akcil et al. 2015).

Apart from this, the remediation strategies as mentioned earlier severely damaged microbial communities, destroyed organic matter, and altered micro- and macropores in the soil, which required additional cost of improvement to support plantation after HMs removal from contaminated soils.

6 Remediation of HMs Contaminated Soils with the Focus on Immobilization Techniques

The process of diminishing the solubilization, mobilization, and bioavailability of HMs in the soils with the assistance of different organic and inorganic additives reagents is known as "Immobilization" (Lwin et al. 2018; Peng et al. 2018; Nejad et al. 2018). Different amendments such as organic (biochar, compost, agricultural wastes), inorganic (phosphates, calcium, iron-based additives), and clay (zeolite, bentonite, etc.) are being used for the effective fixation of HMs in the soil. The working principle of immobilization is to fix and bind the HMs via different mechanisms such as adsorption, oxidation, precipitation, and reduction instead of removing them from the soil (Akcil et al. 2015; Peng et al. 2018). The role of amendments is to transform the mobile phase into a more stable form (Lwin et al. 2018). Usually, this happened due to the higher CEC values of stabilizing additives, which strongly bind HMs, thereby reducing their bioavailability (Akcil et al. 2015). Interestingly, the applications of organic substances as stabilizing additives not only reduce the bioavailability of HMs but also provide essential mineral nutrients in the soil, which improved plant growth as well as microorganism (Lwin et al. 2018). Additionally, it is hard to understand the suitable amendment with its right dose for an efficient immobilization. Hence, exploration of new amendments due to the viability of different raw materials, their continuous testing, and performance monitoring as additives increase the interest of researchers toward immobilization during modern ages (Chiang et al. 2012; Peng et al. 2018; Nejad et al. 2018).

6.1 Immobilization Through Organic Soil Additives

Several organic compounds varying in properties supremely immobilize certain HMs in the environment. Generally, it includes different kinds of manures, biosolids, wood ash, biochar, different composts, and wood chips (Sabir et al. 2013; Peng et al. 2018). Organic amendments significantly bind HMs with the assistance of numerous functional groups present in humic acids, which reduced the bioavailability of HMs to some extent (Lwin et al. 2018). Moreover, organic-based additives not only bind HMs but also nourish the soil. However, the only drawback in the addition of organic amendments is the risk of HMs remobilization due to the decomposition of organic matter (Lwin et al. 2018).

6.1.1 Biochar

The permeable and carbonaceous material formed after pyrolyzing the organic feedstocks like manures, plant-wood biomass, and sludges is termed as "Biochar" (Nejad et al. 2018; Lwin et al. 2018). Biochar has distinctive attributes, i.e., higher CEC values, large surface area, alkaline nature, higher sorption capacity with many functional groups, and presence of humic and fulvic substances (Lin et al. 2012; Lwin et al. 2018). Interestingly, biochar has positively influenced the soil properties via enhancing its microbial activity and carbon sequestration (Paz-Ferreiro and Fu 2014; Nejad et al. 2018; Lwin et al. 2018). The co-effect of biochar with chitosan in the soil irrigated with wastewater containing excessive HMs was also tested to save the quality of brinjal. The combined application of biochar and chitosan significantly reduces the bioavailability of numerous HMs in the soil and promote the safe production of brinjal (Turan et al. 2018).

It acts as an excellent HMs adsorbent due to the presence of diverse functional groups such as hydroxyl, carboxyl, and phenolic hydroxyl, and presence of negative charge sites in its chemical structure, which helped in retaining more HMs in it (Mahar et al. 2015; Kammann et al. 2015; Lwin et al. 2018). Previous studies showed the remarkable immobilization of different HMs with biochar additions. For instance, biochar applications in HMs contaminated soils reduced the bioavailability of different HMs (Mahar et al. 2015; Huang et al. 2017). Likewise, the type of feedstock, as well as pyrolysis temperature, also influenced HMs immobilization in the soil (Igalavithana et al. 2017a, b; Khan et al. 2017, 2020). The application of biochar produced from different feedstock such as hardwood (Beesley et al. 2010), shell and cow bone (Ahmad et al. 2012), chicken manure, and green waste (Park et al. 2011; Lwin et al. 2018) in HMs contaminated soils resulted in the reduction of HMs bioavailability in soil. Likewise, the biochar produced from cottonseed hull feedstock at a higher temperature significantly adsorbs HMs due to change in the composition of oxygen-containing functional groups (Mahar et al. 2015). The reduction in HMs bioavailability could be due to the change in pH of the soil from acidic to alkaline, which increased the concentrations of OH^- ions, thereby reducing HMs bioavailability (Cantrell et al. 2012; Joseph et al. 2015). Additionally, the applications of biochar not only reduced HMs bioavailability but also improved the hydro-infiltration, aeration, and aggregate stability of the soil (Lwin et al. 2018) as well as provide essential nutrients such as Mg, Na, K, S, and Ca linked with biochar (Nejad et al. 2018; Uchimiya et al. 2011; Lu et al. 2012). The biochar produced at low (Uchimiya et al. 2011) and high temperatures (Cao et al. 2011; Beesley et al. 2010) released available Ca, P, and K in the soil (Nejad et al. 2018), which improved overall plant health. Numerous studies reported that biochar could also augment microbial growth owing to its various functions of enhancing soil aeration, retaining nutrients, and providing micro-pores as habitats which increased nutrient cycling (Kong et al. 2018).

6.1.2 Mulching and Composting

The use of organic by-products "Mulching or Composting" for soil nourishment and remediation purposes is an effectually and economically viable option (Lwin et al. 2018; Mahar et al. 2015). Similarly, the peculiar nature of mulching is due to its makeup as it is derived from living waste (farmyard/swine manure, cow/pig/poultry waste, green waste). Exemplary mulching promotes nutritional quality and physical properties (particle size division, porosity, and cracking patterns) of the soil (Lwin et al. 2018; Mahar et al. 2015). Simultaneously, the application of mulching in soil stimulates the pH, boosts surface charges, and precipitation mechanisms for HMs immobiliztion. It can adsorb HMs through metal binders (phosphates or carbonates), but the performance of mulching in the soil completely relies on soil nature, pH, EC, humification, and CEC. Likewise, organic mulching significantly reduces the bioavailability of HMs and their uptake by the plants (Alamgir et al. 2011; Mahar et al. 2015; Lwin et al. 2018). Interestingly, the addition of compost/mulching in contaminated soil increases soil pH, encourages growth and nutrients uptake in plants via enhanced root development, and strengthens the activity of microbes as well as HMs immobilization. Furthermore, mulching also fertilizes the soil and plants by augmenting the concentrations of essential elements like N, P, Ca, Fe, and Mg, organic and microbial C, which influenced the soil respiration and enzymatic activities. The other side of mulching should also be considered because the organic amendments also have disease-causing bacteria and higher concentrations of dissolved salts, which can introduce new pathways of HMs in soil (Mahar et al. 2015; Lwin et al. 2018).

6.1.3 Agricultural Waste

Researchers have continuously discovered the cheap and accessible amendments for the remediation of HMs contaminated soils. Interestingly, agricultural waste is a powerful amendment for HMs immobilization in the soil. Agricultural waste includes rice–wheat husks and brans sawdust of several plants, the bark of trees, groundnut, and coconut shells, hazelnut shells, walnut shells, cotton seeds hull, waste tea leaves, maize corn cob, sugarcane bagasse, fruit and vegetable peels (apple, orange, and banana, etc.), sugar beet pulp, coffee beans, cotton stalks, sunflower stalks, grapes stalks, and Arjun nuts (Sud et al. 2008). The main components of agricultural waste are lignin, hemicellulose, extractives, proteins, sugar, starch, hydrocarbons, cellulose, and functional groups, which participated in the adsorption of HMs. The worthwhile agricultural waste is a highly efficient, nutrient-rich, eco-friendly amendment and used for the removal of HMs present in the soil. Interestingly, several HMs are removed from the environment by applying agro-waste. Under optimum pH conditions, agro-waste such as rice husk, sawdust of rubber, and Indian rosewood successfully stabilized higher Cr concentrations in polluted soils. Interestingly, the effective Pb immobilization was also reported with the assistance of different agro-waste (Sud et al. 2008).

6.2 Geo-Polymers as Stabilizing Additives

The geopolymers are also known as a promising ceramic adsorbent for HMs adsorption from domestic and industrial effluents. It is produced during the reaction between NaOH or KOH with Si or Al and has a great potential to clean the HMs contaminated sites. The divine and multitasking geopolymer are hard, heat and fire resistant, high water retention, and suitable shear stress as well as compressive strengths and used for the adsorption of HMs (Rasaki et al. 2019). Geopolymers comprising minerals, clay, slag, fly ash, and cement, which contains interlinked crossed bonds with cations having larger surface areas, substantially removed numerous HMs (Sturm et al. 2016; Rasaki et al. 2019; Cheng et al. 2012). Geopolymers show excellent adsorbing capabilities due to their larger surface area, high porosity, and strong adhesive forces because of mesopores that tightly bind HMs. Geopolymeric materials have proven a substantial HMs stabilization potential in metal-polluted soils owing to their strong adsorbing as well binding capabilities (Rasaki et al. 2019).

6.2.1 Cement-Based Stabilizing Agents

Cement is a strong binding agent to stabilize HMs in contaminated soils and significantly decreases their leaching as well as bioavailability due to the presence of Ca silicates, sulfates, aluminates, and alumino-ferrite minerals. Immobilization with cement is an interesting way of remediating metal-polluted soils, but its efficiency depends on its composition, chemical structure, temperature, particle size, etc. (Wuana and Okieimen 2011; Mahar et al. 2015).

The applications of Portland cement were being effective in reducing Pb and Cd concentrations in the leachates due to a reduction in their bioavailability (Voglar and Leštan 2011; Mahar et al. 2015). Likewise, magnesium phosphate cement has also outstanding features such as quick setting, strong binder, and excellent stabilization potential (Xu et al. 2015; Wang and Dai 2017; Wang et al. 2018). Magnesium phosphate cement is also used for remediation of Pb polluted soils by forming pyromorphite and lead phosphate complexes (Wang et al. 2018; Debela et al. 2013).

6.2.2 Clay Minerals

Remediation of metal contaminated soils with clay amendments is an innovative idea due to their several advantages such as universal availability, wide acceptability at commercial scale, high efficiency, and economic viability. Bentonite, attapulgite, and sepiolite are known to be the most common clay amendments recommended for remediating agricultural soils contaminated with HMs (Yi et al. 2017; Yuan et al. 2013). Bentonite mainly composed of montmorillonite, useful clay for reducing the bioavailability of HMs in the soil via providing adsorption sites (Yi et al. 2017; Sun et al. 2015). Likewise, attapulgite (palygorskite) is also effectively capable of immobilizing different HMs from contaminated sites. Attapulgite shows the quick and strong fixation of HMs in paddy soils by increasing its pH, thereby decreasing their leaching effect (Yi et al. 2017; Sheikhhosseini et al. 2013). Sepiolite proved to be the highest sorption capabilities of HMs immobilization due to its divine structure made up of one sheet of octahedral magnesium oxide or hydroxide between two sheets of tetrahedral silica (Yi et al. 2017). Previously, the additions of sepiolite not only stabilized various HMs but also reduced their uptake in different plants such as spinach, rice, alfalfa, and ryegrass (Sun et al. 2016; Liang et al. 2016; Wu et al. 2016; Abad-valle et al. 2016). Similarly, bentonite, palygorskite, and attapulgite effectively immobilized different HMs through immobilizing them, which resultantly restricted their uptake (Yi et al. 2017; Sun et al. 2015; Argiri et al. 2013; Houben et al. 2012; Zotiadis et al. 2012; Zhang et al. 2011). Correspondingly, biochar mixed with zeolite effectively reduced Ni mobility and its uptake in maize, sunflower, and red clover and improved the nutrient and health status of these plant species (Shahbaz et al. 2018a, b).

Although these clay minerals have the great immobilizing potential for remediation purposes, however, prior knowledge about their effective dose remarkably influenced the progress of remediation strategy.

6.3 Immobilization Through Inorganic Amendments

The stabilization of HMs with the assistance of cost-effective, energy-efficient, natural, and synthetic inorganic amendments is remarkable. Several inorganic stabilizers used for HMs immobilization are phosphates, calcium, silicon compounds, and mineral amendments (Peng et al. 2018).

6.3.1 Calcium Compounds

The particular calcium forms used for the remediation of HMs contaminated soils are gypsum and liming substances. The ability of gypsum for metal adsorption has already been extensively available in the literature. Gypsum was used for reducing the concentrations of different HMs (Cu, Pb, Cr, and Cd) in polluted soils due to the presence of excessive sulfide formation (Tsunematsu et al. 2012; Vink et al. 2010). Gypsum acts as a soil conditioner, provides nutrients, and enhances soil productivity and soil physical properties as well as improved sulfur and calcium uptake in plants. The HMs contaminated soils have poor structure as well as less aggregation, prone to erosion and infertility but the addition of Ca in gypsum form promote the development of soil structure (Smith 2011). The long-term effects of gypsum application to soil improved the Ca and P status in the soil, which strengthens the soil solution ionic system. On the other side, calcium addition in the form of lime ameliorated the acidity of soil and was effective for HMs immobilization. Lime applications in contaminated soil significantly reduce the DTPA extractable Zn, Fe, and Cu. Interestingly, lime materials induce high pH, which initiates hydrolysis reactions and produces metal precipitates. Interestingly, the lime effect in the soil causes nutrient bioavailability in soil. Although liming application induces better soil environment by fixing HMs, however, too much liming effect can inhibit the availability of beneficial micronutrients (Fe, Zn, and Mn) to plants and soil (Lwin et al. 2018).

6.3.2 Phosphorus Compounds

Both natural and synthetic phosphorous-based amendments effectively remediate metal-polluted soils (Lwin et al. 2018). The eminent nature of phosphorous compounds can be hydrophilic like diammonium phosphate and hydrophobic such as phosphate rocks for stabilizing HMs in soil (Nejad et al. 2018; Lwin et al. 2018; Mahar et al. 2015). The use of P-additives stabilized HMs by forming insoluble metal precipitates or by binding them through adsorption (Lwin et al. 2018; Bolan et al. 2014). Likewise, hydroxypyromorphite and chloropyromorphite as P-amendments were also useful in reducing Pb bioavailability in contaminated soils (Chen and Li 2010; Nejad et al. 2018). Although phosphate-based amendments are adequate for remediating the metal-polluted soils, however, the interaction of phosphate minerals with targeted pollutants is of a great concern due to their remobilization risk in the soil (Mahar et al. 2015; Nejad et al. 2018; Lwin et al. 2018). For example, the additions of single and triple superphosphates (SSP and TSP) enhance the Cd concentrations in soil (Lwin et al. 2018).

6.3.3 Iron Compounds

Immobilization of HMs in contaminated soils with iron-based materials has already been well examined in recent years. It was realized that the zero-valent iron (ZVI) and Fe^{II} tend to remove the HMs concentrations from the environment. Zero-valent iron binds HMs and other pollutants due to its strong oxidizing–reducing nature, which helps in reducing HMs bioavailability. For example, ferrous salt applications such as ferrous sulfate heptahydrate not only produced Cr precipitates but also transformed it into less toxic and more stable form (Hashim et al. 2011).

6.4 Metal Oxides

Metal oxides play an active role in controlling the chemistry of HMs in the soil. Several metal oxides such as Fe, Al, Ti, Ce, and Mn immobilize HMs in the soil due to large surface area, which makes them suitable for immobilization (Hua et al. 2012). These metal oxides bind HMs through co-precipitation, sorption, and forming metal complexes (Bolan et al. 2014). For example, the oxides of Mn such as todorokite, hausmannite, and cryptomelane have been previously used for reducing Pb bioavailability (Bolan et al. 2014). Likewise, iron oxides such as hematite, magnetite, and maghemite significantly reduced the concentrations of exchangeable Zn fractions as well as significantly decreased the DTPA-extractable Zn, Cu, and Cd.

6.5 Industrial By-products

The sustainable use of industrial waste products such as fly ash, red mud, iron and steel slag, sludge, and paper waste discharged from different industries has been well documented in recent years (Dang et al. 2019). Moreover, handling several hundred tons of wastes of such by-products required additional disposal sites; therefore, it is recommended to use them in a sustainable way rather deteriorating environmental quality (Lwin et al. 2018; Sengupta and Tarar 2014). These by-product additives are not only efficient in reducing HMs pollution but also cost effective (Ishak and Abdullah 2014). Previously, the applications of fly ash, slag, and red mud have been reported to reduce the bioavailability of several HMs by enhancing soil pH or by binding them via chemisorption onto the surfaces of oxides and hydroxides of Al and Fe (Lwin et al. 2018; Ning et al. 2016; Valerie 2015). Interestingly, both organic and inorganic industrial waste products also contain other essential nutrients such as P, S, K, B, Mn, Mo, Si, and Fe, which improved plant growth and soil enzymes (Lwin et al. 2018; Teresa and Valentina 2012). Soil structure, CEC, bulk density, water-holding capacity, and aggregate stability are known to be improved after the applications of these industrial waste products in HMs contaminated soils (Stanojković-Sebić et al. 2014). Besides these positive aspects of applying industrial waste products for remediation purposes, the presence of toxic hazardous substances in them should be considered carefully (Klebercz et al. 2012; Lwin et al. 2018).

6.6 Immobilization as a Group Technology

This section provides the recent trends in the field of immobilization coupled with other remediation techniques.

6.6.1 Immobilization Coupled with Phytomanagement

The remediation of HMs contaminated soils with the co-application of immobilization and phytoremediation was considered contradictory because the former reduced the bioavailability while the latter required higher HMs solubilization for plant uptake. Immobilizing additives not only reduced the excessive HMs leaching from the contaminated sites but also supported plant establishment by their limited uptake (Muhammad et al. 2012; Iqbal et al. 2012). For example, during the enhanced phytoextraction, the co-application of elemental sulfur with red mud and gravel sludge not only reduced Cd and Zn leaching but also enhanced their uptake by *S. smithiana* from Pb-Zn smelter contaminated soil (Iqbal et al. 2012). Like enhanced phytoextraction, aided phytostabilization coupled with organic and inorganic amendments has already been reported for the management of soils very highly contaminated with an individual (Lu et al. 2014) or multi-HMs such as military shooting ranges (Lago-Vila et al. 2019; Radziemska et al. 2019, 2020) and mining soils. However, this technique does not work well for such HMs contaminated soils with low pH and high salt contents (Gascó et al. 2019; Wang et al. 2012).

6.6.2 Immobilization Coupled with Microorganisms

The combined applications of amendments with (micro)organisms also attained the interest of researchers worldwide. Bioimmobilization remediation method reduces HMs bioavailability by bioprecipitation, bioaccumulation, and biosorption. For instance, phosphorous solubilizing microorganisms reduced Pb mobility by forming insoluble Pb–phosphate complexes by releasing phosphorus from its sources (Jalili et al. 2020; Khan et al. 2020; Nejad et al. 2018; Mahar et al. 2015). Likewise, the applications of arbuscular mycorrhizal fungi (AMF) with lignin-derived biochar in Pb-acid batteries contaminated soils significantly reduced Pb uptake and its translocation to barley grain. The mechanism associated with this reduction in Pb transport was due to restricted Pb accumulation in root cells, its adsorption with glomalin as well as immobilization in mycelia of AMF (Khan et al. 2020). Also, these microorganisms improved soil health owing to the presence of porous structure which served as a habitat for them as well as through solubilizing essential nutrients from the soil or organic matrix (Peng et al. 2018).

7 The Mechanism Involved in the Immobilization of HMs

Different mechanisms governed the chemistry and stabilization of HMs upon the addition of various organic and inorganic amendments in contaminated soils. Silicon dioxide plays a vital role in HMs immobilization, which strongly binds HMs onto the surfaces of SiO_2, thereby reducing their mobility (Ricou-Hoeffer et al. 2000). The presence of SiO_2 in both organic and inorganic stabilizing agents increased the formation of metal oxides as well as metal silicides. Likewise, the presence of Ca and Si complexes such as calcium silicates are known to have higher efficient adsorption capacity, which enhanced HMs immobilization by forming insoluble surface precipitates. In general, the presence of clay minerals, organic matter, the oxides and hydroxides of Fe and Mn, amorphous aluminosilicates, and calcium carbonates strongly influences the absorption of HMs in the soil. These substances provide phenolic, amino, carboxylic, and alcoholic functional groups in the soil, which strongly bind HMs (Nejad et al. 2018).

8 Recent Advances in Managing HMs Contaminated Soils Through Immobilization

This segment provides the necessary information about recent advancements in the immobilization remediation technique.

8.1 Modifications of Organic Additives

Recent advancements in research have proven that biochar has immense potential as an immobilizing amendment owing to comparatively its low cost and the availability of plenty of raw feedstock resources. Hence, biochar has developed as a practical alternative in remediating and managing different contaminants especially HMs. The physical and chemical characteristics of biochar depend upon several factors such as the type of feedstock as well as pyrolysis conditions such as temperature, residence time, and heating rate (Zhou et al. 2013). The modification and functionalization of biochar through different processes aimed to enhance its surface or interlayer spaces, pore size, molecular weight, CEC, and the type and amount of functional groups, which enhanced the HMs adsorption capacities of engineered biochars as compared to their native biochars (Nazari et al. 2019). Rajapaksha et al. (2016) and references therein comprehensively provided the essential information regarding different modification methods and associated mechanisms for the management of numerous contaminants present in the environment. The applications of magnetically modified poultry litter biochar in multi-HMs contaminated paddy soil collected from near Pb-Zn mine area remarkably increased plant growth while decreased HMs leaching (Lü et al. 2018). Similarly, the additions of modified coconut shell-derived biochar in multi-HMs contaminated soils not only resulted in the better immobilization of HMs but also increased soil biological properties as compared to native coconut shell biochar (Liu et al. 2018). The applications of modified biochar-chitosan-clay nano-amalgams and their applications in the simultaneous stabilization of HMs from the acid mine soil and water have been tested. Results revealed that HMs were efficiently immobilized by forming nanocomposites due to the presence of $-NH_2$ groups (Arabyarmohammadi et al. 2018). Biochar chitosan and hematite composites also resulted in the efficient transformation of Cr^{VI} in contaminated soils. The additions of biochar-chitosan and biochar-hematite resulted in the Cr reduction up to 46% and 38% in contrast to biochar treatment (Zibaei et al. 2020). The application of biochar supported carboxymethyl cellulose nanoscale iron sulfide (FeS) composite has been evaluated for Cr stabilization in the soil. The additions of composite in the soil increased Cr immobilization up to 94% while decreased its concentration up to 95% in $CaCl_2$ extract (Lyu et al. 2018).

8.2 Clay Modifications

Previously, the applications of clay minerals with other organic and inorganic amendments reported to efficiently reduce the bioavailability, mobility, and toxicity of HMs from the ecosystem (Sarkar et al. 2019; Yadav et al. 2019). Recently, the modifications of geomaterials through different methods have been reviewed in detail by Han et al. (2019), Sarkar et al. (2019), and citations therein for the removal of different HMs from the environment (Han et al. 2019; Sarkar et al. 2019). The attapulgite was modified with zero-valent iron and used for the immobilization of different HMs in the soil. Results suggested that the stabilizing additives remarkably reduced the extractable concentrations of HMs in the soil and promoted seed germination, root development as well as the activities of antioxidants by diminishing the contents of ROS (Xu et al. 2019). Sepiolite additions with phosphate fertilizer, limestone, and biochar resulted in the reduced bioavailability of HMs, which revealed its compatibility with other amendments. The successful immobilization of Cd in contaminated paddy soils was observed in sepiolite infused with limestone treatment (Yi et al. 2017). Likewise, the combined applications of bentonite with sodium, sepiolite with bentonite, limestone, and phosphate increased the immobilization of different HMs in the soil (Sun et al. 2016; Montenegro et al. 2015; Zhou et al. 2013; Liang et al. 2011). Although these clay composites are efficient in alleviating HMs toxicity from the soil, however, prior knowledge about their proper dose, the microbial status of the soil, and physicochemical features of the multi−contaminated matrix should be accounted for their better performance at the field scale.

9 Estimating the Efficacy of Immobilizing Agents Through Long-Term Monitoring at Field Scale

The success of immobilization remediation strategies depends upon numerous factors such as the extent and nature of soil contamination, soil pH, the presence of other mineral ions, land-use practices, as well as the economic feasibility of the remediation process. Therefore, sufficient knowledge about soil type taken into consideration before selecting a remediation plan for a specific site (Puschenreiter 2008; Sun et al. 2016). Also, immobilizing agents with strong HMs binding capabilities and long-term durability proven to be effective in immobilization, and the decision-makers allow to use them in field experiments (Xu et al. 2018; Sun et al. 2016). The significant valuable facts about the possibilities of ex-situ immobilization acquired from long-term field experiments in contrast to short-term pot and model experiments (Sun et al. 2016). The Cd contaminated soil was amended with different rates of sepiolite (0.1, 0.5, 1% w/w) to assess its toxicity to rice plants in a field experiment. The significant reduction of up to 49% as well as 50% of Cd concentrations in rice grain was observed during the first- and second-year experiments, respectively. The sepiolite additions for 2 successive years in a field restricted Cd translocation toward rice grain up to 75%, which promotes safe rice production for human consumption (Chen et al. 2020). The co-applications of biochar, as well as sepiolite, increased the growth, biomass, and grain quality of maize when grown in farmlands contaminated with HMs due to mining activity. This improvement in the growth of maize attributed to the reduction in Cd bioavailability, thereby restricting its contents in plants and grain (Zhan et al. 2019). The effectiveness of limestone and sepiolite amendments has been investigated to reduce exchangeable Pb and Cd in a 3-year in situ soil experiment. It was observed that the influences of limestone and sepiolite on exchangeable Cd remain relatively persistent, while a gradual decrease in exchangeable Pb was observed with time (Wu et al. 2016). Likewise, the long-term influence of sepiolite additives in Cd contaminated paddy soil was observed in a 2-year field experiment. A remarkable stabilization effect of sepiolite in Cd contaminated soil was observed, which also persisted during the next year. Results revealed that exchangeable as well as HCl–Cd labile fractions remain reduced, which showed the long-term stabilizing effect of sepiolite (Liang et al. 2016). The changes in the physicochemical properties of the soil due to weathering processes increase the risk of HMs remobilization. Likewise, the breakdown of organic matter also resulted in the release of organic-bound HMs, which increases the risk of their leaching especially from the soils with low pH values. Therefore, the long-term continuous monitoring of such sites is recommended to control other environmental hazards.

10 Environmental Concerns Associated with HMs After Their Fixation

The efficacy and potential dissolution of HMs from the amended soils could be assessed through different physicochemical and biological methods.

10.1 Biological Assessment Method

The phytotoxic assessment method is the most reliable, efficient, and widely acceptable method, performed to test the effectiveness of stabilizing agents. The improved plant height and biomass production was observed in plants grown on amended HMs contaminated soils (Lu et al. 2014). The reduced HMs uptake by the plants due to a reduction in bioavailability after their fixation on to the surfaces of immobilizing agents is another additional parameter of the phytotoxic assessment method. Presently, this trend is gaining the attention of scientists for the management of multicontaminated soils such as military firing range (Lago-Vila et al. 2019; Radziemska et al. 2019, 2020) as well as mining soils (Gascó et al. 2019). Moreover, soil (micro)organisms can also be used as indicators to assess the contamination level before and after the additions of immobilizing agents (Huang et al. 2020; Khan et al. 2020; Tu et al. 2020).

10.2 Physiochemical Assessment

Presently, different microdetection and fraction schemes were used to determine HMs fractions by taking soil water, DTPA, $Ca(NO_3)_2$ (Shahbaz et al. 2018a) as well as HCl extractants (Zeng et al. 2018). Likewise, the DGT (diffusive gradient in thin film) technique is also being used to determine HMs flux released by diffusion from the solid phase to soil solution (Muhammad et al. 2012). Additionally, single extractions through toxicity characteristic leaching procedure (TCLP), as well as sequential extraction methods, were also used to determine the behavior and speciation of HMs through a particular extractant (Guo et al. 2006). Among these methods, the sequential extraction developed by Tessier et al. (1979) has been widely used in soil experiments.

10.3 Release Assessment Approaches

The release of HMs from the immobilizing additives can never be avoided due to change in the soil properties as well as weathering processes. The mechanism associated with the release of HMs after fixation can be assessed by two empirical models.

10.3.1 Bulk Diffusion Model

This model describes the release of HMs from the cement-based stabilization matrix in HMs contaminated soils. The bulk diffusion is the main driving force that enhanced HMs release from monolith due to the breakdown of the outer shell of the stabilizing agent. The bulk diffusion model can be calculated according to the below equation of Fickian diffusion (Baker and Bishop 1997):

$$\frac{\partial C}{\partial t} = D_{\mathrm{e}} \frac{\partial^2}{\partial x^2}.$$

In the above expression, D_{e} represents the effective diffusion coefficient ($\mathrm{cm}^2\ \mathrm{S}^{-1}$) associated with the porosity and tortuosity; C expresses HMs ($\mathrm{g\ cm}^{-3}$) concentrations; t expresses time (s), and x expresses the distance (cm). However, this model was considered failing in predicting the long-term leaching of HMs from contaminated soils under acidic conditions (Guo et al. 2006).

10.3.2 Shrinking Unreacted Core (SUC) Model

The SUC model well explains the release and dissolution mechanisms of HMs from the stabilizing agent under acidic conditions. This release was attributed to the disintegration of outer surfaces of immobilizing agents, which caused leaching of contaminants. The expression of potential release factor (PRF) evolved from the SUC model was proposed as a means of determining HMs mobilizing efficacy from the fixed surface (Baker and Bishop 1997).

$$\mathrm{PRF} = \frac{\sqrt{2D_{\mathrm{e,s}}\ f^2{}_{\mathrm{mo}}\ C^2{}_{\mathrm{m}}}}{\beta_{\mathrm{c.}}}$$

In this equation, PRF expresses potential release factor; $D_{\mathrm{e,s}}$ the coefficient of effective diffusion (under acidic environments); f_{mo} expresses dimensionless leachable fractions; C_{m} denotes the concentrations of solid pollutant ($\mathrm{mol\ cm}^{-3}$), and β_{c} acid neutralization capacity ($\mathrm{kmol\ eq\ m}^{-3}$).

11 Conclusion and Way Forward

The accumulation of HMs in soils due to their release from different anthropogenic sources significantly reduced productive agricultural lands for sustainable food production. Such poorly vegetated HMs contaminated soils are considered to be the continuous release of pollution via leaching or runoff affecting the other natural resources. Therefore, the remediation of HMs contaminated soils is necessary to prevent other additional hazards associated with them. The primary objective of remediation work is to reduce the risk of human exposure, limit the level of contamination, and prevent further deterioration of other environmental resources. In the past, different destructive remediation methods such as soil washing, soil excavation, soil replacement, vitrifying, and electrokinetic methods have been practiced for the management of HMs contaminated soil. Although, these methods are effective in limiting the concentrations of HMs from the soil, however, damaged soil fertility and organic matter alter physicochemical properties of the soil and produced residual wastes that required additional treatment cost. The success of the remediation technique entirely depends upon the speciation of HMs, soil factors as well as the level and depth of contamination. Therefore, the standards for the selection of suitable remediation methods are (1) long-term efficiency and durability of stabilizing agents in achieving the remediation objectives, (2) efficient reduction in the volume of HMs, (3) effective in promoting plant establishment via reducing HMs toxicity, and (4) cost effective. In situ stabilization of HMs in soils by using different organic, inorganic, and other stabilizing agents has gained the attention of scientists worldwide due to its economic feasibility and high efficiency. The additions of amendments in single or co-contaminated HMs not only reduced the mobility, toxicity as well as their labile fractions but also supported plant establishment with maximum production. Among different HMs immobilizing agents, the significant results were acquired in HMs contaminated soils (field and pot trials) when amended with clay minerals, rock phosphate, biochar, calcium hydroxide, phosphates, and hydroxylapatite. The dissolution of HMs due to climatic factors from stabilizing agents may result in their leaching. Therefore, the long-term monitoring of HMs contaminated sites is necessary to control such environmental hazards.

References

Abad-Valle P, Álvarez-Ayuso E, Murciego A, Pellitero E (2016) Assessment of the use of sepiolite amendment to restore heavy metal polluted mine soil. Geoderma 280:57–66

Adeyeye EI (1994) Determination of trace heavy metals in Illisha africana fish and in associated water and soil sediments from some fish ponds. Int J Environ Stud 45:231–238

Ahmad M, Lee SS, Yang JE, Ro HM, Lee YH, Ok YS (2012) Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotoxicol Environ Saf 79:225–231

Akcil A, Erust C, Ozdemiroglu S, Fonti V, Beolchini F (2015) A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J Clean Prod 86:24–36

Alamgir M, Kibria MG, Islam M (2011) Effects of farm yard manure on cadmium and lead accumulation in Amaranth (Amaranthus oleracea L.). J Soil Sci Environ Manag 2(8):237–240

Alshawabkeh AN (2009) Electrokinetic soil remediation: challenges and opportunities. Sep Sci Technol 44(10):2171–2187

Arabyarmohammadi H, Darban AK, Abdollahy M, Yong R, Ayati B, Zirakjou A, van der Zee SE (2018) Utilization of a novel chitosan/clay/biochar nanobiocomposite for immobilization of heavy metals in acid soil environment. J Polym Environ 26(5):2107–2119

Argiri A, Ioannou Z, Dimirkou A (2013) Impact of new soil amendments on the uptake of lead by crops. Commun Soil Sci Plant 44:566–573

Atafar Z, Mesdaghinia A, Nouri J, Homaee M, Yunesian M, Ahmadimoghaddam M, Mahvi AH (2010) Effect of fertilizer application on soil heavy metal concentration. Environ Monit Assess 160(1–4):83

Baker PG, Bishop PL (1997) Prediction of metal leaching rates from solidified/stabilized wastes using the shrinking unreacted core leaching procedure. J Hazard Mater 52(2–3):311–333

Beesley L, Moreno-Jiménez E, Gomez-Eyles JL (2010) Effects of biochar and green waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ Pollut 158:2282–2287

Beiyuan J, Awad YM, Beckers F, Tsang DC, Ok YS, Rinklebe J (2017) Mobility and phytoavailability of As and Pb in a contaminated soil using pine sawdust biochar under systematic change of redox conditions. Chemosphere 178:110–118

Beolchini F, Fonti V, Rocchetti L, Saraceni G, Pietrangeli B, Dell'Anno A (2013) Chemical and biological strategies for the mobilisation of metals/semi-metals in contaminated dredged sediments: experimental analysis and environmental impact assessment. Chem Ecol 29 (5):415–426

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soils—to mobilize or to immobilize? J Hazard Mater 266:141–166

Cai LM, Wang QS, Wen HH, Luo J, Wang S (2019) Heavy metals in agricultural soils from a typical township in Guangdong Province, China: occurrences and spatial distribution. Ecotoxicol Environ Saf 168:184–191

Cantrell KB, Hunt PG, Uchimiya M, Novak JM, Ro KS (2012) Impact of pyrolysis temperature and manure source on physicochemical characteristics of biochar. Bioresour Technol 107:419–428

Cao XD, Ma L, Liang Y, Gao B, Harris W (2011) Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ Sci Technol 45:4884–4889

Chaoua S, Boussaa S, El Gharmali A, Boumezzough A (2019) Impact of irrigation with wastewater on accumulation of heavy metals in soil and crops in the region of Marrakech in Morocco. J Saudi Soc Agric Sci 18(4):429–436

Chrastný V, Komárek M, Hájek T (2010) Lead contamination of an agricultural soil in the vicinity of a shooting range. Environ Monit Assess 162:37-46.

Chen YH, Li FA (2010) Kinetic study on removal of copper (II) using goethite and hematite nano-photocatalysts. J Colloid Interface Sci 347(2):277–281

Chen D, Ye X, Zhang Q, Xiao W, Ni Z, Yang L, Huang M (2020) The effect of sepiolite application on rice Cd uptake–a two-year field study in southern China. J Environ Manag 254:109788

Cheng TW, Lee ML, Ko MS, Ueng TH, Yang SF (2012) The heavy metal adsorption characteristics on metakaolin-based geopolymer. Appl Clay Sci 56:90–96

Cheraghi M, Lorestani B, Merrikhpour H (2012) Investigation of the effects of phosphate fertilizer application on the heavy metal content in agricultural soils with different cultivation patterns. Biol Trace Elem Res 145(1):87–92

Chiang YW, Santos RM, Ghyselbrecht K, Cappuyns V, Martens JA, Swennen R, Meesschaert B (2012) Strategic selection of an optimal sorbent mixture for in-situ remediation of heavy metal contaminated sediments: framework and case study. J Environ Manag 105:1–11

Dang VM, Joseph S, Van HT, Mai TLA, Duong TMH, Weldon S, Taherymoosavi S (2019) Immobilization of heavy metals in contaminated soil after mining activity by using biochar and other industrial by-products: the significant role of minerals on the biochar surfaces. Environ Technol 40(24):3200–3215

Debela F, Arocena JM, Thring RW, Whitcombe T (2013) Organic acids inhibit the formation of pyromorphite and Zn-phosphate in phosphorous amended Pb-and Zn-contaminated soil. J Environ Manag 116:156–162

Ettler V (2016) Soil contamination near non-ferrous metal smelters: a review. Appl Geochem 64:56–74

Fayiga AO (2019) Remediation of inorganic and organic contaminants in military ranges. Environ Chem 81–91

Finzgar N, Tlustos P, Lestan D (2007) Relationship of soil properties to fractionation, bioavailability and mobility of lead and zinc in soil. Plant Soil Environ 53(5):225

Fonti V, Dell'Anno A, Beolchini F (2013) Influence of biogeochemical interactions on metal bioleaching performance in contaminated marine sediment. Water Res 47(14):5139–5152

Gascó G, Álvarez ML, Paz-Ferreiro J, Méndez A (2019) Combining phytoextraction by Brassica napus and biochar amendment for the remediation of a mining soil in Riotinto (Spain). Chemosphere 231:562–570

González-Guerrero M, Melville LH, Ferrol N, Lott JN, Azcon-Aguilar C, Peterson RL (2008) Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus Glomus intraradices. Can J Microbiol 54(2):103–110

Guo G, Zhou Q, Ma LQ (2006) Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: a review. Environ Monit Assess 116:513–528

Han H, Rafiq MK, Zhou T, Xu R, Mašek O, Li X (2019) A critical review of clay-based composites with enhanced adsorption performance for metal and organic pollutants. J Hazard Mater 369:780–796

Hashim MA, Mukhopadhyay S, Sahu JN, Sengupta B (2011) Remediation technologies for heavy metal contaminated groundwater. J Environ Manag 92(10):2355–2388

Hembrom S, Singh B, Gupta SK, Nema AK (2020) A comprehensive evaluation of heavy metal contamination in foodstuff and associated human health risk: a global perspective. In: Contemporary environmental issues and challenges in era of climate change, pp 33–63

Houben D, Pircar J, Sonnet P (2012) Heavy metal immobilization by cost-effective amendments in a contaminated soil: effects on metal leaching and phytoavailability. J Geochem Explor 123:87–94

Hua M, Zhang S, Pan B, Zhang W, Lv L, Zhang Q (2012) Heavy metal removal from water/wastewater by nanosized metal oxides: a review. J Hazard Mater 211:317–331

Huang D, Liu L, Zeng G, Xu P, Huang C, Deng L, Wang R, Wan J (2017) The effects of rice straw biochar on indigenous microbial community and enzymes activity in heavy metal-contaminated sediment. Chemosphere 174:545–553

Huang Y, Chen Q, Deng M, Japenga J, Li T, Yang X, He Z (2018) Heavy metal pollution and health risk assessment of agricultural soils in a typical peri-urban area in Southeast China. J Environ Manag 207:159–168

Huang C, Wang W, Yue S, Adeel M, Qiao Y (2020) Role of biochar and Eisenia fetida on metal bioavailability and biochar effects on earthworm fitness. Environ Pollut 114586

Igalavithana AD, Lee SE, Lee YH, Tsang DC, Rinklebe J, Kwon EE, Ok YS (2017a) Heavy metal immobilization and microbial community abundance by vegetable waste and pine cone biochar of agricultural soils. Chemosphere 174:593–603

Igalavithana AD, Park J, Ryu C, Lee YH, Hashimoto Y, Huang L, Lee SS (2017b) Slow pyrolyzed biochars from crop residues for soil metal(loid) immobilization and microbial community abundance in contaminated agricultural soils. Chemosphere 177:157–166

Iqbal M, Puschenreiter M, Oburger E, Santner J, Wenzel WW (2012) Sulfur-aided phytoextraction of Cd and Zn by Salix smithiana combined with in situ metal immobilization by gravel sludge and red mud. Environ Pollut 170:222–231

Ishak CF, Abdullah R (2014) In-situ immobilization of selected heavy metals in soils using agricultural wastes and industrial by-products. In: Proceedings of MARCO-FFTC joint Int. seminar on management and remediation technologies of rural soils contaminated, pp 22–26

Jalili B, Sadegh-Zadeh F, Jabari-Giashi M, Emadi M (2020) Lead bioimmobilization in contaminated mine soil by Aspergillus Niger SANRU. J Hazard Mater 393:122375

Joseph S, Husson O, Graber ER, van Zwieten L, Taherymoosavi S, Thomas T (2015) The electrochemical properties of biochars and how they affect soil redox properties and processes. Agronomy 5(3):322–340

Johnsen IV, Mariussen E, Voie Ø (2019) Assessment of intake of copper and lead by sheep grazing on a shooting range for small arms: a case study. Environ Sci Pollut Res 26:7337–7346.

Juris B, Karina S, Ikrema H, Reinis J, Sandris L (2015) Removal of heavy metals from contaminated soils by electrokinetic remediation

Kammann CI, Schmidt HP, Messerschmidt N, Linsel S, Steffens D, Müller C, Koyro HW, Conte P, Joseph S (2015) Plant growth improvement mediated by nitrate capture in co-composted biochar. Sci Rep 5:11080

Kelebemang R, Dinake P, Sehube N, Daniel B, Totolo O, Laetsang M (2017) Speciation and mobility of lead in shooting range soils. Chem Spec Bioavailab 29:143–152

Khan W, Ramzani PMA, Anjum S, Abbas F, Iqbal M, Yasar A, Ihsan MZ, Khan SA (2017) Potential of miscanthus biochar to improve sandy soil health, in situ nickel immobilization in soil and nutritional quality of spinach. Chemosphere 185:1144–1156

Khan MA, Ramzani PMA, Zubair M, Rasool B, Khan MK, Ahmed A, Iqbal M (2020) Associative effects of lignin-derived biochar and arbuscular mycorrhizal fungi applied to soil polluted from Pb-acid batteries effluents on barley grain safety. Sci Total Environ 710:136294

Kierczak J, Potysz A, Pietranik A, Tyszka R, Modelska M, Néel C, Mihaljevič M (2013) Environmental impact of the historical Cu smelting in the Rudawy Janowickie Mountains (South-Western Poland). J Geochem Explor 124:183–194

Klebercz O, Mayes WM, Anton AD, Feigl V, Jarvis AP, Gruiz K (2012) Ecotoxicity of fluvial sediments downstream of the Ajka red mud spill, Hungary. J Environ Monit 14:2063–2071

Komárek M, Vaněk A, Ettler V (2013) Chemical stabilization of metals and arsenic in contaminated soils using oxides–a review. Environ Pollut 172:9–22

Kong L, Gao Y, Zhou Q, Zhao X, Sun Z (2018) Biochar accelerates PAHs biodegradation in petroleum-polluted soil by biostimulation strategy. J. Hazard. Mater 343, 276-284.

Kumar V, Singh J, Kumar P (2019) Heavy metals accumulation in crop plants: sources, response mechanisms, stress tolerance and their effects. In: Contaminants in agriculture and environment: health risks and remediation 1, p 38

Kuppusamy S, Palanisami T, Megharaj M, Venkateswarlu K, Naidu R (2016) In-situ remediation approaches for the management of contaminated sites: a comprehensive overview. Rev Environ Contam Toxicol 236:1–115

Lago-Vila M, Rodríguez-Seijo A, Vega FA, Arenas-Lago D (2019) Phytotoxicity assays with hydroxyapatite nanoparticles lead the way to recover firing range soils. Sci Total Environ 690:1151–1161

Li Z, Zhang T (2013) Vitrification. The International Information Center for Geotechnical Engineers

Li P, Lin C, Cheng H, Duan X, Lei K (2015) Contamination and health risks of soil heavy metals around a lead/zinc smelter in southwestern China. Ecotoxicol Environ Saf 113:391–399

Liang X, Xu Y, Wang L, Sun G, Qin X, Sun Y (2011) In-situ immobilization of cadmium and lead in a contaminated agricultural field by adding natural clays combined with phosphate fertilizer. Acta Sci Circum (in Chinese) 31:1011–1018

Liang X, Xu Y, Xu Y, Wang P, Wang L, Sun Y, Huang R (2016) Two-year stability of immobilization effect of sepiolite on Cd contaminants in paddy soil. Environ Sci Pollut Res 23 (13):12922–12931

Lin Y, Munroe P, Joseph S, Henderson R, Ziolkowski A (2012) Water extractable organic carbon in untreated and chemical treated bio-chars. Chemosphere 87:151–157

Liu H, Xu F, Xie Y, Wang C, Zhang A, Li L, Xu H (2018) Effect of modified coconut shell biochar on availability of heavy metals and biochemical characteristics of soil in multiple heavy metals contaminated soil. Sci Total Environ 645:702–709

Lu H, Zhang W, Yang Y, Huang X, Wang S, Qiu R (2012). Relative distribution of Pb2+ sorption mechanisms by sludge-derived biochar. Water Res 46(3):854–862

Lu H, Li Z, Fu S, Méndez A, Gascó G, Paz-Ferreiro J (2014) Can biochar and phytoextractors be jointly used for cadmium remediation? PLoS One 9(4)

Lü J, Jiao WB, Qiu HY, Chen B, Huang XX, Kang B (2018) Origin and spatial distribution of heavy metals and carcinogenic risk assessment in mining areas at You'xi county Southeast China. Geoderma 310:99–106

Lwin CS, Seo BH, Kim HU, Owens G, Kim KR (2018) Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality—a critical review. Soil Sci Plant Nutr 64(2):156–167

Lyu H, Zhao H, Tang J, Gong Y, Huang Y, Wu Q, Gao B (2018) Immobilization of hexavalent chromium in contaminated soils using biochar supported nanoscale iron sulfide composite. Chemosphere 194:360–369

Mariussen E, Heier LS, Teien HC, Pettersen MN, Holth TF, Salbu B, Rosseland BO (2017) Accumulation of lead (Pb) in brown trout (Salmo trutta) from a lake downstream a former shooting range. Ecotoxicol Environ Saf 135:327–336

Mahar A, Ping W, Ronghua LI, Zhang Z (2015) Immobilization of lead and cadmium in contaminated soil using amendments: a review. Pedosphere 25(4):555–568

Marrugo-Negrete J, Pinedo-Hernández J, Díez S (2017) Assessment of heavy metal pollution, spatial distribution and origin in agricultural soils along the Sinú River Basin, Colombia. Environ Res 154:380–388

Montenegro AC, Ferreyroa GV, Parolo ME, Tudino MB, Lavado RS, Molina FV (2015) Copper speciation in soil: time evolution and effect of clay amendment. Water Air Soil Pollut 226:1–10

Muddassir M, Noor MA, Ahmed A, Aldosari F, Waqas MA, Zia MA, Jalip MW (2019) Awareness and adoption level of fish farmers regarding recommended fish farming practices in Hafizabad, Pakistan. J Saudi Soc Agric Sci 18(1):41–48

Muhammad I, Puschenreiter M, Wenzel WW (2012) Cadmium and Zn availability as affected by pH manipulation and its assessment by soil extraction, DGT and indicator plants. Sci Total Environ 416:490–500

Nacke H, Gonçalves AC, Schwantes D, Nava IA, Strey L, Coelho GF (2013) Availability of heavy metals (Cd, Pb, and Cr) in agriculture from commercial fertilizers. Arch Environ Contam Toxicol 64(4):537–544

Naidu G, Ryu S, Thiruvenkatachari R, Choi Y, Jeong S, Vigneswaran S (2019) A critical review on remediation, reuse, and resource recovery from acid mine drainage. Environ Pollut 247:1110–1124

Nazari S, Rahimi G, Nezhad AKJ (2019) Effectiveness of native and citric acid-enriched biochar of Chickpea straw in Cd and Pb sorption in an acidic soil. J Environ Chem Eng 7(3):103064

Nejad ZD, Jung MC, Kim KH (2018) Remediation of soils contaminated with heavy metals with an emphasis on immobilization technology. Environ Geochem Health 40(3):927–953

Ning D, Liang Y, Song A (2016) In situ stabilization of heavy metals in multiple-metal contaminated paddy soil using different steel slag-based silicon fertilizer. Environ Sci Pollut Res 23:23638

Ottosen LM, Jensena PE, Kirkelunda GM, Dias-Ferreirab C, Hansenc HK (2012) Electrodialytic remediation of heavy metal polluted soil–treatment of water saturated or suspended soil. Chem Eng 28:103–108

Ou-Yang X, Chen JW, Zhang XG (2010) Advance in supercritical CO_2 fluid extraction of contaminants from soil. Geol Bull China 29(11):1655–1661

Park JH, Choppala G, Bolan NS, Chung JW, Chuasavathi T (2011) Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348:439–445

Pain DJ, Mateo R, Green RE (2019). Effects of lead from ammunition on birds and other wildlife: a review and update. Ambio 48:935–953

Paz-Ferreiro J, Fu S (2014) Biological indices for soil quality evaluation: perspectives and limitations. Land Degrad Dev

Pedersen KB, Kirkelund GM, Ottosen LM, Jensen PE, Lejon T (2015) Multivariate methods for evaluating the efficiency of electrodialytic removal of heavy metals from polluted harbour sediments. J Hazard Mater 283:712–720

Peng JF, Song YH, Yuan P, Cui XY, Qiu GL (2009) The remediation of heavy metals contaminated sediment. J Hazard Mater 161(2–3):633–640

Peng W, Li X, Xiao S, Fan W (2018) Review of remediation technologies for sediments contaminated by heavy metals. J Soils Sediments 18(4):1701–1719

Puschenreiter M (2008) Final research report

Quenea K, Lamy I, Winterton P, Bermond A, Dumat C (2009) Interactions between metals and soil organic matter in various particle size fractions of soil contaminated with waste water. Geoderma 149(3–4):217–223

Radziemska M, Bęś A, Gusiatin ZM, Cerdà A, Mazur Z, Jeznach J, Brtnický M (2019) The combined effect of phytostabilization and different amendments on remediation of soils from post-military areas. Sci Total Environ 688:37–45

Radziemska M, Bęś A, Gusiatin ZM, Cerdà A, Jeznach J, Mazur Z, Brtnický M (2020) Assisted phytostabilization of soil from a former military area with mineral amendments. Ecotoxicol Environ Saf 188:109934

Rajapaksha AU, Chen SS, Tsang DC, Zhang M, Vithanage M, Mandal S, Ok YS (2016) Engineered/designer biochar for contaminant removal/immobilization from soil and water: potential and implication of biochar modification. Chemosphere 148:276–291

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574

Rasaki SA, Bingxue Z, Guarecuco R, Thomas T, Minghui Y (2019) Geopolymer for use in heavy metals adsorption, and advanced oxidative processes: a critical review. J Clean Prod 213:42–58

Ricou-Hoeffer P, Hequet V, Lecuyer I, Le Cloirec P (2000) Adsorption and stabilization of nickel ions on fly ash/lime mixing. Water Sci Technol 42:79–84

Rieuwerts JS (2007) The mobility and bioavailability of trace metals in tropical soils: a review. Chem Spec Bioavailab 19(2):75–85

Sabir M, Hanafi MM, Aziz T, Ahmad HR, Zia-Ur-Rehman M, Saifullah GM, Hakeem KR (2013) Comparative effect of activated carbon, pressmud and poultry manure on immobilization and concentration of metals in maize (Zea mays) grown on contaminated soil. Int J Agric Biol 15:559–564

Sarkar B, Rusmin R, Ugochukwu UC, Mukhopadhyay R, Manjaiah KM (2019) Modified clay minerals for environmental applications. In: Modified clay and zeolite nanocomposite materials. Elsevier, pp 113–127

Selim HM (2018) Phosphate in soils: interaction with micronutrients, radionuclides and heavy metals. CRC Press

Sengupta I, Tarar JL (2014) Use of fly ash as bio-pesticide for cotton plant. Int J Current Res Life Sci 3(12):087–090

Shahbaz AK, Iqbal M, Jabbar A, Hussain S, Ibrahim M (2018a) Assessment of nickel bioavailability through chemical extractants and red clover (*Trifolium pratense L.*) in an amended soil: related changes in various parameters of red clover. Ecotoxicol Environ Saf 149:116–127

Shahbaz AK, Lewińska K, Iqbal J, Ali Q, Iqbal M, Abbas F, Ramzani PMA (2018b) Improvement in productivity, nutritional quality, and antioxidative defense mechanisms of sunflower

(*Helianthus annuus L.*) and maize (*Zea mays L.*) in nickel contaminated soil amended with different biochar and zeolite ratios. J Environ Manag 218:256–270

Shahbaz AK, Ramzani PMA, Saeed R, Turan V, Iqbal M, Lewińska K, Fatima M (2019) Effects of biochar and zeolite soil amendments with foliar proline spray on nickel immobilization, nutritional quality and nickel concentrations in wheat. Ecotoxicol Environ Saf 173:182–191

Shaheen SM, El-Naggar A, Antoniadis V, Moghanm FS, Zhang Z, Tsang DC, Rinklebe J (2020) Release of toxic elements in fishpond sediments under dynamic redox conditions: assessing the potential environmental risk for a safe management of fisheries systems and degraded waterlogged sediments. J Environ Manag 255:109778

Sharma S, Tiwari S, Hasan A, Saxena V, Pandey LM (2018) Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. Biotech 8(4):216

Sheikhhosseini A, Shirvani M, Shariatmadari H (2013) Competitive sorption of nickel, cadmium, zinc and copper on palygorskite and sepiolite silicate clay minerals. Geoderma 192:249–253

Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharmacol 43(3):246

Smith KL (2011) Gypsum as an agricultural amendment: general use guidelines. TDD no. 800-589-8292 (Ohio only)

Stanojković-Sebić A, Maksimović S, Jošić D, Pivić R (2014) The use of metallurgical slag as a by-product of the steel industry in chemical melioration of acid soils. Metall Mater Eng 20 (3):191–198

Sturm P, Gluth GJG, Brouwers HJH, Kühne HC (2016) Synthesizing one-part geopolymers from rice husk ash. Constr Build Mater 124:961–966

Sud D, Mahajan G, Kaur MP (2008) Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions–a review. Bioresour Technol 99 (14):6017–6027

Sun Y, Li Y, Xu Y, Liang X, Wang L (2015) In situ stabilization remediation of cadmium (Cd) and lead (Pb) co-contaminated paddy soil using bentonite. Appl Clay Sci 105:200–206

Sun Y, Xu Y, Xu Y, Wang L, Liang X, Li Y (2016) Reliability and stability of immobilization remediation of Cd polluted soils using sepiolite under pot and field trials. Environ Pollut 208:739–746

Tandy S, Meier N, Schulin R (2017) Use of soil amendments to immobilize antimony and lead in moderately contaminated shooting range soils. J Hazard Mater 324:617–625

Tauqeer HM, Hussain S, Abbas F, Iqbal M (2019) The potential of an energy crop “Conocarpus erectus” for lead phytoextraction and phytostabilization of chromium, nickel, and cadmium: an excellent option for the management of multi-metal contaminated soils. Ecotoxicol Environ Saf 173:273–284

Teresa AB, Valentina C (2012) Possible uses of steelmaking slag in agriculture: an overview, material recycling – trends and perspectives. ISBN: 978-953-51-0327-1

Tessier A, Campbell PG, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51(7):844–851

Tsunematsu S, Uematsu E, Saito K, Tamura H (2012) Immobilization of arsenic in natural soils by gypsum powder, mechanistic interpretations. Transactions of the Japanese Society of Irrigation. Drainage Rural Eng 80:141–150

Tu C, Wei J, Guan F, Liu Y, Sun Y, Luo Y (2020) Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. Environ Int 137:105576

Turan V, Khan SA, Iqbal M, Ramzani PMA, Fatima M (2018) Promoting the productivity and quality of brinjal aligned with heavy metals immobilization in a wastewater irrigated heavy metal polluted soil with biochar and chitosan. Ecotoxicol Environ Saf 161:409–419

Uchimiya M, Chang S, Klasson KT (2011) Screening biochars for heavy metal retention in soil: role of oxygen functional groups. J Hazard Mater 190(1–3):432–444

Valerie C (2015) Use of red mud in soil remediation: review of applications and challenges. Bauxite Residue Valorization Best Practices 1(1):81–87

Vink JP, Harmsen J, Rijnaarts H (2010) Delayed immobilization of heavy metals in soils and sediments under reducing and anaerobic conditions; consequences for flooding and storage. J Soil Sediments 10(8):1633–1645

Voglar GE, Leštan D (2011) Efficiency modeling of solidification/stabilization of multi-metal contaminated industrial soil using cement and additives. J Hazard Mater 192(2):753–762

Walraven N, Van Os BJH, Klaver GT, Middelburg JJ, Davies GR (2014) The lead (Pb) isotope signature, behaviour and fate of traffic-related lead pollution in roadside soils in the Netherlands. Sci Total Environ 472:888–900

Wang YS, Dai JG (2017) Use of magnesia sand for optimal design of high performance magnesium potassium phosphate cement mortar. Construct Build Mater 153:385–392

Wang K, Zhang J, Zhu Z, Huang H, Li T, He Z, Yang X, Alva A (2012) Pig manure vermicompost (PMVC) can improve phytoremediation of Cd and PAHs co-contaminated soil by Sedum alfredii. J Soils Sediments 12:1089–1099

Wang YS, Dai JG, Wang L, Tsang DC, Poon CS (2018) Influence of lead on stabilization/solidification by ordinary Portland cement and magnesium phosphate cement. Chemosphere 190:90–96

Wu YJ, Zhou H, Zou ZJ, Zhu W, Yang WT, Peng PQ, Liao BH (2016) A three-year in-situ study on the persistence of a combined amendment (limestone+ sepiolite) for remedying paddy soil polluted with heavy metals. Ecotoxicol Environ Saf 130:163–170

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011, Article ID 402647

Xu B, Ma H, Li Z (2015) Influence of magnesia-to-phosphate molar ratio on microstructures, mechanical properties and thermal conductivity of magnesium potassium phosphate cement paste with large water-to-solid ratio. Cem Concr Res 68:1–9

Xu Y, Liang X, Wang L, Sun Y, Huang Q (2018) Stability and universal applicability of immobilization effect of sepiolite on cadmium in acid paddy soil. In: Luo Y, Tu C (eds) Twenty years of research and development on soil pollution and remediation in China. Springer, pp 399–411

Xu C, Qi J, Yang W, Chen Y, Yang C, He Y, Lin A (2019) Immobilization of heavy metals in vegetable-growing soils using nano zero-valent iron modified attapulgite clay. Sci Total Environ 686:476–483

Yadav VB, Gadi R, Kalra S (2019) Clay based nanocomposites for removal of heavy metals from water: a review. J Environ Manag 232:803–817

Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729

Yi X, Xuefeng L, Yingming X, Xu Q, Qingqing H, Lin W, Yuebing S (2017) Remediation of heavy metal-polluted agricultural soils using clay minerals: a review. Pedosphere 27(2):193–204

Yuan GD, Theng BKG, Churchman GJ, Gates WP (2013) Clays and clay minerals for pollution control. Dev Clay Sci 5:587–644

Yeung AT, Gu YY (2011) A review on techniques to enhance electrochemical remediation of contaminated soils. J Hazard Mater 195:11–29

Zeng F, Ali S, Zhang H, Ouyang Y, Qiu B, Wu F, Zhang G (2011) The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ Pollut 159(1):84–91

Zeng G, Wan J, Huang D, Hu L, Huang C, Cheng M, Jiang D (2017) Precipitation, adsorption and rhizosphere effect: the mechanisms for phosphate-induced Pb immobilization in soils—a review. J Hazard Mater 339:354–367

Zeng X, Xiao Z, Zhang G, Wang A, Li Z, Liu Y, Zou D (2018) Speciation and bioavailability of heavy metals in pyrolytic biochar of swine and goat manures. J Anal Appl Pyrolysis 132:82–93

Zhan F, Zeng W, Yuan X, Li B, Li T, Zu Y, Li Y (2019) Field experiment on the effects of sepiolite and biochar on the remediation of cd-and Pb-polluted farmlands around a Pb–Zn mine in Yunnan Province, China. Environ Sci Pollut Res 26(8):7743–7751

Zhang G, Lin Y, Wang M (2011) Remediation of copper polluted red soils with clay materials. J Environ Sci 23:461–467

Zhou DM, Hao XZ, Xue Y, Cang L, Wang YJ, Chen HM (2004) Advances in remediation technologies of contaminated soils. Ecol Environ Sci 13(2):234–242

Zhou Y, Gao B, Zimmerman AR, Fang J, Sun Y, Cao X (2013) Sorption of heavy metals on chitosan-modified biochars and its biological effects. Chem Eng J 231:512–518

Zibaei Z, Ghasemi-Fasaei R, Ronaghi A, Zarei M, Zeinali S (2020) Improvement of biochar capability in Cr immobilization via modification with chitosan and hematite and inoculation with Pseudomonas putida. Commun Soil Sci Plant Anal:1–13

Žibret G, Van Tonder D, Žibret L (2013) Metal content in street dust as a reflection of atmospheric dust emissions from coal power plants, metal smelters, and traffic. Environ Sci Pollut Res 20 (7):4455–4468

Zotiadis V, Argyraki A, Theologou E (2012) Pilot-scale application of attapulgitic clay for stabilization of toxic elements in contaminated soil. J Geotech Geoenviron 138:633–637

Inhibition of Donor and Acceptor Side of Photosystem II by Cadmium Ions

Roberto Barbato

Contents

Abstract

The effect of different concentrations of cadmium (5, 50, and 500 μM) on photosystem II of *Thellungiella halophyla* leaves was investigated in vivo by means of chlorophyll *a* fluorescence transients, PAM fluorometry, and fluorescence decay after a single saturating flash. Our results indicated that cadmium affects both the donor and acceptor side of the photosystem; the ability to donate electrons to $P_{680}{}^{+}$ is adversely affected by cadmium, limiting the ability to oxidize water. In addition, cadmium affects the acceptor side of photosystem II by slowing down the electron transfer between Q_A and Q_B, likely by inducing a modification of the Q_B site.

Keywords

Photosynthesis · Photosystem II · Fluorescence · Cadmium · *Thellungiella*

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1 Introduction

Heavy metal contamination is one of the major environmental problems nowadays, causing many concerns. It is due to industrial and agricultural activities, traffic, dispersal of mining wastes, use of contaminated sewage sludges, manures, phosphate fertilizers, etc. (Sanità di Toppi and Gabbrielli 1999; Wu et al. 2007). Among different heavy metals, cadmium, Cd^{2+}, is known as one of the most toxic, both for plants and animals (Wagner 1993). In plants, no biological function has so far been described for cadmium. Cadmium ions are readily taken up by roots, via the same transporters involved in the uptake of Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+} (Clemens 2006) and then translocated to shoots to different extents, depending on the plant species and even on genotype within a single species (Hart et al. 1998; Guo et al. 1999). Cadmium in known to affect many aspects of the physiology of plants (Ismael et al. 2019); among them, the inhibitory effects on photosynthesis are well documented, both at the level of photosystems (PS) and light harvesting (Küpper et al. 2007). The donor side of photosystem II, particularly at the level of water oxidation by the Mn-cluster, has been shown to be one main target for Cd^{2+}, leading to inactivation of photosystem by a donor side-mediated inhibitory effect (Sigfridsson et al. 2004; Pagliano et al. 2006). To further contribute to the understanding of this topic, here we report additional data on the effect of Cd^{2+} on photosystems II (PSII), by using chlorophyll *a* fluorescence transient analysis (OJIP), and Q_A^- reoxidation after a single saturating flash and pulse amplitude modulated (PAM) fluorometry. Our results confirmed the donor side of PSII as one main Cd^{2+} target but indicated also that Q_A^- reoxidation is affected as well.

2 Materials and Methods

Thellungiella halophyla was grown as described by Goussi et al. (2018). Leaves from 4- to 5-week-old plants were detached and vacuum infiltrated with a solution containing different amounts of Cd^{2+}, 0 μM (control), 5 μM, 50 μM, and 500 μM in water. Cadmium was administrated as $Cd\ (NO_3)_2{\cdot}7H_2O$. Control samples were infiltrated with water. After 30 min of dark incubation, leaves were analyzed for: (1) chlorophyll *a* fluorescence transients, by using a Hanly-PEA as described by Goussi et al. (2018); (2) Q_A^- reoxidation kinetics by using a PSI FL-3500 fluorometer as described by Vass et al. (1999); (3) PAM fluorometry as reported in Goussi et al. (2018). Data were exported to and analyzed with the Origin 2019 software.

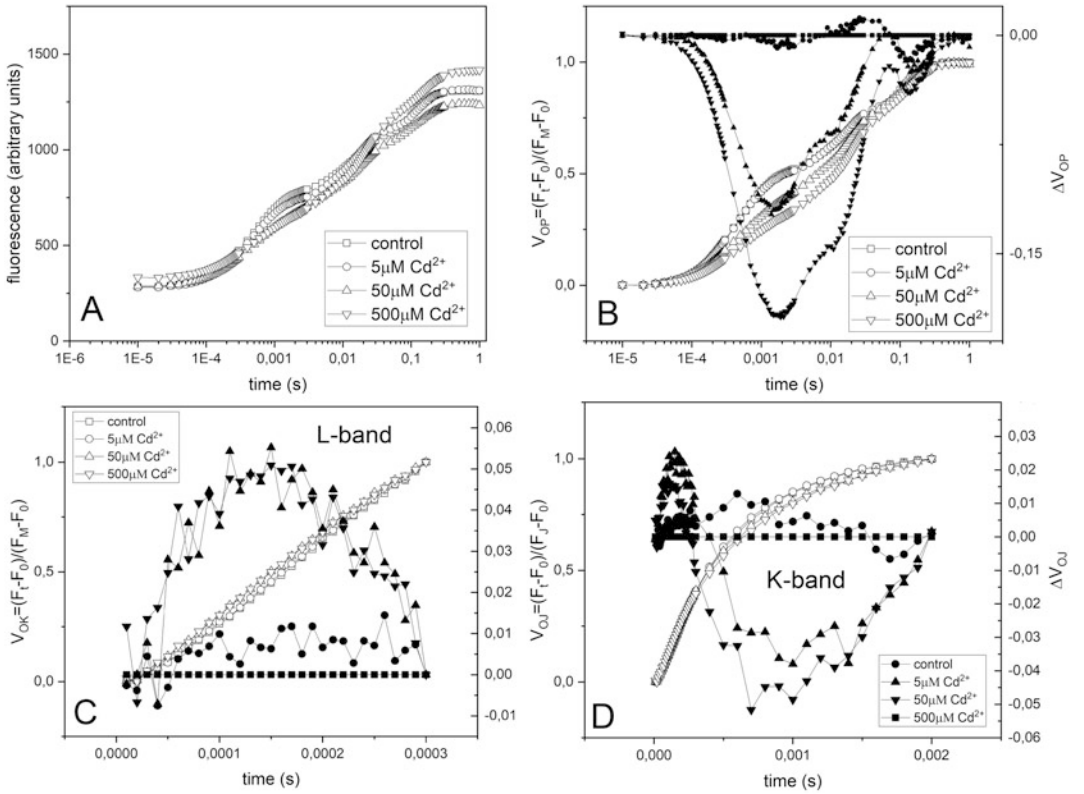

Fig. 1 Effect of treatment with Cd^{2+} on chlorophyll *a* fluorescence transients. (**a**) chlorophyll fluorescence transients, row data; (**b**) same data as in A after double normalization at F_0 and F_M (open symbols); the transient from control sample was then subtracted to other samples (including the control one, closed squares) and plotted as ΔV_{OP}; (**c**) the part of the chlorophyll transient between 0 and 0.3 ms was double-normalized (open symbols) and the control was subtracted from other samples, as above described; the resulting difference is the so-called L-band; (**d**) the part of the chlorophyll transient between 0 and 2 ms was double-normalized (open symbols) and the control was subtracted from other samples, as above described; the resulting difference is the so-called K-band

3 Results and Discussion

3.1 OJIP Analysis

Infiltration of leaves with Cd^{2+} solutions of different concentrations brought about some modifications in the chlorophyll *a* fluorescence transients (Fig. 1a), resulting in the lowering of the F_V/F_M ratio, from 0.801 ± 0.005 of the control to 0.767 ± 0.005, 0.775 ± 0.004, and 0.777 ± 0.005 of samples treated, respectively, with 5, 50, and 500 μM Cd^{2+}. These differences were significative at the level of $p < 0.005$. Chlorophyll transients were double-normalized and plotted as relative V_{OP} (Fig. 1b, open symbols). A change in the shape of transients was clearly observed, characterized by a loss of fluorescence in the JI phase, particularly pronounced at higher Cd^{2+} concentrations (50 and 500 μM). To further highlight these differences, the differential curves ΔV_{OP} (resulting from subtracting the control from the Cd^{2+}-infiltrated double-normalized fluorescence values) were calculated and presented in Fig. 1b (closed symbols). A negative band peaking at 2 ms was clearly observed, the intensity of which increased with Cd^{2+} concentration. This finding suggested a lower accumulation of the primary quinone acceptor Q_A^{-}, which could be due either to a slowing down of electron coming from water oxidation system or to an acceleration in the electron transfer to the second acceptor of the electron transport chain, Q_B (Strasser 1997; Chen and Cheng 2010). To get additional insights on changes induced by cadmium treatment, transients were further analyzed by considering respective ranges of time, i.e., 0–300 μs (phase OK, L-band) and 0–2 ms (phase OJ, K-band). After normalization in the respective ranges of time (open symbols in Fig. 1c, d), differential curves were constructed (closed symbols in Fig. 1c, d). The L-band, which appears at approximately 150 μs, indicates energetic connectivity (grouping) of the PSII units (Strasser et al. 2004). Therefore, a positive L-band, such as that observed in plants treated with Cd^{2+} (Fig. 1c), indicated that the energetic cooperation among the PSII units became less stable (Pollastrini et al. 2017) upon cadmium treatment. Such phenomenon is likely associated with disorganization of the thylakoid membranes, which has already been observed by electron microscopy in rice (Pagliano et al. 2006). The K-band (closed symbols in Fig. 1c) appears at approximately 300 μs (Fig. 1d) and is considered indicative of the electron donation activity from the oxygen-evolving complex (OEC) to the PSII, in competition with electrons not coming from water (Pollastrini et al. 2017). A positive K-band, such as the one observed in plants treated with Cd^{2+} (Fig. 1d), indicates a loss of OEC integrity, likely because of an impaired stability of the Mn_4CaO_5 complex: this is the physiological electron donor to the PSII but, in the presence of Cd^{2+}, it could suffer the competition from other unspecific electron donors (Gururani et al. 2012). Positive values for the K-band may also indicate an increase in the functional antenna size associated with the PSII (Yusuf et al. 2010). However, taking into account results from our short-time experiments, the observed positive K-band is likely to derive from destabilization of donor side components rather than to a change in antenna size. This view agrees with the loss of Mn-related EPR signal previously reported by us in rice (Pagliano et al. 2006) and in PSII particles

(Sigfridsson et al. 2004). Thus, our results are consistent with the possibility that a damage to water oxidation complex is induced by treatment with Cd^{2+}, limiting the amount of electrons extracted from water; in turn, this could limit the accumulation of Q_A in the reduced form.

3.2 Fast Fluorescence Decay Analysis

To further investigate this point, fast fluorescence decay was used to investigate Q_A^- reoxidation kinetics (Fig. 2). Results from analysis of fast fluorescence decay after a single saturating flash are reported in Fig. 3. Treatment with Cd^{2+}, particularly at high concentration (50 and 500 μM), brought about a slowing down of fluorescence decay, suggesting the impairment of Q_A^- reoxidation. In particular, as reported in Table 1, the amplitude of A1 phase (describing electron transfer between Q_A^- and a plastoquinone bound to the Q_B site in the dark) showed a significative decrease in the 500 μM Cd^{2+} and 50 μM Cd^{2+} treated samples with respect to the control ($p < 0.05$). Moreover, also the time constant of reactions was decreased, particularly in the 500 μM Cd^{2+} treated sample ($p < 0.05$). The effect of Cd^{2+} on the second phase A2 (originating from reoxidation of Q_A^- by plastoquinone molecules in reaction centers with empty Q_B site at the time of the flash) is pronounced as well, as its amplitude

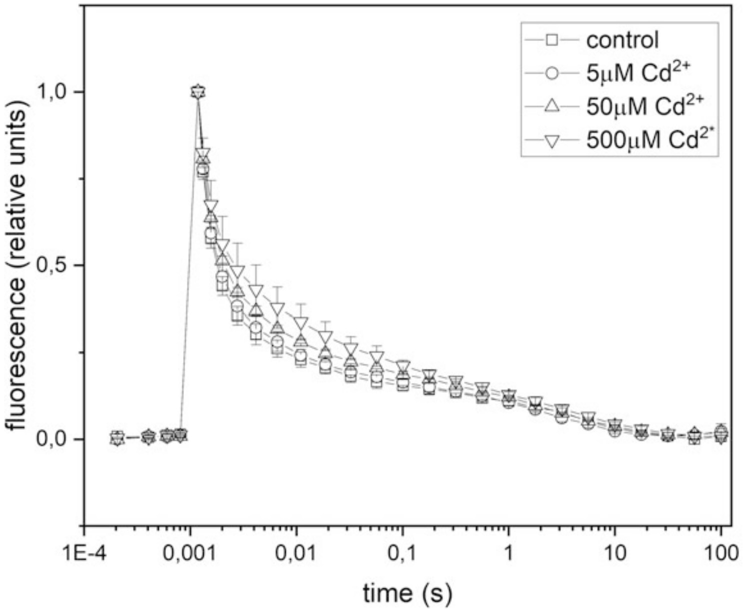

Fig. 2 Fluorescence decay kinetics in control and Cd^{2+} after a single saturating flash

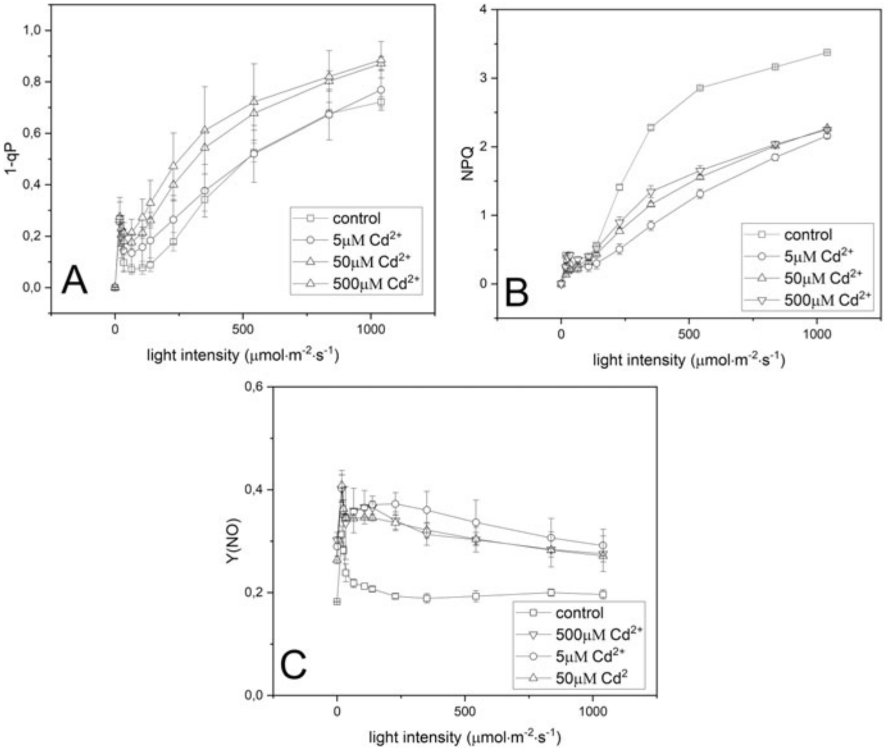

Fig. 3 Effect of Cd^{2+} treatment on some quenching parameters: (**a**) 1-*qP*; (**b**) NPQ; (**c**) Y(NO). Quenching parameters are defined as follows: $qP = (F_M' - F)/(F_M' - F_0')$; NPQ $= (F_M - F_M')/F_M'$; Y(NO) $= F/F_M$. Leaves were irradiated for 3 min at the indicated light intensity, and then a saturating pulse was fired (300 ms at 5000 μmol s^{-1} m^{-2}), allowing determination of quenching parameters

was significatively decreased in 500 μM Cd^{2+} with respect to the control. Differences were instead not statistically significative between control and 5 and 50 μM Cd^{2+} samples; at variance with amplitudes, all time constants were instead significatively modified ($p < 0.05$). These findings suggest that Cd-treatments likely affected the Q_B site, thus influencing electron transfer from Q_A^- both when a plastoquinone is already bound to the site (phase A1) and when the site was empty at the time of the flash (phase A2). Similar results were reported also by Faller et al. (2005) and Sigfridsson et al. (2004) by using an in vitro approach with highly resolved PSII preparation. The phase A3, describing charge recombination between Q_A^- and the donor side components of PSII, in terms of amplitude, is the most affected one, with a pronounced increase in all Cd^{2+}-treated samples. This finding agrees with data reported above obtained from OJIP analysis, confirming that cadmium adversely affect reactions involved in oxidation of water.

Table 1 Calculated values for amplitudes (*A*1–*A*3) and relative time constants (*T*1–*T*3) for control and Cd^{2+}-treated samples

	*A*1	*T*1 (ms)	*A*2	*T*2 (s)	*A*3	*T*3(s)
Control	$1.023 \pm 0.045^{a,b}$	0.348 ± 0.031^{a}	0.272 ± 0.029^{a}	$0.0076 \pm 0.0004^{a,b,c}$	$0.167 \pm 0.009^{a,b,c,}$	2.185 ± 0.416
5 μM Cd^{2+}	0.968 ± 0.053	0.333 ± 0.012	0.253 ± 0.024	0.0069 ± 0.0004^{a}	0.190 ± 0.009^{a}	2.176 ± 0.794
50 μM Cd^{2+}	0.875 ± 0.011^{a}	0.377 ± 0.0156	0.258 ± 0.013	0.0077 ± 0.0006^{b}	0.202 ± 0.003^{b}	1.194 ± 0.182
500 μM Cd^{2+}	0.781 ± 0.099^{b}	0.421 ± 0.037^{a}	0.226 ± 0.011^{a}	0.0129 ± 0.0023^{c}	0.224 ± 0.015^{c}	1.684 ± 0.523

In the different columns, same letter indicates that observed differences are significative at the level of 0.05

3.3 PAM Fluorometry

The effect of Cd^{2+} on PSII was further investigated by PAM fluorometry (Fig. 3a–c). To investigate the effect of Cd^{2+} on PSII photochemistry, the 1-qP parameter was used. This parameter is related to the "excitonic pressure" to which PSII centers are subjected at any given light intensity and is proportional to the amount of centers with a reduced Q_A (Demmig-Adams et al. 1990). As shown in Fig. 3a, treatment with Cd^{2+} brought about a marked increase of the excitonic pressure, as the fraction of centers with a reduced Q_A increased at any light intensity. This condition could promote double reduction of Q_A leading to an increased probability of chlorophyll triplet formation, making possible the occurrence of acceptor side-type photoinhibition (Vass et al. 1992). A decrease of qP (that is, an increase of 1-qP) has also been reported both in tomato (Lopez-Millan et al. 2009) and wheat (Ci et al. 2010). Thermal dissipation of absorbed light was investigated by calculating NPQ (i.e., the sum of: (1) qE, PSBS-dependent NPQ; (2) qT, state transition dependent on LHCII phosphorylation; (3) qI, related to the amount of photoinhibited PSII centers) and Y(NO) (the amount of absorbed energy passively dissipated (Kramer et al. 2004; (Klughammer and Schreiber 2008). Treatment with Cd^{2+} brought about a marked loss of NPQ (Fig. 3b), and at a cadmium concentration as low as 5 μM, most of its inhibitory effect was already observed. A decrease in NPQ upon cadmium treatment has also been reported by Lysenko et al. (2015) and linked to a loss of the PSBS-dependent qE component. Whether this effect could be due to binding of cadmium to the PSBS protein should be investigated. The loss of NPQ (and qP, Fig. 3a) is paralleled by a marked increase of Y(NO) (Fig. 3c). As stated above, this parameter reflects the fraction of energy that is passively dissipated in form of heat and fluorescence, mainly due to closed PS II reaction centers (Klughammer and Schreiber 2008) and its increase is usually interpreted as a symptom of PSII damage (Klughammer and Schreiber 2008; Tikkanen et al. 2015), confirming the adverse effect of Cd^{2+} on PSII.

4 Conclusion

The results of this study clearly point to the donor side of PSII as one main target of Cd^{2+}, confirming our previous results with rice. However, an important effect of Cd^{2+} was found also on the acceptor side of the photosystem, where reoxidation of Q_A^- is impaired.

References

Chen LS, Cheng L (2010) The acceptor side of photosystem II is damaged more severely than the donor side of photosystem II in 'Honeycrisp' apple leaves with zonal cholorosis. Acta Physiol Plant 32:253–261

Ci D, Jiang D, Wollenweber B, Dai T, Jing Q, Cao W (2010) Cadmium stress in wheat seedlings: growth, cadmium accumulation and photosynthesis. Acta Physiol Plant 32:365–373

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88:1707–1719

Demmig-Adams B, Adams WW, Czygan F-C, Schreiber U, Lange OL (1990) Differences in the capacity for radiationless energy dissipation in the photochemical apparatus of green and blue-green alga1 lichens associated with differences in carotenoid composition. Planta 180:582–589

Faller P, Kienzler K, Krieger-Liszka A (2005) Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of photosystem II by competitive binding to the essential Ca^{2+} site. Biochim Biophys Acta 1706:158–164

Goussi R, Manaa A, Derbali W, Cantamessa S, Abdelly C, Barbato R (2018) Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in *Thellungiella salsuginea*. J Photochem Photobiol B 183:275–287

Guo YL, Schulz R, Marshner H (1999) Genotypic differences in uptake and distribution of cadmium and nickel in plants. Angew Bot 69:42–48

Gururani MA, Upadhyaya CP, Strasser RJ, Woong YJ, Park SW (2012) Physiological and biochemical responses of transgenic potato plants with altered expression of PSII manganese stabilizing protein. Plant Physiol Bioch 58:182–194

Hart JJ, Welch RM, Norvel WA, Sulivan LA, Kochian LV (1998) Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiol 116:1413–1420

Ismael MA, Elyamine AM, Moussa MG, Cai M, Zhao X, Hu C (2019) Cadmium in plants: uptake, toxicity, and its interactions with selenium fertilizers. Metallomics 11:255–277

Klughammer C, Schreiber U (2008) Complementary PSII quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. PAM Application Notes 1:27–35

Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Photosynth Res 79:209–218

Küpper H, Parameswaran S, Leitenmaier B, Trtílek M, Šetlík I (2007) Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator Thlaspi caerulescens. New Phytol 175:655–674

Lopez-Millan AF, Sagardoy R, Solanas M, Abadia A, Abadia J (2009) Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. Environ Exp Bot 65:376–385

Lysenko EA, Klaus AA, Pshybytko NL, Kusnetsov VV (2015) Cadmium accumulation in chloroplasts and its impact on chloroplastic processes in barley and maize. Photosynth Res 125:291–303

Pagliano C, Raviolo M, Dalla Vecchia F, Gabbrielli R, Gonnelli C, Rascio N, Barbato R, La Rocca N (2006) Evidence for PSII donor-side damage and photoinhibition induced by cadmium treatment on rice (*Oryza sativa* L.). J Photochem Photobiol B 84:70–78

Pollastrini M, Nogales AG, Benavides R, Bonal D, Finer L, Fotelli M, Gessler A, Grossiord G, Radoglou K, Strasser RJ, Bussotti F (2017) Tree diversity affects chlorophyll *a* fluorescence and other leaf traits of tree species in a boreal forest. Tree Physiol 37:199–208

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Sigfridsson KGV, Bernart G, Mamedov F, Styring S (2004) Molecular interference of Cd^{2+} with photosystem II. Biochim Biophys Acta 1659:19–31

Strasser RJ (1997) Donor side capacity of Photosystem II probed by chlorophyll *a* fluorescence transients. Photosynth Res 52:147–155

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou GC, Govindjee (eds) Chlorophyll *a* fluorescence: a signature of photosynthesis. Advances in photosynthesis and respiration. Springer, Dordrecht, pp 321–362

Tikkanen M, Rantala S, Aro E-M (2015) Electron flow from PSII to PSI under high light is controlled by PGR5 but not by PSBS. Front Plant Sci 6:521–523

Vass I, Styring S, Hundall T, Koivuniemi A, Aro E-M, Andersson B (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced QA species promote chlorophyll triplet formation. Proc Natl Acad Sci U S A 89:1408–1412

Vass I, Kirilovsky D, Etienne AL (1999) UV-B radiation-induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium Synechocystis sp. PCC 6803. Biochemistry 38:12786–12794

Wagner GJ (1993) Accumulation of cadmium in crop plants and its consequences to human health. Adv Agron 51:173–212

Wu F, Zhang G, Dominy O, Wu H, Bachir M (2007) Differences in yield components and kernel Cd accumulation in response to Cd toxicity in four barley genotypes. Chemosphere 70:83–92

Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee SNB (2010) Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: Physiological and chlorophyll *a* fluorescence measurements. Biochim Biophys Acta 1797:1428–1438

Physiological and Molecular Mechanism of Metalloid Tolerance in Plants

Roseline Xalxo, Jipsi Chandra, Neha Pandey, and Keshavkant Sahu

Contents

Abstract

Since the onset of the industrial revolution, metalloids are an exceptional class of toxicants that adversely affects plant growth and productivity when present in high concentrations in the agricultural soil. Various transporters are accountable

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for the entry and distribution of different elements inside the plant. Due to similar structural properties, many toxic metalloid ions share the similar transport network like phosphate transporters, aquaglyceroporins, hexose transporters, sulfate transporters, etc. Incidence of surplus quantity of toxic metalloid ions inside the plant tissues causes severe damages to cellular biomolecules, affects key metabolic processes, and hampers plant growth, ultimately leading to reduced crop productivity. Therefore, detoxification strategies of metalloids at the physiological and molecular level are crucial in order to curtail their toxic consequences. Implementing genetic engineering techniques, efforts have been made by various scientists to impede the uptake of toxic metalloids by plants by reducing the transporters activities and to upregulate metalloid binding peptides and proteins such as metallothioneins and phytochelatins, for sequestration of toxic metalloids in the tissues. Reduced transport of metalloids in the tissues together with their augmented sequestration inside the cells would result in production of metalloid-tolerant plants. The present chapter summarizes current status of knowledge related to transport mechanisms and detoxification strategies of metalloids in plants in relation to plant-metalloid tolerance.

Keywords

Detoxification · Metalloids · Phytochelatins · Sequestration · Transporters

1 Introduction

In the present era, due to rapid Industrial Revolution and urbanization, considerable amount of toxic heavy metals (HMs) and metalloids are liberated in the environment which are accountable for polluting environment (Talukdar 2013; Chandrakar et al. 2018; Xalxo and Keshavkant 2018). Metalloids are those elements which are neither categorized under metals or nonmetals and have intermediate properties of both metals and nonmetals. Among various metalloids, boron (B), silicon (Si), germanium (Ge), arsenic (As), antimony (Sb), tellurium (Te), and polonium (Po) are some of the toxic metalloids arranged on the basis of their increasing atomic number (Brown et al. 2002). These metalloids are enormously reactive and cause phytotoxicity and are considered as the chief environmental pollutant that limits world agricultural production. However, degree of phytotoxicity depends on various factors like their types, nature, and amount of metalloids that encounter plants. Presence of some metalloids in soil like B and Si is crucial, or at least profitable, for plants but a few of them such as As and Sb are toxic to plants and can interfere and disrupt their normal functioning (Chandrakar et al. 2018). Toxic metalloids are released into the environment through various sources, some of the them include, mining and refining of ores, utilization of fertilizers and pesticides, combustion of fossil fuels, exhaust emissions from vehicles, power stations, solid wastes disposal, use of wastewater for irrigation of soil, and contiguous industrial activities (Finnegan and Chen 2012; Chandrakar et al. 2016). Contamination of metalloids in the agricultural land leads to amplified accumulation of these in plants and their parts as well as assists their entry into the human food cycle. Additionally, these severely amend the normal growth and alter various physiological parameters, and biochemistry of plants (Finnegan and Chen 2012; Chandrakar et al. 2017a). The simplest adjustment that can be executed by the plant to resist the consequences of metalloid is to manage the transportation of these toxic metalloids into (uptake or influx) and out of (efflux) the cell. Various transporters in roots are responsible for the uptake of metalloids from the soil and subsequently they are transferred to various plant organs. These transporters comprise variety of genome encoded transporter proteins. To regulate the transportation of metalloids in the plants these proteins have different specificity, particular expression patterns, and cellular localizations. Different groups of proteins like sulfate transporters, phosphate transporters, Nodulin 26-like intrinsic proteins, aquaglyceroporins, silicon influx transporters**,** hexose transporters, etc., are accountable for the metalloid transport (Farooq et al. 2009). For instance, from soil arsenate (As^{V}) is taken inside the plant by root system taken up via phosphate transporter, arsenite (As^{III}) and Sb^{III} by means of hexose transporter, while selenate is taken up via sulfate transporters. These transporters are expressed during normal condition but when the metalloid concentration exceeds the permissible limit in the soil, the expression levels of transporters genes increase. Plants have their own tolerance mechanism against these toxic metalloids. One of the strategies includes elimination of the toxic metalloids by active efflux or through sequestration, when metalloids are relocated to adjacent cells of different plant parts (Fu et al. 2000). Secondly, efforts have been done by researchers to use different potent compounds or chemical coated nanoparticles (NPs) to lessen the negative consequences caused by metalloids, HMs, and radiations (Singh et al. 2015; Tripathi et al. 2015, 2017; Chandrakar et al. 2017a, b; Xalxo and Keshavkant 2019). One of the central mechanisms associated with metalloid tolerance is compartmentalization and its subsequent sequestration by formation of metalloid complex with proteins. Increase in modernization, industrialization, and utilization of HMs and metalloids in farming sector led to higher accumulation of these in the environment. Therefore, there is an urgent need of thorough understanding regarding uptake and transport of metalloids in plants, their sequestration, and tolerance mechanisms (Dhankher 2005). Furthermore, the recent advancement of nanotechnology applications to mitigate the noxious effects of metalloid toxicity has led the researchers into new field to investigate the involvement of synthesized NPs and to explore the mechanism involved in mitigation processes. Therefore, metalloid toxicity has become a major problem and researchers are paying massive attention in recent years. The primary objective of this chapter is to focus predominantly on the mechanisms of metalloid-induced oxidative stress related responses, and tolerance mechanisms in plants.

2 Metalloids and Biological Interference

Metalloids, also accredited as semimetals, comprise physical and chemical properties between metals and nonmetals (Coyle et al. 2002). In appearance, it resembles metals, brittle and excellent electricity conductor but their chemical nature resembles nonmetals. In plants and animals, various intrinsic proteins localized on membranes like water transporting aquaporins and glycerol transporting aquaglyceroporins assist the uptake and transport of metalloids like Si, B, As, and Sb (Hermans et al. 2006). The detrimental consequences caused by these toxic metalloids are also due to their unique properties which show resemblance with biological components that are involved in vital biological processes. For instance, as As^V mimic phosphate, it substitutes the phosphate during ATP synthesis and decreases the ATP content inside the living system (Chandrakar et al. 2016; Huang et al. 2016). Arsenate also inhibits respiration by decreasing the activity of pyruvate dehydrogenase. Arsenite, the trivalent form of As, has a high affinity for –SH group of cysteine residues and various other enzymes. It inhibits the catalytic properties of vital enzymes. Arsenite also diminishes carbohydrate content and decreases the production of various coenzymes, consequently leading to altered tricarboxylic acid or Krebs cycle and disturbed pyruvate metabolism (Höller et al. 2014). Since, As^{III} and As^V, both have the capability to stimulate excess generation of reactive oxygen species (ROS) in the cells, these cause oxidative damage to vital biomolecules (proteins, lipids, and carbohydrate) and nucleic acids (DNA and RNA) (Kushwaha et al. 2019). In plants, when As gets accumulated to toxic levels, it terrorizes the health of human and animal by entering into food chain. Antimony, upon entering plant cells form complexes with biomolecules, intergate with the sulfhydryl group of key proteins and modifies their activities, hampers glucose metabolism through glycolytic pathway and negatively influence the formation of ATP (Kagi and Schaffer 1988). Plants on encountering Se in elevated amount induce oxidative stress inside the cell through Se-induced ROS production and lead to distortion of protein structure and function (Gupta and Gupta 2017). Boron when present in lower concentration shows extensive array of advantageous consequences like carbohydrate and protein metabolism, maintains integrity of plasma membrane and cell wall (Kumar and Trivedi 2016). But, when present in higher concentration, B disrupts various key cellular processes and its phytotoxicity is not easy to deal with (Polsia et al. 2016). Silicon is a biologically potent molecule which has the potential to activate defense mechanisms against various abiotic stresses. It has shown to prevent fungal attack in plants by increasing the enzymatic activities of chitinase, peroxidase, flavonoid phytoalexins, and polyphenol oxidase. Germanium is widely used in industries and chemically, it is an analogue of Si. When plants take up excess Ge, it negatively influences various biological pathways and stimulates aging (Halperin et al. 1995). Various advancements in omics like transcriptomics, metabolomics, and proteomics have facilitated scientists, the better understanding of metalloids toxicity and its tolerance mechanisms in agricultural crops (Singh et al. 2016).

3 Metalloid Stress and Plant Responses

3.1 Morphological Effects of Metalloids

Availability of metalloid in the soil above the permissible limit leads to various modifications in the plant metabolism which causes curling, wilting, chlorosis, necrosis of leaf blades, diminution in the leaf number and consequently diminished photosynthetic rate and biomass accretion, decreased rate of cell elongation, proliferation and nodulation in roots, diminutive growth, and low productivity (Talukdar 2013; Chandrakar et al. 2017b). Metalloids toxicity also leads to distortion of cellular membranes by binding with the sulfhydryl groups of various key enzymes and proteins which results in loss of turgor and rigidity ultimately leading to wilting of plants. Presence of metalloids in agricultural soil restricts/limits the uptake and transport of water and vital minerals. Limitation of water and minerals in the cells leads to dehydration which causes leaf curling. Also, metalloid toxicity leads to shortage of essential nutrients in plants that causes necrosis, subsequently affects plant parts, particularly the green leaves turn brownish or blackish. Metalloids accretion also results in the necrotic patches in specific regions, especially leaf outline and can led to the gradual fatality of the entire leaf. Ultrastructure analysis by Farnese et al. (2017) revealed that necrosis was the outcome of cytoplasm leakage, membrane vesiculation, and cellular disorganization. Reduction in root length due to the metalloid toxicity was observed by Reid et al. (2004), Wang et al. (2010), and Farooq et al. (2017) in *Hordeum vulgare* L., *Cucumis sativus* L., and *Brassica napus* L. seedlings, respectively. Various morphological symptoms like necrosis, wilting, and shriveling were observed by Campos et al. (2018) in *Pityrogramma calomelanos* L. under As toxicity.

3.2 Physiological Effects of Metalloids

3.2.1 Growth and Productivity

Root Plasmolysis

Toxic metalloid, being non-essential elements significantly affects plants growth, development, and yield responses. Metalloids are taken up and then transported by means of various transporters which are localized in plasma membranes of roots (Chandrakar et al. 2016). When plants encounter metalloid toxicity, roots are the primary organ to come across this contaminant, where this toxic metalloid results in discoloration and plasmolysis of cells (Shaibur and Kawai 2011; Talukdar 2013). Plasmolysis is an active biological process and a distinctive characteristic of living cells and hence, used as a marker to check the cell viability under various abiotic stresses (Lang et al. 2014). Lou et al. (2015) revealed that exposure of plant roots to As causes augmentation in root lignification and degeneration of microtubules, subsequently an abrupt increase in the cell width consequently increased root diameter.

Biomass Accumulation

Diminution in germination rate, root elongation, and biomass accumulation are the most sensitive parameters of metalloid toxicity. Many scientists revealed that the principal consequences of metalloid toxicity are inhibited germination rate, biomass accumulation, root elongation, and plant growth (Reid et al. 2004; Wang et al. 2010; Chandrakar et al. 2016). Decreases in germination percentage and growth responses may be the outcome of harmful consequences of metalloids on cellular functioning where maximum accessible energy is used for the production of stress linked vital compounds like phytochelatins (PCs), antioxidants, etc. (Chandrakar et al. 2018). Various researches have revealed rigorous decline in the biomass, which eventually reduces crop yield under metalloid toxicity (Reid et al. 2004; Chandrakar et al. 2017a, b). Lessening in biomass accumulation may also be because of amplified permeability of cell membranes thereby augmented leakage of cellular constituents/fundamental nutrients which are primary components for the generation of energy and plant development (Farooq et al. 2015; Chandrakar et al. 2018). Also, water loss is amplified in parallel to reduction in water uptake by the roots resulting in inhibited biomass accumulation (Chandrakar et al. 2018). Further, metalloid toxicity leads to protein degradation that may be due to the insufficiency of carbohydrate, an adaptation strategy by cells resulting in diminished protein level and biomass accretion (Agnihotri and Seth 2016). So, it is obvious that metalloid phytotoxicity is well acknowledged to cause detrimental consequences to plant growth and development when present in higher concentration (Talukdar 2013; Chandrakar et al. 2016).

Cell Division, Elongation, and Expansion

Exposure of plants to toxic metalloids results in suppressed mitotic activity, most particularly in the meristematic zone of roots consequently reduced growth. Metalloids hinder the normal cell division and thus diminish the growth of meristematic cells of root and shoot. Metalloid-induced reduction in mitotic activity thus decreased the rate of cellular division in the root apical meristem consequently leading to inhibited expansion and elongation of the novel cells (Farooq et al. 2015; Chandrakar et al. 2018). Metalloids also diminish root turgor pressure causing inhibition in cell enlargement. Exposure to metalloids negatively affects the cell division machinery that is responsible for the cell cycle regulation in plants (Sharma 2012). Metalloids are also acknowledged to decrease the production of proteins like cyclins and cdks that are responsible for regulating cell cycle (Reichard and Puga 2010; Chandrakar et al. 2018). Patra et al. (2004) revealed that As binds to the thiol groups and results in inhibited activity of those enzymes which are mainly involved in DNA repair system, consequently DNA damage. Sharma et al. (2012) performed genomic analyses of As exposed *Oryza sativa* L. expansion genes (*Os01g14660* and *Os04g46650*) and observed that expression of these genes was downregulated. Also, metalloid attacks the thiol group of various proteins, particularly tubulin proteins which are responsible for microtubule formation during cell division thereby altering elongation process. Likewise, Norton et al. (2008) revealed that exposure of As to *Oryza sativa* L. decreased the transcript level of two tubulin genes (*Os03g45920* and *Os03g56810*) and two microtubule genes (*Os03g13460* and *Os09g27700*). Arsenic toxicity has also led to disorganization and abnormal functions of spindle machinery during cell division and may possibly be the outcome of stimulation of lagging chromosomes and/or loss of microtubule of spindles. Arsenic-induced chromosomal anomalies with decreased mitotic index were also reported by Patra et al. (2004). Other detrimental consequences of metalloid toxicity are the chromosome stickiness together with breakage and joining, which might possibly be due to the formation of chromosomal bridges. Metalloid mediated chromosomal aberrations are grouped into two categories: clastogenic effect like fragments, micronuclei, and ring chromosome bridges. While, other one is the precocious chromosomal movement during early phase of cell cycle, more particularly at anaphase stage which may lead to early terminalization and chromosome stickiness (Mumthas et al. 2010). Chromosomal anomalies with decreased mitotic index, modified cell divisions (mitotic and meiotic), disruption of nuclear and plasma membranes, distortion in thylakoid structure, intensification in quantity of nucleoli, starch granules, vacuoles and plastoglobuli, severe plasmolysis, deterioration of chromatin materials, etc., are few of the ultramorphological alterations reported under various metalloids toxicity (Patra et al. 2004; Chandrakar et al. 2018).

3.2.2 Photosynthetic System

Plant species when encounters abiotic stresses causes varied detrimental consequences ranging from reduced growth and development to diminished photosynthetic rate, finally leading to cell death (Gusman et al. 2013a; Xalxo and Keshavkant 2018). Photosynthesis is a basic physio-chemical process to maintain plants life and various activities from which the syntheses of organic compounds (carbohydrates) occur through use of light energy. Metalloids are considered as serious environmental stresses due to their unfavorable effects on bioenergetic processes of photosynthesis. The photosynthetic pigments are susceptible to metalloid contamination which possibly may alter the photosynthetic rate. Decreased concentration of chlorophyll upon metalloid stress might be the outcome of decreased activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and/or its biosynthetic enzymes: δ-aminolevulinic acid dehydratase and protochlorophyllide reductase (Gusman et al. 2013a; Chandrakar et al. 2016, 2018; Farooq et al. 2016). Gene expression analysis of biosynthetic enzymes illustrated that under abiotic stress the transcript level of δ-aminolevulinic acid dehydratase gene was decreased significantly (Agnihotri and Seth 2016). Stoeva and Bineva (2003) and Mateos-Naranjo et al. (2012) demonstrated that As exposure to *Avena sativa* L. and *Spartina densiflora* seedlings showed significant diminution in the chlorophyll fluorescence ratio, photosynthetic efficiency, rate of CO_2 fixation, and functioning of photosystem-II.

Metalloid toxicity also results in structural disorganization of chloroplasts membranes and perturbations in functioning of integral photosynthetic activity, consequently breakage and bulging of thylakoid membranes and decreased biosynthesis of photosynthetic pigment (Stoeva and Bineva 2003; Chandrakar et al. 2016). Inside the cell, apart from mitochondria, peroxisomes and other organelles like chloroplasts are few of the chief sites for the formation of free radicals, and are pretty susceptible to HM injury (Chandrakar et al. 2016). Predominantly, this free radical modifies the function of chloroplasts membranes, and elements of the electron transport chain that operate in chloroplast, thereby damaging the light reaction of photosynthesis (Talukdar 2013). Li et al. (2006) and Schneider et al. (2013) revealed that *Pteris vittata* and *Leucaena leucocephala* when exposed to As toxicity, resulted in structural injury to the chloroplasts internal membranes. Metalloid-imposed degradation and modification in internal membranes of chloroplasts have consequences on pace of photosynthesis which is linked with decreased level of photosynthetic pigments and rate of carbon assimilation (Stoeva and Bineva 2003; Chandrakar et al. 2018). Reduction in pigment synthesis may possibly be due to the absence of suitable adaptive regulation of pigment synthesis system due to higher concentrations of metalloids (Sharma et al. 2012; Chandrakar et al. 2016). Metalloid-induced overproduced ROS mediated lipid peroxidation reaction may also be accountable for destruction of chloroplasts membranes (Chandrakar et al. 2016).

In plants, metalloid toxicity also negatively influences the photochemical efficiency and heat dissipation ability, thereby altering rates of gas exchange and fluorescence emission (Gusman et al. 2013a). Ahsan et al. (2010) showed that leaves of *Oryza sativa* L. treated with As^V resulted in decreased level of large subunit of RuBisCo which indicates that As^V not only hampers the CO_2 fixation but also alters the expression of chloroplastic DNA. In contrary, an amplification in small subunit of RuBisCo transcripts was noticeable in As^V exposed *Arabidopsis thaliana* (Abercrombie et al. 2008). In regard to As, as it is an analogue of phosphate, As^V disturbs the process of oxidative phosphorylation by dislocating one molecule of phosphate from ATP, thus leading to the formation of unstable ADP-As^V complex. This uncoupling reaction causes interruption in the normal cellular energy flow and, rate of ATP synthesis gets suppressed (Meharg and Hartley-Whitaker 2002).

3.2.3 Nutrient and Water Uptake

Various vital functions of plant roots include nutrients uptake from the soil, provide support to the plant, and affix it to the substrate through root system. Moreover, in case of HMs/metalloids roots are the first organ, which comes in contact with the metalloid, where they accumulate inside the cells which causes disturbance in the nutrient uptake and transport (Stoeva et al. 2005). Metalloids toxicity causes detrimental consequences to plant root system, due to which selectivity and permeability of root cell membrane get altered, causing lesser nutrients uptake (Gusman et al. 2013b). Also, metalloid ions compete for binding to transport protein, and thus block the binding of micro and macronutrients to transport protein, hence alter the nutrient uptake (Gusman et al. 2013b). Furthermore, alterations in the uptake and transport of ions lead to lower stomatal conductance due to metalloids-imposed interruption in the membrane integrity. A remarkable change was observed in nutrients uptake and their transport accompanied with reduced rate of net photosynthesis in *Triticum aestivum* L. and *Lactuca sativa* L. under metalloid stress (Liu et al. 2008; Gusman et al. 2013b). Mallick et al. (2011) revealed that As toxicity caused reduction in the potassium content which was associated with the aggregation of plaques in the root that led to decreased transportation of vital nutrients. Metalloid toxicity also reduces the pigment levels in the plants that may be due to the disturbed status of nutrients (Siddiqui et al. 2015a, b). Plants absorb nitrogen from soil, principally in form of nitrate or ammonium. Upon As exposure, the genes which are accountable for nitrate and ammonium transport were found to be downregulated consequently disrupting nitrogen assimilation (Norton et al. 2008). Micronutrients, for example, manganese (Mn), copper (Cu), and iron are the major components of superoxide dismutase (SOD) enzyme and reduction in the micronutrients concentration results in decreased antioxidant capacity (Farnese et al. 2014). Kumar et al. (2015) showed a decrease in uptake of magnesium, which is the essential atom of chlorophyll and acts as enzyme cofactor that triggers phosphorylation process and sulfur, which forms the important constituent of glutathione (GSH), PCs, and non-protein thiols. Arsenate and phosphorous (vital plant nutrient) both competes for the same transporters across the membranes of root cells and As^V dislocate phosphate from ATP and thus obstructs the rate of ATP synthesis and energy flow (Garg and Singla 2011). The expression of enzymes like H^+ATPases is amended under As stress, which is accountable for the transport of ions and nutrients across the cells (Pathare et al. 2016).

4 Mechanism of Metalloid Tolerance in Plants

4.1 Restriction of Uptake and Transport of Metalloids

4.1.1 Exclusion of Metalloid from the Plants

Heavy metals/metalloids enter inside the plants either by symplastic (intracellular) or via apoplastic (extracellular) pathways (Chandrakar et al. 2016). There is an energy-dependent process for the entry of HMs/metalloids into the plant cells via specific HMs/metalloids-ion carriers or channels. By preventing the excess entry of HMs/metalloids inside the plant its toxic effect can be lessened. Basic approach in this regard, starts by analyzing the ability of plants to assimilate metalloid ions. Generally, ecotypes adapted to HM/metalloid contaminated environments accumulate low amount of toxicant; however, non-resistant ecotypes accumulate comparatively high amount of toxicants. This is known as exclusion or avoidance phenomena, where the plant shows high tolerance to the HM/metalloid ions.

4.1.2 Cellular Exclusion of Metalloids

Cellular exclusion of HM/metalloid is a vital adaptive strategy for plant tolerance against phytotoxicity. After the uptake of HMs/metalloids they are transported to the aerial parts through apoplastic movement and hence a large amount of HMs/metalloids are accumulated in apoplastic space. However, upon exposure to aluminum (Al), a sensitive *Triticum aestivum* L. cultivar showed higher accumulation of Al in symplastic space than the tolerant cultivar signifying its exclusion property (Tice et al. 1992). Heavy metals/metalloids transporter proteins are potentially engaged in the exclusion of lethal HMs/metalloid ions from the symplastic to the apoplastic space.

4.1.3 Metalloid Complexation at the Cell Wall-Plasma Membrane

The boundary between the cell wall and plasma membrane is the region where cell accumulates high amount of HMs/metalloids and for that reason it is assumed that this could be the location for HMs/metalloids tolerance. In *Lolium multiflorum*, high concentration of HM was found to be bounded at the cell wall and plasma membrane (Iwasaki et al. 1990). Though, there is a need for better understanding of the cell wall and plasma membrane function in HMs/metalloids tolerance.

4.1.4 Distribution of Metalloids

Plants seek to diminish the negative consequences exerted by HMs/metalloids by altering the distribution and translocation of HMs/metalloids within their organs or cells. The most common mechanism includes accumulating excess HMs/metalloids in plant roots; however, hyperaccumulators transfer HMs/metalloids to their aerial parts like, shoots and leaves. (Zheng et al. 1997). Elevated accretion of HMs/metalloids in plant roots thus decreases the transfer of HMs/metalloids to the shoots, and can be regarded as a tolerance strategy.

4.2 Complexation and Compartmentation of Metalloid Within the Plant Cells

4.2.1 Intracellular Sequestration or Compartmentation Within the Vacuoles

Vacuolar sequestration of metallic contaminants is considered to be a major molecular mechanism for phytoremediation and safer crop productivity (Zhang et al. 2018). Vacuole acts as a store house for a number of protein transporters [H^+-ATPase; H^+-pumping pyrophosphatase; Na^+/H^+ antiporter; Ca^{2+}/H^+ antiporters; Cu transporter COPT5; multidrug and toxic compound extrusion (MATE) transporters; vacuolar sugar transporters (AtSuc4); and vacuole iron transporter (VIT)] for the stress tolerance/phytoremediation upon exposure to environmental contaminants (Tan et al. 2019). The characteristic property of hyperaccumulators is to detoxify and sequester the metalloid and to accumulate high concentration of it on the aerial parts without causing destruction to the plants. Detoxification/sequestration of the HMs/metalloids takes places at various locations like, trichomes, epidermis, and cuticle (Küpper et al. 2000; Robinson et al. 2003; Freeman et al. 2006) where the injury caused by the HMs/metalloids to photosynthetic machinery is at lesser extent. Few instances have shown exclusion of HM/metalloid from stomatal guard cell as well as subsidiary cells (Frey et al. 2000; Cosio et al. 2005). This exclusion from the stomatal cells protects them from HMs/metalloid-induced detrimental consequences. The detoxification/sequestration mechanisms include HMs/metalloid complex formation with ligands and/or elimination of these complexes from cytoplasm by sequestering them into inactive organelles, particularly vacuoles and cell walls. Comparative transcriptome analyses of hyperaccumulator and related non-hyperaccumulator species have illustrated that HMs/metalloid sequestration depends on the constitutive overexpression of genes that encode proteins accountable for HMs/metalloids transport across the vacuole membrane, i.e., tonoplast and/or plasma membrane that are responsible for HMs/metalloid exclusion from the cytoplasm.

4.2.2 Formation of Metalloid Complex by Phytochelatins

In plants, PCs are the primary HMs/metalloid binding thiol peptides having a general structure [γ-glutamyl(Glu)-cysteinyl(Cys)]n-glycine, where n ranges between 2 to 11. Various amino acids like glutamic acid, serine, glutamine, or β-alanine can be the terminal amino acid but mainly glycine acts as a primary amino acid (Anjum et al. 2015). Plants exposure to HM/metalloid induces syntheses of PCs in various plant organs. Glutathione acts as a precursor for the syntheses of PCs and is done in collaboration with the enzyme phytochelatin synthase (PCS) (Anjum et al. 2015). The enzyme PCS is also known by the name γ-Glu-Cysdipeptidyl transpeptidase (EC 2.3.2.15). Various metals and metalloids regulate the activity of this enzyme. Phytochelatins have been recognized in various plants and microorganisms and amplified production of these is regarded as marker of metalloid contamination (Pandey et al. 2019). Availability of surplus metalloid in plant growth medium reduced the glutathione (GSH) content while amplified the synthesis of PCs. Experiment conducted by Sui et al. (2001) illustrated that transgenic *Brassica juncea* plants with intensified expression of GSH showed enormous biosyntheses of PCs and hence tolerance against metalloid toxicity. Expression analysis of PCS showed stimulation upon As^{III} and As^{V} toxicity (Sundar et al. 2000). The PC forms complex with the metalloid and thereafter the PC-metalloid complex is moved to vacuoles with the help of proton gradient across the tonoplast vesicles (Sweet et al. 1990). Because of the presence of Cys residues in PCS, As has high affinity for –SH groups and As forms strong PC-metalloid complexes. These complexes with the help of various transporters are then sequestered into vacuoles (Anjum et al. 2015). Overexpression of PCS in transgenic plants has been acknowledged as an appropriate approach for the phytoremediation of As (Li et al. 2004). In *Arabidopsis thaliana* overexpression of PCS enzyme enhanced As tolerance against toxic pollutants (Li et al. 2004).

4.2.3 Complexing by Metallothioneins

The metallothioneins (MTs) are sulfhydryl-rich metal binding proteins present in the cytosol and serve as HMs/metalloid chelators (Anjum et al. 2015). Plant MTs comprise four subfamilies: p1, p2, p3, and pec. The p1 subfamily consists of MT1a and MT2; p2 subfamily involves MT2a and MT2b; p3 subfamily includes MT3, and pec subfamily contains MT4a and MT4b (Masters et al. 1994). Metalloid toxicity stimulates MTs encoding genes in plants (Karin et al. 1983). Apart from acting as chelators, MTs also play major role in regulating cell growth and its proliferation, and act as ROS scavengers (Klaassen et al. 2009). Metallothioneins combine with various metalloids by forming mercaptide bonds between metalloids and Cys residues of MTs (Moffatt and Denizeau 1997) and therefore the capability of MTs to sequester HMs/metalloids relies upon distribution, organization, and expression of Cys residues. Depending on their localization, MTs are expressed at different sites, for instance, MT1 and MT2 are expressed in roots, MT3 in leaves and MT4 in seeds. Major functions performed by MT1a and MT2b are redistribution of metals through phloem, whereas MT2a and MT3 function as chaperons. Localization study of MTs showed that these MTs are involved in detoxification mechanisms of HMs/metalloids. The immunoblot analyses of *Arabidopsis thaliana* MTs (MT1 and MT2) proteins demonstrated that higher accumulation of these proteins during metalloid stress may possibly be due to overexpression of corresponding mRNAs (Kagi and Schaffer 1988). Overexpression of MT1 and MT2 proteins was also observed by Nath et al. (2014) in *Oryza sativa* L. under As^V exposure. Gautam et al. (2012) also identified various MTs that participated in As detoxification in *Oryza sativa* L.

4.3 Hyperaccumulating Mechanism

The other main strategy for decontamination of environment from the metalloids is hyperaccumulation (Ernst 2000; Lasat 2002). Phytoremediation is an eco-friendly and cost-effective method that uses plants to remove contamination from environments. Plants having extraordinary metal-accumulating ability are categorized under hyperaccumulator plants (Cho-Ruk et al. 2006). In the present era, biotechnologists are showing extensive interest in plants that have the capacity to translocate and accumulate high amount of metalloids from substrate to shoots. Phytoremediation process takes the benefit of exceptional and selective uptake ability of metal/metalloids from root systems, and simultaneously their translocation, bioaccumulation, and degradation (Tangahu et al. 2011). Hyperaccumulating plants show a shoot-to-root element concentration ratio greater than one, which dictates that there is a higher translocation of element from root to shoot. Non-accumulating plants show restricted translocation of element from root to shoot, therefore have a shoot-to-root ratio significantly less than unity. Preferably, hyperaccumulators should flourish in contaminated environments, need minimal maintenance, and lead to high biomass accrual (Salido et al. 2003). This environmental friendly technique is used to decontaminate the soils, sediments, and sludge medium from metals, metalloids, radionuclides, nonmetals, and organic contaminants (Prasad and De Oliveira Freitas 2003). Among various plant species recognized as hyperaccumulators, most of the species belong to family Pteridaceae (Xie et al. 2009). *Isatis cappadocica*, a plant found in Iran is considered as As hyperaccumulator. Under As stress, this plant produces excess thiol and chelate As with GSH and PCs (Karimi et al. 2009). However, *Pteris vittata*, a fern is also well equipped with proficient systems for uptake, translocation, and sequestration of As^V/As^{III} (Xie et al. 2009; Danh et al. 2014). Experiment conducted by Sridhar et al. (2011) revealed that upon As exposure, *Pteris vittata* showed higher accumulation of As along the walls of vascular bundles of stems and to some extent in roots, therefore, no noteworthy alteration was seen in cell structure. Energy-dispersive X-ray microanalysis illustrated that As was primarily found in the epidermal cell vacuoles of *Pteris vittata* fronds (Lombi et al. 2002).

To overcome various limitations with hyperaccumulator plants, this technique can further be improved through genetic engineering (genoremediation). Additionally, improved understanding of molecular mechanisms of transgenic plants needs to be explored for improving the proficiency of phytotechnologies. Thus, in current era, advancement in the field of biotechnology through genetic engineering is explored extensively to increase the use of phytotechnology for HMs/metalloid remediation. In regard to this, gene expression of transporters proteins and molecular mechanisms involved in remediation process are being studied to increase the genoremediation (Cherian and Oliviera 2005). On the other hand, detrimental consequences of HMs/metalloids on living creature can also be alleviated through genetically engineered hyperaccumulators (Kumar et al. 2015; Rai et al. 2015). Molecular mechanisms involved in phytoremediation and gene manipulations for engineering transgenic hyperaccumulators is being examined via overexpression of PCs, MTs and GSH, transporter genes, and diminished oxidative injury or phytotoxicity (Rai et al. 2015; Gerhardt et al. 2017).

4.4 Chemical Modification

Various engineering approaches are being employed to develop plants resistant against abiotic stresses. One strategy includes biochemical transformation of HMs/metalloids to less toxic, volatile forms. In plants, chemical modification of As by reducing As^{V} to $\mathrm{As}^{\mathrm{III}}$ and regulating the $\mathrm{As}^{\mathrm{III}}$ level intracellularly may considerably influence As tolerance and detoxification efficiency (Bleeker et al. 2006). In this regard, functional analysis of genes is important, particularly those genes which are accountable for regulating As^{V} reduction (e.g., arsenate reductase; AR) and As levels cellularly. Dhankher et al. (2006) silenced the expression of an AR gene (AtACR2; As compounds resistance 2) of *Arabidopsis thaliana* roots and the result revealed that in the deficiency of As^{V} reduction in roots, less As was retained in roots whereas high amount of As was accumulated in shoots of transgenic plants when compared to the wild type. Chao et al. (2014) and Sanchez-Bermejo et al. (2014) recognized As-reducing enzymes namely HAC1/ATQ1 from the root epidermis and root hair cells, respectively, that provided As stress tolerance to plants by reducing As^{V} into $\mathrm{As}^{\mathrm{III}}$. Moreover, Meadows (2014) also revealed that HAC1/ATQ1 are engaged in diminishing As concentration in the different tissues of plants.

4.5 Modulation of Transcription Factor

It is well known that metalloids stimulate signaling pathways and regulate various transcription factors. The metalloids influence epidermal growth factor receptors (EGFR) which are induced in response to binding of metalloids to epidermal growth factor (EGF) (Farooq et al. 2009). Epidermal growth factor receptors negatively regulate the protein tyrosine phosphatases (PTPs). The thiol groups of PTPs are engaged in its dephosphorylation activity (Fu et al. 2000). Because, the thiol group of PTPs gets oxidized, the PTPs become inactivated and the transactivation of EFGR takes place (Ghosh et al. 1999). The activated EFGR stimulates phosphorylation of mitogen activated protein kinase (MAPK). The MAPKs are categorized in three groups such as extracellular signal regulated kinase1/2 (ERK1/2), stress activated protein kinases/Jun-N-terminal protein kinases, and p38 MAPK. The ERK1/2 modulates the growth and differentiation, whereas, SAPK/JUN and p38 MAPK control stress associated signal transduction pathway. Arsenite and Sb^{III} responsive repressor ArsR, govern the regulation of arsABC resistance genes (Ha et al. 2012). When As gets attached to repressor molecules, conformational alteration of ArsR takes place that leads to disintegration of ArsR from the promoter site thus permitting its transcription and it acts as an intracellular metalloid sensor (Haeder and Beringer 1981). Metalloids are also acknowledged to stimulate the MAPKs that modulate genes responsible for stress tolerance. Sodium arsenite induces stress responsive genes via AP-1 transcription factors. The Hog 1 MAPK regulates transcription of genes in collaboration with AP-1 transcription factors linked with metalloid associated stress response (Höller et al. 2014). In *Saccharomyces cerevisiae* yeast AP-1-like proteins Yap1p and Yap8p have been recognized as transcription factors which are related with metalloid (As and Sb) tolerance (Wysocki et al. 2001). Among these, Yap1p modulates the expression of genes which are accountable for oxidative stress. Upon As and Sb exposure, overexpression of Yap1p was observed and also increased expression of antioxidant genes was found in Yeast cells. In the budding yeast *Saccharomyces cerevisiae* Yap8p is shown to present in the nucleus where it mediates increased expression of the As detoxification genes ACR2 and ACR3 (Wysocki et al. 2001). These findings imply that Yap1p and Yap8p mediate tolerance to metalloids toxicity in yeast cells by activating transcription of separate defensive genes. Song et al. (2014) revealed that OsABCC1, a protein of Ctype ATP-binding cassette transporter (OsABCC) family, diminish the As concentration in the *Oryza sativa* L. grain by sequestering As in the vacuoles and restricting its distribution to other plant parts.

4.6 Induction of Antioxidant Defense and Glyoxalate Systems

Exposure of metalloids leads to excess production of ROS in plants (Finnegan and Chen 2012; Chandrakar et al. 2016). Additionally, metalloid toxicity modifies various vital plant functions at physiological, biochemical, and molecular levels. Metalloids are shown to inactivate or downregulate various enzymes, denature proteins, and disrupt cell membrane integrity which obstructs normal metabolism, photosynthesis, and respiration along with excess generation of free radicals. Reactive oxygen species negatively influences various cellular functions like damage nucleic acids, oxidize proteins, and cause lipid peroxidation (LPO) (Sigfridsson et al. 2004; Chandrakar et al. 2016). Additionally, methylglyoxal (MG), a cytotoxic reactive oxidative compound is impetuously generated when plants are exposed to abiotic stresses including metalloid toxicity which results in cellular damage ultimately leading to mutation and even cause cell death (Hasanuzzaman et al. 2014). Plant cells and their organelles like chloroplasts, mitochondria, peroxisomes, etc., employ antioxidant defense systems and glyoxalase systems to defend themselves against metalloid-induced toxic free radicals and MG. Extensive research has shown that the stimulation of the cellular antioxidant machinery is vital for protection against various stresses (Chandrakar et al. 2017a, b; Xalxo and Keshavkant 2018). The components of antioxidant defense system are enzymatic and non-enzymatic agents. Enzymatic antioxidants include SOD, catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate ascorbate (DHAR), and glutathione reductase (GR), while GSH, ascorbate (AsA) (both water soluble), carotenoids, and tocopherols (lipid soluble) are categorized as non-enzymatic members (Gill and Tuteja 2010; Chandrakar et al. 2017a, b; Yadu et al. 2017; Xalxo and Keshavkant 2018). The enzymatic and non-enzymatic antioxidant systems maintain intracellular redox homeostasis, thus preventing excess accumulation of toxic ROS inside the cells (Gill and Tuteja 2010). Glyoxalase or MG detoxification system is comprised of two efficient vital enzymes, glyoxalase I (Gly I) and glyoxalase II (Gly II) (Hasanuzzaman et al. 2014). Apart from these two systems, chelating agents like PCs, MTs, etc., are synthesized in plant cells to protect itself from various abiotic stresses (Cobbett and Goldsbrough 2002; Singh et al. 2015). Therefore, efficient functioning of plant defense systems (antioxidant defense and glyoxalase systems) against ROS and MG, and production of different chelating agents dictate the tolerance capability of plants under various abiotic conditions. On the other hand, the proficiency of defense system shows a discrepancy with plant genotypes and degree of toxicity. As the concentration of metalloid increases in the soil, plants lose their survival capacity. Hence, augmentation of abovementioned molecular approaches is one of the chief strategies to manage metalloid toxicity. Nowadays, exogenous application of various phytoprotectants like plant hormones, organic acids, signaling molecules, and trace elements are common in research and are anticipated to metalloid stress tolerance.

4.7 Synthesis of Proline

Proline (Pro) acts as a marker of various abiotic stresses and plays a vital role against stress tolerance. Various functions of Pro include osmoprotection, reorganization of chlorophyll, protection of enzymes from denaturation and protein stabilization, etc. It also acts as an energy source during abiotic stress condition to restore the growth and development. Proline accumulation in plants has been found to be amplified during various stress conditions and was associated with HMs/metalloid tolerance. Accumulation of Pro is common phenomenon that occurs in variety of plants during stress conditions (Dhir et al. 2004; Ahmad et al. 2012) and aids to overcome such situations by osmotic adjustment, enzyme protection, and stabilizing the structure of various organelles and macromolecules. Although, Pro does not directly chelate the HMs/metalloid, it stabilizes native protein structure by controlling their water content and thus this osmoprotectant facilitates in maintaining the proteins integrity. Studies have also shown that Pro acts as ROS scavenger and detoxifies it. Importantly, Pro also modulates the activities of antioxidant and glyoxalase pathway enzymes and makes the plant more tolerant to stress-induced oxidative stress (Hossain et al. 2010). Various researchers have demonstrated that, exogenous application of Pro showed increased tolerance against metal/metalloid-induced oxidative injury that was due to the stimulation of defensive proteins (Hossain et al. 2010; Kumchai et al. 2013; Irfan et al. 2014). One vital function of Pro includes intensified synthesis of GSH in cells that ultimately leads to generation of PCs (Zenk 1996). Ahmad and Gupta (2013) demonstrated that As-induced excess Pro accretion was found to be linked with augmented defense system and regulation of expressions of PCs, PCS, GR, and glutathione synthetase genes that makes the plant more tolerant to As stress.

4.8 Synthesis of Nitric Oxide and Metalloids Tolerance

In plants, nitric oxide (NO), a gaseous molecule, functions as signaling molecule and has significant role in growth and development. It has shown to modulate various physiological and molecular processes in plants, such as, protects plants against pathogen invasion and also helps plants to manage HMs/metalloid-imposed phytotoxicity (Bellin et al. 2013; Singh et al. 2016). Depending upon its concentration and localization, NO can stimulate beneficial as well as detrimental consequences within the cells (Leitner et al. 2009). Intensive research has been done to explore the function of NO as signaling molecule and stress alleviating agent in both plants and animals. For instance, NO functions as secondary messenger during neuronal signal transduction pathway, immune response, and cardiovascular homeostasis, and simultaneously can act as causative agent for various patho-physiological anomalies (Tuteja et al. 2004). Nitric oxide is well acknowledged to provide tolerance against HMs/metalloid-induced oxidative injury in plants and this involves the intensified expression of antioxidant gene (Liu et al. 2011). Nitric oxide protects plants from oxidative damage via (1) reacting with lipid radicals, which ceases elongation step of lipid peroxidation reaction, (2) by scavenging superoxide anion and peroxynitrite thus neutralizing them, which would otherwise have led to the generation of other reactive intermediates, (3) stimulation of enzymatic and non-enzymatic antioxidant, and (4) serving as a signal molecule in various biological processes, leading to alteration in gene expression (Bai et al. 2011; Hasanuzzaman et al. 2011, 2012; Ha et al. 2012). Various researchers have revealed that exogenous application of NO has been shown to provide a tolerance against HMs/metalloid such as As, Al, Cu, and Mn (Yu et al. 2005; Singh et al. 2009; Srivastava and Dubey 2012; Sun et al. 2014). Singh et al. (2009) observed that exposure of As^{V} amplified the oxidative damage in *Oryza sativa* L. which was evident by excess accrual of increased concentration of ROS and lipid peroxidized products (Malondialdehyde;

MDA). On the other hand, NO application to As^V stressed *Oryza sativa* L. plants showed reduced content of ROS and MDA, which depicts the protective role of NO against metalloid stress. Similar observation was also reported by Singh et al. (2016) in *Oryza sativa* L. under As stress. In similar study, there was a less membrane damage and that may be due to NO that neutralized ROS, which are responsible for membrane damage. Decreased content of As into roots and shoots in NO added As^V stressed plants may also be responsible for reduced oxidative stress. Although NO is a stable molecule it can react and scavenge free radicals like, ROS, and neutralize them, thus preventing the plant from oxidative stress (Liu et al. 2011). Several other researchers have also revealed the mitigation property of NO against wide range of environmental stresses (Bai et al. 2011; Hasanuzzaman et al. 2011, 2012).

5 Potential Biotechnological Strategies for Remediation of Metalloids

Various biotechnological advancements are employed for the removal of HM/metalloid containment from the ecosystem. One of the strategies used for cleaning environment from toxic pollutants is bioremediation, which utilizes natural and genetically modified microorganisms. It is appraised as a cost-effective and eco-friendly method. This method depends on advanced detoxification and degeneration of noxious contaminants either via excess accumulation of toxicant intracellularly or through enzymatic conversion of toxic compounds to lesser or completely non-toxic form (Brar et al. 2006). Many naturally or modified microbes acquire the capacity of degradation, transformation, or chelation of various toxic contaminants and thus offer improved strategies to fight against environmental pollution. Scientists deploy either natural or genetically manipulated microbes to eliminate HMs, metalloids, radioactive waste, and oil products from contaminated locations (Dixit et al. 2015).

Phytoremediation is another economical, green-clean tool used for removal of HMs/metalloids contaminations from environment. Studies have illustrated the ability to detoxify and transform the toxic pollutants to non-toxic products by plants and rhizospheric bacteria that can be removed without their accumulation in these. Moreover, in the presence of highly noxious metals and metalloids, plants uptake, transfer, and accrue them in the aboveground biomass, followed by subsequent harvesting for recovery of metal. Various researchers have studied and published the HM/metalloid uptake, detoxification, and its accrual but mainly these studies have been performed in model plants in laboratory at small scale (Ovecka and Takac 2014; Dhankher et al. 2011). Investigation at metabolomics level can facilitate the scientists to identify the metabolites that are related with HM/metalloid stress, and subsequent mapping of their metabolic pathways to recognize the associated genes (Kumar et al. 2014). One fascinating approach to increase the understanding of plant metabolism during HM/metalloid stress is to develop appropriate imaging techniques. In this regard, scientists have implemented Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), Matrix Assisted Laser Desorption

Ionization (MALDI), and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) (Jones et al. 2015). Still, intensive efforts are required to facilitate imaging visualization to determine the localization and distribution of HM/metalloid inside tissues. In spite of latest advancements made in biotechnology, like accessibility of entire genome sequences of numerous plants species, the potential of plants for remediation of contaminated site on large scale still needs attention. Complete genomes and transcriptomes of various HM/metalloid-tolerant organisms are exploited via next generation sequencing (He et al. 2011; Peña-Montenegro and Dussán 2013). Mass spectrometry-based proteomics is widely employed to study the HM, metalloid, and other stresses in plants, bacteria, and marine organisms (Zakeri et al. 2012; Muralidharan et al. 2012; Hossain and Komatsu 2013; Cvjetko et al. 2014). Altogether these approaches guarantee the use of genetically modified microbes with amplified phytoremediation proficiency. In upcoming years, more attention should be given to improve the phytoremediation method using genomic and metabolic engineering approaches to develop tolerant plant against HM/metalloids toxicity. One other approach is gene stacking method by which plants can be engineered for regulation of biological pathways that are involved in the uptake, detoxification, transport, and translocation to aerial parts and higher accretion of HMs/metalloids. Furthermore, more emphasis should be given to develop breeding programs to improve growth habits of naturally occurring hyperaccumulators and to breed with those traits that fasten their growth for commercial phytoremediation of HM/metalloid. Additionally, phytoremediation method can be used in collaboration with biofuel production in contaminated sites. This approach would be beneficial to eliminate the contaminants and concurrently generate renewable bioenergy.

6 Conclusions and Future Prospects

It is evident from various researches that non-judicious use and excessive presence of HMs/metalloids have lethal consequences on flora and fauna. These metalloids with the aid of various transporters protein like phosphate transporters, aquaglyceroporins, hexose transporters, sulfate transporters, etc., enter inside the plant cell. Some metalloids enter the plant via transporters of vital nutrients like phosphate, sulfate, etc., which are obligatory for plants' normal growth and metabolism. Metalloids stimulate antioxidant gene expression and complex signaling pathways, crucial for mediating cellular response against metalloid toxicity. Furthermore, PCs and MTs play vital role in metalloid sequestration inside the vacuoles and cell walls and render tolerance against metalloids toxicity. Biotechnological approaches are in progress to intensify plant tolerance against metalloids stress via, altering genes which are accountable for metalloid uptake and transport and by enhanced synthesis of GSH, PCs, and MTs that function as metalloid chelators and sequester. Increasing such genes responsible for metalloid sequestration leads to increased tolerance against metalloids toxicity. However, extensive progression in the field of genomics, transcriptomics, proteomics, and metabolomics will facilitate the researchers to investigate the details of intriguing pathways connected with plant molecular responses against metalloids stress.

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References

Abercrombie JM, Halfhill MD, Ranjan P, Rao MR, Saxton AM, Yuan JS, Stewart CN (2008) Transcriptional responses of *Arabidopsis thaliana* plants to arsenate As(V) stress. BMC Plant Biol 8:87–101

Agnihotri A, Seth CS (2016) Exogenously applied nitrate improves the photosynthetic performance and nitrogen metabolism in tomato (*Solanum lycopersicum* L. cv Pusa Rohini) under arsenic (V) toxicity. Physiol Mol Biol Plants 22:341–349

Ahmad MA, Gupta M (2013) Exposure *of Brassica juncea* (L) to arsenic species in hydroponic medium: comparative analysis in accumulation and biochemical and transcriptional alterations. Environ Sci Pollut Res 20:8141–8150

Ahmad MA, Gaur R, Gupta M (2012) Comparative biochemical and RAPD analysis in two varieties of rice (*Oryza sativa*) under arsenic stress by using various biomarkers. J Hazard Mater 217-218:141–148

Ahsan N, Lee DG, Kim KH, Alam I, Lee SH, Lee KW, Lee H, Lee BH (2010) Analysis of arsenic stress induced differentially expressed proteins in rice leaves by two-dimensional gel electrophoresis coupled with mass spectrometry. Chemosphere 78:224–231

Anjum NA, Hasanuzzaman M, Hossain MA, Thangavel P, Roychoudhury A, Gill SS, Rodrigo MA, Adam V, Fujita M, Kizek R, Duarte AC, Pereira E, Ahmad I (2015) Jacks of metal/metalloid chelation trade in plants-an overview. Front Plant Sci 6:192

Bai X, Yang L, Tian M, Chen J, Shi J, Yang Y, Hu X (2011) Nitric oxide enhances desiccation tolerance of recalcitrant *Antiaris toxicaria* seeds via protein *S* nitrosylation and carbonylation. PLoS ONE 6:e20714

Bellin D, Asai S, Delledonne M, Yoshioka H (2013) Nitric oxide as a mediator for defense responses. Mol Plant-Microbe Interact 26:271–277

Bleeker PM, Hakvoort HWJ, Bliek M, Souer E, Schat H (2006) Enhanced arsenate reduction by a CDC25-like tyrosine phosphatase explains increased phytochelatin accumulation in arsenate-tolerant *Holcus lanatus*. Plant J 45:917–929

Brar SK, Verma M, Surampalli RY, Misra K, Tyagi RD, Meunier N (2006) Bioremediation of hazardous wastes-a review. Pract Period Hazard Toxic Radioact Waste Manag 10:59–72

Brown JR, Theodore L, Le May HE, Bursten BE, Burdge JR (2002) Chemistry: the central science, 9th edn. The Central Science Prentice Hall, Upper Saddle River, p 0130669970

Campos NV, Arcanjo-Silva S, Freitas-Silva L, de Araujo TO, Souza-Fernande DP, Azevedo AA (2018) Arsenic hyperaccumulation in *Pityrogramma calomelanos* L. (Link): adaptive traits to deal with high metalloid concentrations. Environ Sci Pollut Res 25:10720–10729

Chandrakar V, Naithani SC, Keshavkant S (2016) Arsenic-induced metabolic disturbances and their mitigation mechanisms in crop plants: a review. Biologia 71:367–377

Chandrakar V, Yadu B, Meena RK, Dubey A, Keshavkant S (2017a) Arsenic-induced genotoxic responses and their amelioration by diphenylene iodonium, 24-epibrassinolide and proline in *Glycine max* L. Plant Physiol Biochem 112:74–86

Chandrakar V, Parkhey S, Dubey A, Keshavkant S (2017b) Modulation in arsenic-induced lipid catabolism in *Glycine max* L. using proline, 24-epibrassinolide and diphenylene iodonium. Biologia 72:292–299

Chandrakar V, Pandey N, Keshavkant S (2018) Plant responses to arsenic toxicity: morphology and physiology. In: Hasanuzzaman M, Nahar K, Fujita M (eds) Mechanisms of arsenic toxicity and tolerance in plants. Springer, Singapore. https://doi.org/10.1007/978-981-13-1292-2_2

Chao DY, Chen Y, Chen J, Shi S, Chen Z, Wang C, Danku JM, Zhao FJ, Salt DE (2014) Genome-wide Association Mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. PLoS Biol 12:e1002009

Cherian S, Oliviera M (2005) Transgenic plants in phytoremediation: recent advances and new possibilities. Environ Sci Technol 39:9377–9390

Cho-Ruk K, Kurukote J, Supprung P, Vetayasuporn S (2006) Perennial plants in the phytoremediation of lead-contaminated soils. Biotechnology 5:1–4

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cosio C, De Santis L, Frey B, Diallo S, Keller C (2005) Distribution of cadmium in leaves of *Thlaspi caerulescens*. J Exp Bot 56:565–575

Coyle P, Philcox JC, Carey LC, Rofe AM (2002) Metallothionein: the multipurpose protein. Cell Mol Life Sci 59:627–647

Cvjetko P, Zovko M, Balen B (2014) Proteomics of heavy metal toxicity in plants. Arh Hig Rada Toksikol 65:1–18

Danh LT, Truong P, Mammucari R, Foster N (2014) A critical review of the arsenic uptake mechanism and phytoremediation potential of *Pteris vittata*. Int J Phytoremed 16:429–453

Dhankher OP (2005) Arsenic metabolism in plants: an inside story. New Phytol 168:503–505

Dhankher OP, Rosen BP, McKinney EC, Meagher RB (2006) Hyperaccumulation of arsenic in the shoots of *Arabidopsis* silenced for arsenate reductase (ACR2). Proc Natl Acad Sci U S A 103:5413–5418

Dhankher OP, Pilon-Smits EAH, Meagher RB, Doty S (2011) Biotechnological approaches for phytoremediation. In: Altman A, Hasegawa PM (eds) Plant biotechnology and agriculture. Academic, Oxford, pp 309–328

Dhir B, Sharmila P, Saradhi PP (2004) Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. Aquat Toxicol 66:141–147

Dixit R, Wasiullah E, Malaviya D, Pandiyan K, Singh U, Sahu A (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7:2189–2212

Ernst WHO (2000) Evolution of metal hyperaccumulation and phytoremediation type. New Phytol 146:357–358

Farnese FS, Oliveira JA, Gusman GS, Leao GA, Silveira NM, Silva PM, Ribeiro C, Cambraia J (2014) Effects of adding nitroprusside on arsenic stressed response of *Pistia stratiotes* L. under hydroponic conditions. Int J Phytoremediation 16:123–137

Farnese FS, Oliveira JA, Paiva EAS, Menezes-Silva PE, da Silva AA, Campos FV, Ribeiro C (2017) The involvement of nitric oxide in integration of plant physiological and ultrastructural adjustments in response to arsenic. Front Plant Sci 8:516

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

Farooq MA, Gill RA, Ali B, Wang J, Islam F, Ali S, Zhou W (2015) Oxidative injury and antioxidant enzymes regulation in arsenic-exposed seedlings of four *Brassica napus* L. cultivars. Environ Sci Pollut Res 22:10699–10712

Farooq MA, Gill RA, Ali B, Wang J, Islam F, Ali S, Zhou W (2016) Subcellular distribution, modulation of antioxidant and stress related genes response to arsenic in *Brassica napus* L. Ecotoxicology 25:350–366

Farooq MA, Islam F, Yang C, Nawaz A, Athar H, Gill RA, Ali B, Song W, Zhou W (2017) Methyl jasmonate alleviates arsenic-induced oxidative damage and modulates the ascorbate-glutathione cycle in oilseed rape roots. Plant Growth Regul 84:135–148

Finnegan P, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. Front Physiol 3:118

Freeman JL, Zhang LH, Marcus MA, Fakra S, McGrath SP, Pilon-Smits EA (2006) Spatial imaging, speciation, and quantification of selenium in the hyperaccumulator plants *Astragalus bisulcatus* and *Stanley apinnata*. Plant Physiol 142:124–134

Frey B, Keller C, Zierold K, Schulin R (2000) Distribution of Zn in functionally different epidermal cells of the hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 23:675–687

Fu D, Libson A, Miercke LJ, Weitzman C, Nollert P, Krucinski J, Stroud RM (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. Science 290:481–486

Garg N, Singla P (2011) Arsenic toxicity in crop plants, physiological effects and tolerance mechanisms. Environ Chem Lett 9:303–321

Gautam N, Verma PK, Verma S, Tripathi RD, Trivedi PK, Adhikari B, Chakrabarty D (2012) Genome-wide identification of rice class I metallothionein gene: tissue expression patterns and induction in response to heavy metal stress. Funct Integr Genomics 12:635–647

Gerhardt KE, MacNeill GJ, Gerwing PD, Greenberg BM (2017) Phytoremediation of salt impacted soils and use of plant growth-promoting rhizobacteria (PGPR) to enhance phytoremediation. In: Phytoremediation. Springer, New York, pp 19–51

Ghosh M, Shen J, Rosen BP (1999) Pathways of As(III) detoxification in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 96:5001–5006

Gill S, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Gupta M, Gupta S (2017) An overview of selenium uptake, metabolism, and toxicity in plants. Front Plant Sci 7:2074

Gusman GS, Oliveira JA, Farnese FS, Cambraia J (2013a) Arsenate and arsenite: the toxic effects on photosynthesis and growth of lettuce plants. Acta Physiol Plant 35:1201–1209

Gusman GS, Oliveira JA, Farnese FS, Cambraia J (2013b) Mineral nutrition and enzymatic adaptation induced by arsenate and arsenite exposure in lettuce plants. Plant Physiol Biochem 71:307–314

Ha S, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2012) Cytokinins: metabolism and function in plant adaptation to environmental stresses. Trends Plant Sci 17:172–179

Haeder HE, Beringer H (1981) Influence of potassium nutrition and water stress on the content of abscisic acid in grains and flag leaves of wheat during grain development. J Sci Food Agric 32:552–556

Halperin S, Barzilay A, Carson M, Roberts C, Lynch J (1995) Germanium accumulation and toxicity in Barley. J Plant Nutr 18:1417–1426

Hasanuzzaman M, Hossain MA, Fujita M (2011) Nitric oxide modulates antioxidant defense and methylglyoxal detoxification system and reduces salinity induced damage in wheat seedling. Plant Biotechnol Rep 5:353–365

Hasanuzzaman M, Nahar K, Alam MM, Fujita M (2012) Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum*) seedlings by modulating the antioxidant defense and glyoxalase system. Aust J Crop Sci 6:1314–1323

Hasanuzzaman M, Nahar K, Hossain MS, Mahmud JA, Rahman A, Inafuku M, Oku H, Fujita M (2014) Coordinated actions of glyoxalase and antioxidant defense systems in conferring abiotic stress tolerance in plants. Int J Mol Sci 18:1. https://doi.org/10.3390/ijms18010200

He M, Li X, Liu H, Miller SJ, Wang G, Rensing C (2011) Characterization and genomic analysis of a highly chromate resistant and reducing bacterial strain *Lysinibacillus fusiformis* ZC1. J Hazard Mater 185:682–688

Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci 11:610–617

Höller S, Hajirezaei MR, Von WN, Frei M (2014) Ascorbate metabolism in rice genotypes differing in zinc efficiency. Planta 239:367–379

Hossain Z, Komatsu S (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. Front Plant Sci 3:310

Hossain MA, Hasanuzzaman M, Fujita M (2010) Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiol Mol Biol Plants 16:259–272

Huang K, Chen C, Zhang J, Tang Z, Shen Q, Rosen BP (2016) Efficient arsenic methylation and volatilization mediated by a novel bacterium from an arsenic- contaminated paddy soil. Environ Sci Technol 50:6389–6396

Irfan M, Ahmad A, Hayat S (2014) Effect of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. Saudi J Biol Sci 21:125–131

Iwasaki K, Sakurai K, Takahashi E (1990) Copper binding by the root cell walls of Italian ryegrass and red clover. Soil Sci Plant Nutr 36:431–439

Jones OAH, Dias DA, Callahan DL, Kouremenos KA, Beale DJ, Roessner U (2015) The use of metabolomics in the study of metals in biological systems. Metallomics 7:29–38

Kagi JHR, Schaffer A (1988) Biochemistry of Metallothionein. Biochemistry 27:8509–8515

Karimi N, Ghaderian SM, Raab A, Feldmann J, Meharg AA (2009) An arsenic accumulating, hyper-tolerant *brassica*, *Isatis cappadocica* Desv. New Phytol 184:41–47

Karin M, Cathala G, Nguyenhuu MC (1983) Expression and regulation of a human metallothionein gene carried on an autonomously replicating shuttle vector. Proc Natl Acad Sci U S A 80:4040–4044

Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238:215–220

Kumar S, Trivedi PK (2016) Heavy metal stress signaling in plants. In: Ahmad P (ed) Plant metal and interaction. Elsevier, New York, pp 585–603

Kumar A, Singh RP, Singh PK, Awasthi S, Chakrabarty D, Trivedi PK, Tripathi RD (2014) Selenium ameliorates arsenic induced oxidative stress through modulation of antioxidant enzymes and thiols in rice (*Oryza sativa* L.). Ecotoxicology 23:1153–1163

Kumar D, Singh VP, Tripathi DK, Prasad SM, Chauhan DK (2015) Effect of arsenic on growth, arsenic uptake, distribution of nutrient elements and thiols in seedlings of *Wrightia arborea* (Dennst.) Mabb. Int J Phytoremediation 17:128–134

Kumchai J, Huang JZ, Lee CY, Chen FC, Chin SW (2013) Proline partially overcomes excess molybdenum toxicity in cabbage seedlings grown in vitro. Genet Mol Res 12:5589–5601

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75–84

Kushwaha BK, Singh S, Tripathi DK, Sharma S, Prasad SM, Chauhan DK, Kumar V, Singh VP (2019) New adventitious root formation and primary root biomass accumulation are regulated by nitric oxide and reactive oxygen species in rice seedlings under arsenate stress. J Hazard Mater 361:134–140

Lang I, Sassmann S, Schmidt B, Komis G (2014) Plasmolysis: loss of turgor and beyond. Plants 3:583–593

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120

Leitner M, Vandelle E, Gaupels F, Bellin D, Delledonne M (2009) No signals in the haze: nitric oxide signaling in plant defence. Curr Opin Plant Biol 12:451–458

Li Y, Dhankher OP, Carreira L, Lee D, Chen A, Schroeder JI (2004) Overexpression of phytochelatin synthase in *Arabidopsis* leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiol 45:1787–1797

Li WX, Chen TB, Huang ZC, Lei M, Liao XY (2006) Effect of arsenic on chloroplast ultrastructure and calcium distribution in arsenic hyperaccumulator *Pteris vittata* L. Chemosphere 62:803–809

Liu Q, Hu C, Tan Q, Sun X, Su J, Liang Y (2008) Effects of As on As uptake, speciation, and nutrient uptake by winter wheat (*Triticum aestivum* L.) under hydroponic conditions. J Environ Sci 20:326–331

Liu X, Wang L, Liu L, Guo Y, Ren H (2011) Alleviating effect of exogenous nitric oxide in cucumber seedling against chilling stress. Afr J Biotechnol 10:4380–4386

Lombi E, Zhao FJ, Fuhrmann M, Ma LQ, MacGrath SP (2002) Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata*. New Phytol 156:195–203

Lou LQ, Shi GL, Wu JH, Zhu S, Qian M, Wang HZ, Cai QS (2015) The influence of phosphorus on arsenic uptake/efflux and As toxicity to wheat roots in comparison with sulfur and silicon. J Plant Growth Regul 34:242–250

Mallick S, Sinam G, Sinha S (2011) Study on arsenate tolerant and sensitive cultivars of *Zea mays* L.: differential detoxification mechanism and effect on nutrient status. Ecotoxicol Environ Saf 74:1316–1324

Masters BA, Quaife CJ, Erickson JC, Kelly EJ, Froelick GJ, Zambrowicz BP, Brinster RL, Palmiter RD (1994) Metallothionein- III is expressed in neurons that sequester zinc in synaptic vesicles. J Neurosci 14:5844–5857

Mateos-Naranjo E, Andrades-Moreno L, Redondo-Gómez S (2012) Tolerance to and accumulation of arsenic in the cordgrass *Spartina densiflora* Brongn. Bioresour Technol 104:187–194

Meadows R (2014) How plants control arsenic accumulation. PLoS Biol 12:e1002008

Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and non resistant plant species. New Phytol 154:29–43

Moffatt P, Denizeau F (1997) Metallothionein in physiological and physiopathological processes. Drug Metab Rev 29:261–307

Mumthas S, Chidambaram AA, Sundaramoorthy P, Ganesh KS (2010) Effect of arsenic and manganese on root growth and cell division in root tip cells of green gram (*Vigna radiata* L.). Emir J Food Agric 22:285–297

Muralidharan S, Thompson E, Raftos D, Birch G, Haynes PA (2012) Quantitative proteomics of heavy metal stress responses in Sydney rock oysters. Proteomics 12:906–921

Nath S, Panda P, Mishra S, Dey M, Choudhury S, Sahoo L, Panda SK (2014) Arsenic stress in rice: redox consequences and regulation by iron. Plant Physiol Biochem 80:203–210

Norton GJ, Lou-Hing DE, Meharg AA, Price AH (2008) Rice-arsenate interaction in hydroponics: whole genome transcriptional analysis. J Exp Bot 59:2267–2276

Ovecka M, Takac T (2014) Managing heavy metal toxicity stress in plants: biological and biotechnological tools. Biotechnol Adv 32:73–86

Pandey AK, Gautam A, Dubey RS (2019) Transport and detoxification of metalloids in plants in relation to plant-metalloid tolerance. Plant Gene 17:100171

Pathare V, Srivastava S, Sonawane BV, Suprasanna P (2016) Arsenic stress affects the expression profile of genes of 14-3-3 proteins in the shoot of mycorrhiza colonized rice. Physiol Mol Biol Plants 22:515–522

Patra M, Bhoumik N, Bandopadhyay B, Sharma A (2004) Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ Exp Bot 52:199–223

Peña-Montenegro TD, Dussán J (2013) Genome sequence an description of the heavy metal tolerant bacterium *Lysinibacillus sphaericus* strain OT4b.31. Stand Genomic Sci 9:42–56

Polsia PM, Lupini A, Araniti F, Longo C, Mauceri A, Sunseri F, Abenavoli MR (2016) Boron toxicity and tolerance in plants: recent advances and future perspectives. In: Ahmad P (ed) Plant metal interaction: emerging remediation techniques. Elsevier, Amsterdam

Prasad MNV, De Oliveira Freitas HM (2003) Metal hyperaccumulation in plants biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:110–146

Rai A, Bhardwaj A, Misra P, Bag SK, Adhikari B, Tripathi RD, Trivedi PK, Chakrabarty D (2015) Comparative transcriptional profiling of contrasting rice genotypes shows expression differences during arsenic stress. Plant Genome 8:1–14

Reichard JF, Puga A (2010) Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. Epigenomics 2:87–104

Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004) A critical analysis of the causes of boron toxicity in plants. Plant Cell Environ 25:1405–1414

Robinson BH, Lombi E, Zhao FJ, McGrath SP (2003) Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*. New Phytol 158:279–285

Salido AL, Kelly HL, Lim JM, Butcher DJ (2003) Phytoremediation of arsenic and lead in contaminated soil using Chinese brake ferns (*Pteris vittata*) and Indian mustard (*Brassica juncea*). Int J Phytoremed 5:89–103

Sanchez-Bermejo E, Castrillo G, del Llano B, Navarro C, Zarco-Fernandez S, Martinez-Herrera DJ (2014) Natural variation in arsenate tolerance identifies an arsenate reductase in *Arabidopsis thaliana*. Nat Commun 5:4617

Schneider J, Labory CR, Rangel WM, Alves E, Guilherme LR (2013) Anatomy and ultrastructure alterations of *Leucaena leucocephala* (Lam.) inoculated with mycorrhizal fungi in response to arsenic-contaminated soil. J Hazard Mater 262:1245–1258

Shaibur MR, Kawai S (2011) Arsenic toxicity in Akitakomachi rice in presence of Fe^{3+}-citrate. Adv Environ Biol 5:1411–1422

Sharma I (2012) Arsenic induced oxidative stress in plants. Biologia 67:447–453

Sharma P, Jha A, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. https://doi.org/10.1155/2012/217037

Siddiqui F, Tandon PK, Srivastava S (2015a) Analysis of arsenic induced physiological and biochemical responses in a medicinal plant, *Withania somnifera*. Physiol Mol Biol Plants 21:61–69

Siddiqui F, Tandon PK, Srivastava S (2015b) Arsenite and arsenate impact the oxidative status and antioxidant responses in *Ocimum tenuiflorum* L. Physiol Mol Biol Plants 21:453–458

Sigfridsson KGV, Bernát G, Mamedov F, Styring S (2004) Molecular interference of Cd^{2+} with Photosystem II. Biochim Biophys Acta 1659:19–31

Singh HP, Kaur S, Batish DR, Sharma VP, Sharma N, Kohli RK (2009) Nitric oxide alleviates arsenic toxicity by reducing oxidative damage in the roots of *Oryza sativa* (rice). Nitric Oxide 20:289–297

Singh VP, Singh S, Kumar J, Prasad SM (2015) Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate-glutathione cycle: possible involvement of nitric oxide. J Plant Physiol 181:20–29

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6:1143

Song WY, Tomohiro YT, Yamaji N, Ko D, Jung KH, Fujii-Kashino M, An G, Martinoia E, Lee Y, Ma JF (2014) A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. Proc Natl Acad Sci 111:15699–15704

Sridhar BBM, Han FX, Diehl SV, Monts DL, Su Y (2011) Effect of phytoaccumulation of arsenic and chromium on structural and ultrastructural changes of Brake Fern (*Pteris vittata*). Braz J Plant Physiol 23:1677–9452

Srivastava S, Dubey RS (2012) Nitric oxide alleviates manganese toxicity by preventing oxidative stress in excised rice leaves. Acta Physiol Plant 34:819–825

Stoeva N, Bineva T (2003) Oxidative changes and photosynthesis in oat plants grown in As-contaminated soil. Bulg J Plant Physiol 29:87–95

Stoeva N, Berova M, Zlatev Z (2005) Effect of arsenic on some physiological parameters in bean plants. Biol Plant 49:293–296

Sui H, Han BG, Lee JK, Walian P, Jap BK (2001) Structural basis of water-specific transport through the AQP1 water channel. Nature 414:872–878

Sun C, Lu L, Liu L, Liu W, Yu Y, Liu X, Hu Y, Jin C, Lin X (2014) Nitrate reductase-mediated early nitric oxide burst alleviates oxidative damage induced by aluminum through enhancement of antioxidant defenses in roots of wheat (*Triticum aestivum*). New Phytol 201:1240–1250

Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, Kumar PC, Murray HW (2000) Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. Clin Infect Dis 31:104–1107

Sweet G, Gandor C, Voegele R, Wittekindt N, Beuerle J, Truniger V, Lin EC, Boos W (1990) Glycerol facilitator of Escherichia coli: cloning of glpF and identification of the glpF product. J Bacteriol 172:424–430

Talukdar D (2013) Arsenic induced changes in growth and antioxidant metabolism of Fenugreek. Russ J Plant Physiol 60:652–660

Tan X, Li K, Wang Z, Zhu K, Tan X, Cao J (2019) A review of plant vacuoles: formation, located proteins, and functions. Plants 8:327

Tangahu BV, Abdullah SRS, Barsi H, Idris M, Anuar N, Mukhlisin M (2011) A review of heavy metals (As, Pb and Hg) uptake by plants through phytoremediation. Int J Chem Eng. https://doi.org/10.1155/2011/939161

Tice KR, Parker DR, DeMason DA (1992) Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. Plant Physiol 100:309–318

Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK, Rai AK (2015) Silicon-mediated alleviation of Cr (VI) toxicity in wheat seedlings as evidenced by chlorophyll florescence, laser induced breakdown spectroscopy and anatomical changes. Ecotoxicol Environ Saf 113:133–144

Tripathi DK, Singh S, Singh VP, Prasad SM, Dubey NK, Chauhan DK (2017) Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. Plant Physiol Biochem 110:70–81

Tuteja N, Chandra M, Tuteja R, Misra MK (2004) Nitric oxide as a unique bioactive signaling messenger in physiology and pathophysiology. J Biomed Biotechnol 4:227–237

Wang BL, Shi L, Li YX, Zhang WH (2010) Boron toxicity is alleviated by hydrogen sulfide in cucumber (*Cucumis sativus* L.) seedlings. Planta 231:1301–1309

Wysocki R, Chery CC, Wawrzycka D, Van Hulle M, Cornelis R, Thevelein JM, Tamas MJ (2001) The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*. Mol Microbiol 40:1391–1401

Xalxo R, Keshavkant S (2018) Hydrolytic enzymes mediated lipid-DNA catabolism and altered gene expression of antioxidants under combined application of lead and simulated acid rain in Fenugreek (*Trigonella foenum graecum* L.) seedlings. Ecotoxicology 27:1404–1413

Xalxo R, Keshavkant S (2019) Melatonin, glutathione and thiourea attenuates lead and acid rain induced deleterious responses by regulating gene expression of antioxidants in *Trigonella foenum graecum* L. Chemosphere 221:1–10

Xie QE, Yan XL, Liao XY, Li X (2009) The arsenic hyperaccumulator fern *Pteris vittata* L. Environ Sci Technol 43:8488–8495

Yadu B, Chandrakar V, Keshavkant S (2017) Glycinebetaine reduces oxidative injury and enhances fluoride stress tolerance via improving antioxidant enzymes, proline and genomic template stability in *Cajanus cajan* L. South Afr J Bot 111:68–75

Yu CC, Hung KT, Kao CH (2005) Nitric oxide reduces Cu toxicity and Cu-induced NH_4^+ accumulation in rice leaves. J Plant Physiol 162:1319–1330

Zakeri F, Sadeghizadeh M, Kardan MR, Shahbani Zahiri H, Ahmadian G, Asoumi F (2012) Differential proteome analysis of a selected bacterial strain isolated from a high background radiation area in response to radium stress. J Proteome 75:4820–4832

Zenk MH (1996) Heavy metal detoxification in higher plants-a review. Gene 179:21–30

Zhang J, Martinoia E, Lee Y (2018) Vacuolar transporters for cadmium and arsenic in plants and their applications in phytoremediation and crop development. Plant Cell Physiol 59:1317–1132

Zheng S, Ma JF, Matsumoto H (1997) High aluminum resistance in buckwheat: I. Al induced specific secretion of oxalic acid from root tip tips. Plant Physiol 117:745–751

Heavy Metals-Induced Morphophysiological and Biochemical Changes in *Mentha piperita* L.

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Abstract

Numerous anthropogenic activities had caused adulteration to normal soil, water, and air composition that directly and indirectly damaging the human environment. Environmental contamination from heavy metals is a major concern for the world community dealing in environmental management. High concentration of heavy metal concentrations in soil can destroy organic macronutrients, morphological variations plant growth inhibition. Heavy metals inhibit the physiological activities of plants; for instance, carbon dioxide (CO_2) fixation, gaseous exchange, photosynthetic translocation, nutrient absorption, and respiration. Normally plants develop antioxidant defense mechanisms against reactive oxygen species (ROS) in low concentration whereas higher concentration of heavy metals disrupts the balance between detoxification and ROS production. During heavy metal stress and accumulation in plants permanent stomatal closure takes place which may enhance ethylene production. Naturally, plants tolerate the stress having vital defensive mechanism against heavy metals. Mainly there are two main strategies used by plants to prevent accumulation of heavy metal in cytoplasm: tolerance and avoidance. Green plant with the premier defense mechanisms can be used for the removal of heavy metals from litho and hydro mediums potentially. This chapter reports the effect of heavy metals on the morphophysiological characters of continentally useful *Mentha piperita* L. in biomedicine and functional foods.

Keywords

Heavy metals · Physiological activities · Defense mechanisms · Contamination

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
Cd	Cadmium
CO_2	Carbon dioxide
Cr	Chromium
Cu	Copper
Fe	Iron
K^+	Potassium ion
Mn	Manganese
Ni	Nickel
O_2	Oxygen
Pb	Lead
PRX	Peroxiredoxins
ROS	Reactive oxygen species
SOD	Superoxide dismutase
Zn	Zinc

1 Introduction

The anthropogenic activities such as rapid urbanization, expanding human population, and unplanned industrial establishment had caused contamination in soil, air, and water. This contamination directly or indirectly harms the environment (Malik et al. 2010; Farid et al. 2017a). The effluents from processing industries with different composition when mixed with fresh water resources convert it into highly contaminated wastewater. The use of contaminated wastewater leads to many diseases, infections, and allergies not only for humans but also for crops, plants, and animals (Sood et al. 2012).

Pollution is classified majorly into land, air, and water, but water pollution leads the tables to degrade the environment (Sood et al. 2012). Water is the basic need of living organism, secondly it transports the contaminants. The major problem of the recent time is that world will face water scarcity and Pakistan is among those countries having challenge of drastic climate change (Ahmad et al. 2007; Jabeen et al. 2015). In the last few decades the ground water structure and landscape of Pakistan have been injudicious and damaged due to rapid urbanization, modern industrialization, and intensive agriculture practices (Azam et al. 2015).

Major three soil remediation categories are chemical remediation, physical remediation, and lastly biological remediation. First category, chemical remediation involved in chemical fixation, chemical leaching, vitrify technology, and in last electro-kinetic remediation (Yao et al. 2012). Second category, physical remediation includes soil washing, soil spading, and soil replacement. Physical remediation such as soil washing where the large amount of water used to remove contaminants from the soil surface. Soil spading and replacement are almost same to dig the contaminated soil area and help to dilute the contamination replacing the upper contaminated layer with fresh layer of soil (Ehsan et al. 2014; Khalid et al. 2017). Another mechanism of biological remediation includes plants, animals, and microorganisms that are used to remove contamination from soil. Phytoremediation is the sub-category of biological remediation. Phytoremediation is defined as using living plants to adsorb, mobilize, and clean contaminants and reduce the risk of heavy metal pollution (Jabeen et al. 2016). As it is a plant-based approach, therefore, phytostabilization may be defined as a process to immobilize the toxic chemicals and compounds in soil, so that plants could not been able to uptake them later (Parmar and Singh 2015). This technique is applied to decrease the bioavailability and mobility of contaminants in environment. This is helpful to stop migration of pollutants into food chain. The plants can stabilize contaminants through sorption by roots in rhizosphere (Wuana and Okieimen 2011).

1.1 Background of Heavy Metal

Environmental contamination from heavy metals is a hot discussion at international, regional, and local level (Malik and Zeb 2009) that largely impact the structural and functional integrity of a food web and ecosystem (Qadir and Malik 2009). The heavy metals are defined as transition metals having specific density ranges from 3.5 to 5.0 g cm^{-3} with ability of higher persistance and long half-lives (Appenroth 2010; Singh et al. 2011). The most common heavy metals are Fe, Co, Cu, Mn, Ni, Cr, Pb, Zn, and Cd (Singh et al. 2011; Farid et al. 2017b, 2018a). At particular, concentrations of heavy metals can affect central nervous system and other body organs of humans and animals (Lee et al. 2006) and pose threat to high trophic level species with great bioaccumulation and bio-magnification capacities (Malik and Zeb 2009).

As an example, toxicity of Ni depends upon its concentration in soil. The growth of roots is highly retarded by Ni as compared to shoot growth (Malarkodi et al. 2008). In acidic conditions Ni becomes more bio-available and increases metal mobility for plant in soil (Chardot et al. 2005). Heavy metals mostly affect the plants in two ways, first by interfering with other essential nutrients and second by causing oxidative stress. As the concentration of heavy metals increases the toxic effects become more apparent. If the heavy metal concentration remains high in soil, it can destroy organic macromolecules (Rahman et al. 2005; Krämer et al. 2007). Heavy metal accumulation may also replace other important macronutrients of soil such as Zn and Mn that may disturb the plant metabolism (Woolhouse 1983; Gonnelli et al. 2001). In plant cytoplasm usually high level of any heavy metal is avoided by organic acids. It also inhibits the mitotic activities, declines plant growth, and results in low fruit yield and quality (Madhava Rao and Sresty 2000; Molas 2002; Duarte et al. 2007).

Some of the heavy metals are present in the soil crust layer. Due to anthropogenic activities and some natural phenomenon, heavy metals are released in the environment that leads to soil contamination and increased toxicity in soil. The most toxic heavy metals which are present in soil are As, Cd, Ni, Cr, Pb, Hg, and Zn (Rizwan et al. 2017a). There are different sources of heavy metal such as magmatic, sedimentary, and metamorphic rocks weathering and soil formation. Heavy metals also present in mineral ores after mining and mineral processing operations. In bedrock heavy metal is present in different chemical composition and forms as ores. The metals generally present in the form of oxides and sulfides, mostly in soil sulfides of Hg, Cd, As, and Pb occur together with sulfides of Fe such as pyrite and sulfides of Cu such as chalcopyrite. Sometimes these heavy metals produced as by-product of metallurgical processes after mining. For example, Zn refining process produced Cd as by-product. Large amount of heavy metals is redistributed from one environment compartments to other such as water, soil, and air from contaminated soil to aquifers (Khalid et al. 2017).

Major anthropogenic sources of heavy metals are pesticides, insecticides, tanneries, batteries, mining and refining of ores, fertilizers and paper industries, waste disposal including solid waste, sludge and waste water irrigation, vehicular and industrial exhausts (Sallah-ud-Din et al. 2017). Heavy metals usually discharge in the environment in both organic and inorganic compositions and compound form. Furthermore, heavy metal resources are categorized into five major classes which are water disposal, agriculture, mining and smelting, industrial processes, and atmospheric disposition (Gajewska et al. 2006).

1.2 Background of Plants

Peppermint belongs to *Lamiaceae* family of plants with taxonomic name as *M. piperita*. It is a perennial herb local to Europe, particularly Greek origin. The term *Mentha* came from Greek mythology (Nair 2001). But currently cultivated around the world. *M. piperita* is a hybrid of water mint and spearmint (McKay and Blumberg 2006). Peppermint is a popular herb and it is used in many forms such as leaf extract, oil extract, and leaf water. This herb has a wide range of application and preferably used in food industry, hygiene products, pharmaceutical, and cosmetic products (Herro and Jacob 2010).

2 Availability and Uptake of Heavy Metals

Plants uptake the heavy metals from soil through roots through cortical tissues. Plants uptake the heavy metals because of their similarities with essential metals such as Zn and Fe and use apoplastic or symplastic pathway to assess xylem transportation system (Sarwar et al. 2017).

Heavy metals can affect plant growth by reducing the chlorophyll content and photosynthesis rate. Metals can lead to water stress in plants by reducing transpiration rate, stomatal conductance, and also reduce the relative leaf water content due to decrease in numbers of cell chloroplasts in xylem vessel. The concentration of heavy metals that present in water or soil is not freely available for plant uptake (Farid et al. 2018b, 2020a). Some heavy metals ions have faster mobility than others such as Pb is considerably immobile than Zn and Cd (Lasat 1999). For metal ions converting into available form are taken up by roots of plants and converted into soil solution. The mobility of ions depends upon on the pH of soil and of chelating agents. Other factors like temperature, root length and size, metal interaction, salinity, external metal concentration, and addition of nutrients are very important to affect the heavy metal ions mobility in soil (Rieuwerts et al. 1998; Olaniran et al. 2013).

Plants have numerous strategies to enhance the bioavailability of nutrients such as secretions of carboxylates, phytosiderophore, and acidification in rhizosphere to create chelate complex ions to bound metals in soil. If water concentration in soil is very low (Rieuwerts et al. 2006), it leads to strong binding with soil particles and many other organic contaminants of soil. Microorganisms play their role to increase metal bioavailability (Sarwar et al. 2017). In rhizosphere microorganisms secreted the enzymes, which are crucial to control the bioavailability of metal ions and their absorption into roots (Ghosh and Singh 2005). After metal ions are absorbed by roots, they contact the cell wall result into an ion exchanger. The uptake of heavy metal ions by roots is either by active transport or passive diffusion or electrochemical potential gradient by carrier. The carriers are complex agents, for example proteins or organic acids. For example, under A1 stress, the buckwheat's roots have been recorded to secret oxalic acid and create a non-oxalate complex which is then translocated into the leaves (Hall 2002).

3 Toxicity of Heavy Metal to Plant

Heavy metal enters in soil through various anthropogenic actions, and constitutes one of the major contaminants that limit the productivity of plants (Woolhouse 1983). Heavy metals are non-biodegradable and persistent in nature which leads to bio-magnification of toxic substance into food chain (Perfus-Barbeoch et al. 2002). Some heavy metals such as Cd and Pb are pure metals, whereas some are known as metalloids, for example As, which generally intrude into plant cells, because of its similar properties to other nutrients (Lombi et al. 2001). Sometimes particular transporter might be involved in uptake of heavy metal ions, for example in case of Cd in *Thlaspi caerulescens*. If heavy metals are allowed to accumulate in plants and crops, these toxic metals build threat to human health (Faller et al. 2005; Fig. 1).

There are three main recognized reasons of metal toxicity: (1) Direct interface with proteins due to their reaction for thioyl-, histidyl-, and carboxyl-groups, affecting the metals to target structural, catalytic, and transport sites of the cell. (2) Generation of ROS that can stimulate the antioxidant defense and play role in oxidative stress. (3) Dislocation of essential cations from particular binding sites leads to collapse the functions (Schützendübel et al. 2002; Fig. 2).

In different biological systems metals can show different chemical properties and distinct behaviors. However, these mechanisms might not be the cause of toxicity in plants. The role of oxidative stress in plants has been studied by assessing alteration in redox metabolic components of affected plants. These approaches gave proper insight into relationship between cellular redox imbalance and metal sensitivity (Farid et al. 2020b; Romero-Puertas et al. 2002).

It was established that heavy metal pollution of soil and air at a distance of 400 m from the source of pollution decreased the yields of fresh herbage by 9–16% and the yield of essential oil by up to 14% compared to the control, but did not negatively affect the essential oil content and its quality (Zheljazkov and Nielsen 1996).

4 Effects of Heavy Metal on Growth and Development of Plants

Peppermint a member of *Lamiaceae* family is a medicinal plant known internally as *M. piperita*. This plant has worldwide recognition as therapeutic agent used in herbal medicines and pharmaceutical and in food industries because of its novel health benefits. It is used for the treatment of nervous system and digestive disorders because of its anti-allergic, anti-tumor, and anti-microbial activities. It is also used in anorexia, cramping, diarrhea, and nausea. People use all forms of this plant like water, leaves, and leaf extract but most common form is used for essential oil (Farid et al. 2015; Habiba et al. 2015). Essential oil is extracted by distillation from healthy, fresh, and dried grounded leaves. Plant essential oil has a large number of chemical fractions with major ones are menthone and menthol, limonene, pulegone, and menthofuran. But chemical composition has variation according to geographical

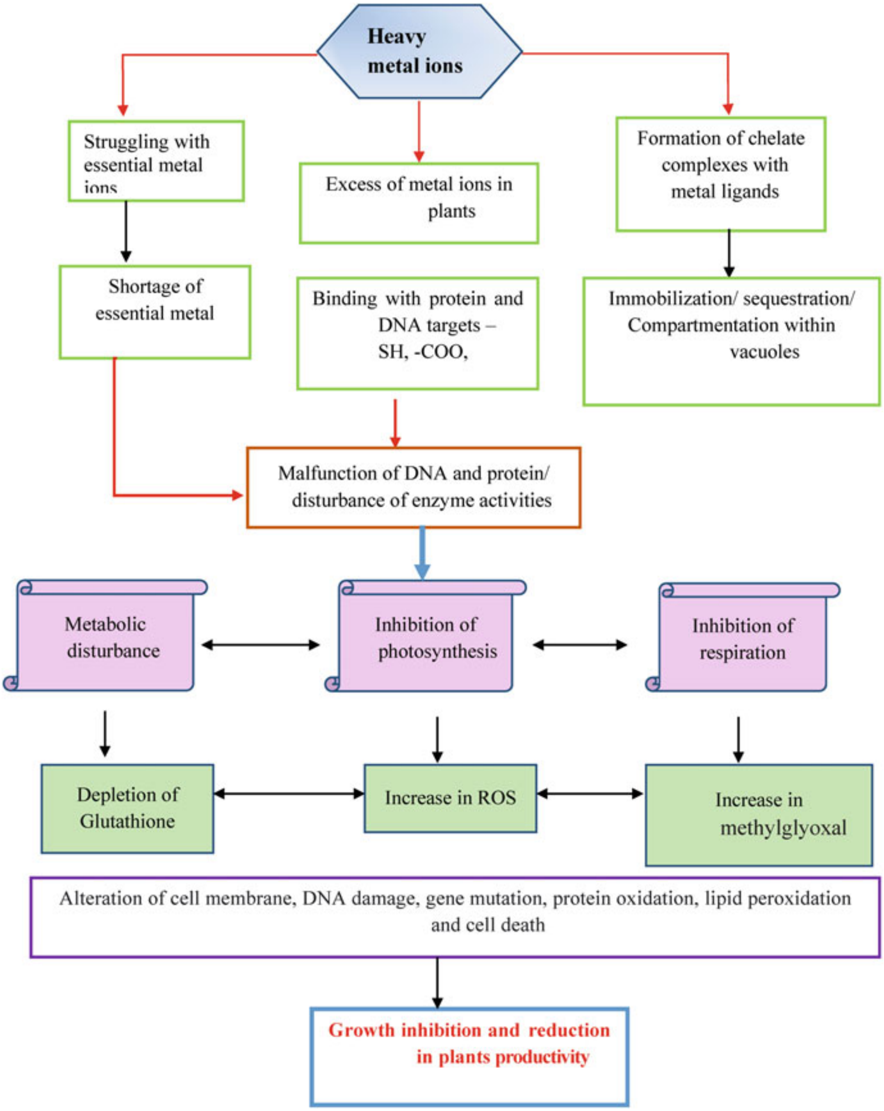

Fig. 1 Different toxic effects of heavy metals in biochemical and physiological activities in plants

region and maturity of plant. There are almost 23 species of *Mentha* across the world (Rasouli et al. 2017).

Mentha crispa also known as a garden plant and being used for the phytoextraction of Pb. They dosed the plants by Pb to investigate the effect of different doses as such as 900, 1800, 3600, 7200, and 9000 mg/kg. Study shows that aerial part and roots are not much affected as compared to control group. But green mass, number of leaves, and budding negatively influenced by Pb. Mostly metal

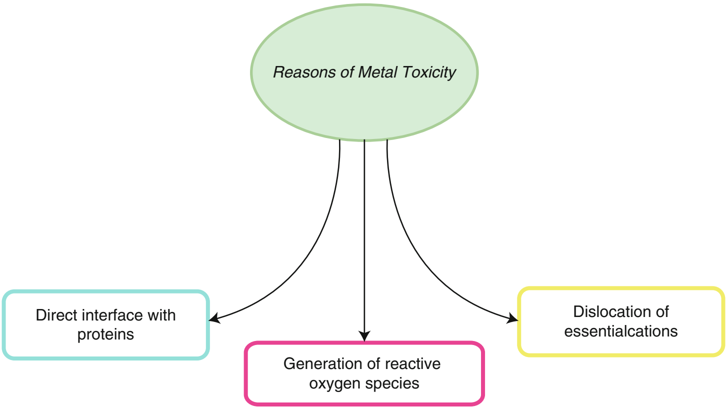

Fig. 2 Three main recognized reasons of metal toxicity

accumulates in aerial and root parts. However, *M. crispa* accumulates and tolerates Pb high concentrations but on the other half it is counted as hyperaccumulator species of Pb.

M. spicata has efficacy for phytoextraction of Pb and Zn in hydroponic solution. In two weeks experiment, plant remained in solution and after that measured its chlorophyll content, lower and upper leaves which proved that Zn and Pb reduced the chlorophyll content and root growth. The results further showed that less Zn entered in plant as compared to Pb however Zn do not enter in leaves tissues of plant (Bekiaroglou and Karataglis 2002). Heavy metal cause structural damage, growth inhibition and reduction in biochemical and physiological activities of plant. Both photosynthetic and growth pigments are pretentious by occurrence of heavy metal (Oancea et al. 2005).

Fe, Zn, Mn, and Cu decline the growth of plant at high concentrations; however, Ni, Cd, Cr, and Pb decline the growth of plants even in lower concentration in soil. Heavy metal inhibits the physiological activities; for instance, CO_2 fixation, gaseous exchange, photosynthate translocation, nutrient absorption, and respiration. The difference in metal accumulation is not correlated with tolerance of heavy metal (Sharma and Agrawal 2005).

5 Effect of Heavy Metal Stress on Anti-Oxidative Enzyme

Antioxidant defense mechanisms reduced the ROS concentration and prevent them from beyond toxic threshold (Mittler et al. 2004). But abiotic stress disrupts the balance between detoxification and ROS production (Møller et al. 2007). There are two components in antioxidant network which consist of non-enzymatic and enzymatic components: Hydrogen peroxide decomposition by APX, O_2 scavenging by SOD, CAT and peroxiredoxins are chiefly related with maintenance of cellular redox steady state (Mittler 2002). Isoforms of SOD, PRX, and APX are localized in several subcellular compartment; however, CAT is mainly localized in peroxisome. Many other enzymes such as glutathione-S transferases and glutathione peroxidase also play role in redox poise of cell (Smith et al. 2004). Heavy metal stresses to *Mentha* plants cause change in antioxidant enzyme functions such as exposure duration and metal ion concentration. Despite lack of discrete patterns the responses show the changes in redox status of cells caused by heavy metals (Farid et al. 2017c; Sharma and Dietz 2009).

6 Physiology Attributes

Heavy metals such as Ni, Cd, and Cu are reported to disturb the photosynthesis function in plants, crops, and vegetations in direct and indirect way. Some heavy metals disturb the enzymes of photosynthetic carbon reduction cycle (Hossain and Komatsu 2013). It is also recorded that heavy metals are reason for decline in the total chlorophyll content in pigeon pea (Zhang et al. 2007). Research study has revealed that carotenoids are usually less affected by heavy metal, causing in lower chlorophyll and carotenoid ratio in plants. Under Cu toxicity, decrease in chlorophyll content and photosynthetic electron transportation was observed (Gwóźdź et al. 1997). Due to heavy metal stress the net photosynthesis rate declines in mature leaves. Cd can cause reduction in transpiration rate, leaf area, and photosynthesis in maize (Pál et al. 2006). Plants exposed to heavy metal show decrease in transpiration rate and increase in stomatal resistance. Cd can increase stomatal and mesophyll resistance to carbon dioxide uptake in leaves which leads to inhibit net photosynthesis (Fediuc and Erdei 2002). High heavy metal stress and accumulation in plants leads to permanent stomatal closure and enhances ethylene production (Rizwan et al. 2017b; Ahmad et al. 2020).

Toxic heavy metals such as Co, Cd, Cr, Ni, and Pb are recorded to cause reduced uptake by immobilization in roots. Heavy metal also causes water stress in crops. Heavy metal such as Cr affects the pigment status, seed growth and germination, senescence, and nutrient content (Sharma and Agrawal 2005).

7 Major Defense Mechanisms Used by Plants Against Heavy Metal Stress

Plants tolerance is the key defensive mechanism against heavy metals. Naturally plants are equipped with various defense mechanisms to tolerate against the stress of heavy metals (Sharma and Dietz 2009). Plants have the natural ability to keep the heavy metals concentrations below toxicity level. Mainly there are two main strategies which are used by plants to prevent accumulation of heavy metal in cytoplasm: tolerance and avoidance. Avoidance is defined as the ability of plants to obstruct unnecessary metal uptake (Tong et al. 2004). However, tolerance is defined as the ability of plants to cope after accumulation of heavy metals in tissues.

The tolerance mainl depends on the type of metal and its concentration, plant species and its growth and development stage (Navari-Izzo and Quartacci 2001).

7.1 Avoidance Mechanisms

The mechanism of avoidance used chelation, exclusion of metal, and translocation in plants (Navari-Izzo and Quartacci 2001). X-ray diffraction analysis has revealed interactions of varying magnitude, namely major, minor, trace, and rare between the soil carbohydrates and minerals that support the concept of metal chelation (Zubair et al. 2012). By preventing the metal ion transport across plasma membrane of plant, the chelating agent enhanced the K^+ efflux and accumulation of heavy metals in cytoplasm and alternating metal cell binding capability that case change in membrane permeability (Yang et al. 2005).

Plasma membrane is the preferred place for heavy metals toxicity execution. If plant tends to control transportation of metal ions but fails, then that will lead to disruption in whole plants functions. Toxicity of heavy metal in plasma membrane causes oxidation and cross linking of thiols protein, K^+ leakage, and inhibited the action of membrane protein and also variation in membrane lipid as well as fluidity (Thakur et al. 2016).

7.2 Chelation and Compartmentalization

If ions pass into cytosol, they can be eradicated by chelation. The compartmentalization and chelation of heavy metal ions are the strongest defense mechanism which is used by plants to prevent from metal toxicity (Callahan et al. 2006; Farid et al. 2019). There are many organic and inorganic ligands present in cytoplasm which can be used for compartmentalization (Krämer et al. 2007). The main organic compounds that can used as chelating agent are, for example, metallothioneins, phytochelatins, amino acids, organic acids, and carbohydrates. However, some inorganic compounds such as silicates and phosphate are also used for chelation (Haydon and Cobbett 2007).

8 Conclusion

It is proven through literature that heavy metals of soil are managed either by antioxidant enzymes which play a major role in stress induced by heavy metals or in uptake of heavy metals using plants which is known as phytoremediation. The tolerance mechanism in plant was stimulated by antioxidant enzymes that helped in deceleration the oxidation of biomolecules and block the process the chain oxidation reactions. Antioxidant enzymes' major function is to survive the plants in stress condition when metal ions excessively accumulate in body of plants. Due to the natural defense mechanisms of plant, the heavy metal from soil and water removed off. These factors include plant growth rate, concentration of heavy metal ions in soil, tolerance during stress, temperature, pH, and binding properties. The factors like translocation, uptake, and sequestration of heavy metal ions in plant with fast growth rate, high biomass, and hyperaccumulator genes for metal. For the remediation of soil high biomass hyperaccumulator plants are used widespread. Soils contaminated with heavy metals have gained alarming and have gained attention of the world because of enhanced geologic and anthropogenic activities. Research studies demonstrated that plants cultivated on metal contaminated soils usually showed reduction in growth, performance, and yield. Here it is concluded that like many dysfunctions of plants under the influence of heavy metals cultivars of *Mentha* species could be grown such polluted areas, but the yield reduction due to heavy metals contamination. It may be suggested that although mentha affected by heavy metals but still remained a very profitable crop and it could be used as substitute than most contaminated crops.

References

Ahmad MSA, Hussain M, Saddiq R, Alvi AK (2007) Mungbean: a nickel indicator, accumulator or excluder? Bull Environ Contam Toxicol 78:319–324. https://doi.org/10.1007/s00128-007-9182-y

Ahmad R, Ali S, Rizwan M, Dawood M, Farid M, Hussain A, Wijayae L, Alyemenie MN, Ahmad P (2020) Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. Physiol Planta 168(2):289–300

Appenroth KJ (2010) Soil heavy metals. Crossroads 19:1–18. https://doi.org/10.1007/978-3-642-02436-8

Azam I, Afsheen S, Zia A et al (2015) Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. Biomed Res Int 2015:942751. https://doi.org/10.1155/2015/942751

Bekiaroglou P, Karataglis S (2002) The effect of lead and zinc on *Mentha spicata*. J Agron Crop Sci 188:201–205. https://doi.org/10.1046/j.1439-037X.2002.00559.x

Callahan DL, Baker AJM, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12. https://doi.org/10.1007/s00775-005-0056-7

Chardot V, Massoura ST, Echevarria G et al (2005) Phytoextraction potential of the nickel hyperaccumulators *Leptoplax emarginata* and *Bornmuellera tymphaea*. Int J Phytorem 7:323–335. https://doi.org/10.1080/16226510500327186

Duarte B, Delgado M, Caçador I (2007) The role of citric acid in cadmium and nickel uptake and translocation, in *Halimione portulacoides*. Chemosphere 69:836–840. https://doi.org/10.1016/j.chemosphere.2007.05.007

Ehsan S, Ali S, Noureen S, Mehmood K, Farid M, Ishaque W, Shakoor MB, Rizwan M (2014) Citric acid assisted phytoremediation of Cd by *Brassica napus* L. Ecotoxicol Environ Saf 106:164–172

Faller P, Kienzler K, Krieger-Liszkay A (2005) Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of photosystem II by competitive binding to the essential Ca 2+ site. Biochem Biophys Acta-Bioenerg 1706:158–164. https://doi.org/10.1016/j.bbabio.2004.10.005

Farid M, Ali S, Ishaque W, Shakoor MB, Niazi NK, Bibi I, Dawood M, Gill RA, Abbas F (2015) Exogenous application of EDTA enhanced phytoremediation of cadmium by *Brassica napus* L. Int J Environ Sci Technol 12(12):3981–3992

Farid M, Ali S, Rizwan M, Ali Q, Abbas F, Bukhari SAH, Saeed R, Wu L (2017a) Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. Ecotoxicol Environ Saf 145:90–102

Farid M, Ali S, Rizwan M, Saeed R, Tauqeer HM, Sallah-Ud-Din R, Azam A, Raza N (2017b) Microwave irradiation and citric acid assisted seed germination and phytoextraction of nickel (Ni) by *Brassica napus* L.; morpho-physiological and biochemical alterations under Ni stress. Environ Sci Pollut Res 24(25):2150–2164

Farid M, Ali S, Akram NA, Rizwan M, Abbas F, Bukhari SAH, Saeed R (2017c) Phyto-management of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. Environ Sci Pollut Res 24(20):16845–16859

Farid M, Ali S, Zubair M, Saeed R, Rizwan M, Sallah-Ud-Din R, Azam A, Ashraf R, Ashraf W (2018a) Glutamic acid assisted phyto-management of silver contaminated soils through sunflower; physiological and biochemical response. Environ Sci Pollut Res 25(25):25390–25400

Farid M, Ali S, Rizwan M, Ali Q, Saeed R, Nasir T, Abbasi GH, Rehmani MIA, Ata-Ul-Karim ST, Bukhari SAH (2018b) Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. Ecotoxicol Environ Saf 151(30):255–265

Farid M, Ali S, Saeed R, Rizwan M, Ali B, Azam A, Ashraf Hussain A, Ahmad I (2019) Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. Int J Phytorem 21(8):760–767

Farid M, Ali S, Rizwan M, Yasmeen T, Arif MS, Riaz M, Saqib M, Zia ur Rehman M, Ayub MA (2020a) Combined effects of citric acid and 5-aminolevulinic acid in mitigating chromium toxicity in sunflower (*Helianthus annuus* l.) grown in Cr spiked soil. Pak J Agri Sci 57(3):1–11

Farid M, Farid S, Zubair M, Rizwan M, Ishaq HK, Ali S, Ashraf U, Alhaithloul HAS, Gowayed S, Soliman MH (2020b) Efficacy of *Zea mays* L. for the management of marble effluent contaminated soil under citric acid amendment; morpho-physiological and biochemical response. Chemosphere 240:124930

Fediuc E, Erdei L (2002) Physiological and biochemical aspects of cadmium toxicity and protective mechanisms induced in *Phragmites australis* and *Typha latifolia*. J Plant Physiol 159:265–271. https://doi.org/10.1078/0176-1617-00639

Gajewska E, Skłodowska M, Słaba M, Mazur J (2006) Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biol Plant 50:653–659. https://doi.org/10.1007/s10535-006-0102-5

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18. https://doi.org/10.15666/aeer/0301_001018

Gonnelli C, Galardi F, Gabbrielli R (2001) Nickel and copper tolerance and toxicity in three Tuscan populations of *Silene paradoxa*. Physiol Plant 113:507–514. https://doi.org/10.1034/j.1399-3054.2001.1130409.x

Gwóźdź EA, Przymusiński R, Rucińska R, Deckert J (1997) Plant cell responses to heavy metals: molecular and physiological aspects. Acta Physiol Plant 19:459–465. https://doi.org/10.1007/s11738-997-0042-5

Habiba U, Ali S, Farid M, Shakoor MB, Rizwan M, Ibrahim M, Abbasi GH, Hayat T, Ali B (2015) EDTA enhanced plant growth, antioxidant defense system and phytoextraction of copper by *Brassica napus* L. Environ Sci Pollut Res 22(2):1534–1544

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11. https://doi.org/10.1093/jxb/53.366.1

Haydon MJ, Cobbett CS (2007) Transporters of ligands for essential metal ions in plants: research review. New Phytol 174:499–506. https://doi.org/10.1111/j.1469-8137.2007.02051.x

Herro E, Jacob SE (2010) *Mentha piperita* (Peppermint). Dermatitis 21:327–329. https://doi.org/10.2310/6620.2011.10080

Hossain Z, Komatsu S (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. Front Plant Sci 3:1–12. https://doi.org/10.3389/fpls.2012.00310

Jabeen A, Huang X, Aamir M (2015) The challenges of water pollution, threat to public health, flaws of water laws and policies in Pakistan. J Water Resour Prot 7:1516–1526. https://doi.org/10.4236/jwarp.2015.717125

Jabeen N, Abbas Z, Iqbal M, Rizwan M, Jabbar A, Farid M, Ali S, Ibrahim M, Abbas F (2016) Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. Arch Agr Soil Sci 62(5):648–652

Khalid S, Shahid M, Niazi NK et al (2017) A comparison of technologies for remediation of heavy metal contaminated soils. J Geochem Explor 182:247–268. https://doi.org/10.1016/j.gexplo.2016.11.021

Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Lett 581:2263–2272. https://doi.org/10.1016/j.febslet.2007.04.010

Lasat MM (1999) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res 2(1):5

Lee CSL, Li X, Shi W et al (2006) Metal contamination in urban, suburban, and country park soils of Hong Kong: a study based on GIS and multivariate statistics. Sci Total Environ 356:45–61. https://doi.org/10.1016/j.scitotenv.2005.03.024

Lombi E, Zhao FJ, McGrath SP et al (2001) Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. New Phytol 149:53–60. https://doi.org/10.1046/j.1469-8137.2001.00003.x

Madhava Rao KV, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci 157:113–128. https://doi.org/10.1016/S0168-9452(00)00273-9

Malarkodi M, Krishnasamy R, Chitdeshwari T (2008) Phytoextraction of nickel contaminated soil using castor phytoextractor. J Plant Nutr 31:219–229. https://doi.org/10.1080/01904160701853654

Malik RN, Zeb N (2009) Assessment of environmental contamination using feathers of *Bubulcus ibis* L., as a biomonitor of heavy metal pollution, Pakistan. Ecotoxicology 18:522–536. https://doi.org/10.1007/s10646-009-0310-9

Malik RN, Jadoon WA, Husain SZ (2010) Metal contamination of surface soils of industrial city Sialkot, Pakistan: a multivariate and GIS approach. Environ Geochem Health 32:179–191. https://doi.org/10.1007/s10653-009-9274-1

McKay DL, Blumberg JB (2006) A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phyther. Res 20(8):619–633

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498. https://doi.org/10.1016/j.tplants.2004.08.009

Molas J (2002) Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni(II) complexes. Environ Exp Bot 47:115–126. https://doi.org/10.1016/S0098-8472(01)00116-2

Møller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Nair B (2001) Final report on the safety assessment of Mentha Piperita (peppermint) oil, *Mentha Piperita* (peppermint) leaf extract, *Mentha Piperita* (peppermint) leaf, and *Mentha Piperita* (peppermint) leaf water. Int J Toxicol 20(3):61–73

Navari-Izzo F, Quartacci MF (2001) Phytoremediation of metals tolerance mechanisms against oxidative stress. Minerva Biotecnol 13(2):73

Oancea S, Foca N, Airinei A (2005) Effects of heavy metals on plant growth and photosynthetic activity. Analele Ştiinţifice Ale Univ:3–6

Olaniran AO, Balgobind A, Pillay B (2013) Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. Int J Mol Sci 14:10197–10228. https://doi.org/10.3390/ijms140510197

Pál M, Horváth E, Janda T et al (2006) Physiological changes and defense mechanisms induced by cadmium stress in maize. J Plant Nutr Soil Sci 169:239–246. https://doi.org/10.1002/jpln.200520573

Parmar S, Singh V (2015) Phytoremediation approaches for heavy metal pollution : a review. J Plant Sci Res 2:1–8

Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548. https://doi.org/10.1046/j.1365-313X.2002.01442.x

Qadir A, Malik RN (2009) Assessment of an index of biological integrity (IBI) to quantify the quality of two tributaries of river Chenab, Sialkot, Pakistan. Hydrobiol 621:127–153. https://doi.org/10.1007/s10750-008-9637-0

Rahman H, Sabreen S, Alam S, Kawai S (2005) Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. J Plant Nutr 28:393–404. https://doi.org/10.1081/PLN-200049149

Rasouli H, Adibi H, Moasefi N (2017) Peppermint and its functionality: a review. Arch Clin Microbiol 8:1–16

Rieuwerts JS, Thornton I, Farago ME, Ashmore MR (1998) Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem Speciat Bioavailab 10:61–75. https://doi.org/10.3184/095422998782775835

Rieuwerts JS, Ashmore MR, Farago ME, Thornton I (2006) The influence of soil characteristics on the extractability of Cd, Pb and Zn in upland and moorland soils. Sci Total Environ 366:864–875. https://doi.org/10.1016/j.scitotenv.2005.08.023

Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017a) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere 187:35–42

Rizwan M, Ali S, Qayyum MF, Ok YS, Adress M, Ibrahim M, Zia-ur-Reham M, Farid M, Abbas F (2017b) Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: a critical review. J Hazard Mater 322:2–16. https://doi.org/10.1016/j.jhazmat.2016.05.061

Romero-Puertas MC, Palma JM, Gómez M et al (2002) Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ 25:677–686. https://doi.org/10.1046/j.1365-3040.2002.00850.x

Sallah-Ud-Din R, Farid M, Saeed R, Ali S, Rizwan M, Tauqeer HM, Bukhari SAH (2017) Citric acid enhanced the antioxidant defense system and chromium uptake by Lemna minor L. grown in hydroponics under Cr stress. Environ Sci Pollut Res 24(21):17669–17678. https://doi.org/10.1007/s11356-017-9290-0

Sarwar N, Imran M, Shaheen MR et al (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116

Schützendübel A, Nikolova P, Rudolf C, Polle A (2002) Cadmium and H2O2-induced oxidative stress in *Populus x canescens* roots. Plant Physiol Biochem 40:577–584. https://doi.org/10.1016/S0981-9428(02)01411-0

Sharma RK, Agrawal M (2005) Biological effects of heavy metals: an overview. J Environ Biol 26:301–313

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50. https://doi.org/10.1016/j.tplants.2008.10.007

Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharmacol 43:246–253. https://doi.org/10.4103/0253-7613.81505

Smith AP, DeRidder BP, Guo WJ et al (2004) Proteomic analysis of Arabidopsis glutathione S-transferases from benoxacor- and copper-treated seedlings. J Biol Chem 279:26098–26104. https://doi.org/10.1074/jbc.M402807200

Sood A, Uniyal PL, Prasanna R, Ahluwalia AS (2012) Phytoremediation potential of aquatic macrophyte, Azolla. Ambio 41:122–137. https://doi.org/10.1007/s13280-011-0159-z

Thakur S, Singh L, Wahid ZA et al (2016) Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. Environ Monit Assess 188:206. https://doi.org/10.1007/s10661-016-5211-9

Tong YP, Kneer R, Zhu YG (2004) Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. Trends Plant Sci 9:7–9. https://doi.org/10.1016/j.tplants.2003.11.009

Woolhouse HW (1983) Toxicity and tolerance in the responses of plants to metals. Physiol Plant Ecol 12:245–300. https://doi.org/10.1007/978-3-642-68153-0_8

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:1–20. https://doi.org/10.5402/2011/402647

Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353. https://doi.org/10.1016/j.jtemb.2005.02.007

Yao Z, Li J, Xie H, Yu C (2012) Review on remediation technologies of soil contaminated by heavy metals. Procedia Environ Sci 16:722–729. https://doi.org/10.1016/j.proenv.2012.10.099

Zhang FQ, Wang YS, Lou ZP, De Dong J (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere 67:44–50. https://doi.org/10.1016/j.chemosphere.2006.10.007

Zheljazkov VD, Nielsen NE (1996) Effect of heavy metals on peppermint and cornmint. Plant Soil 178(1):59–66

Zubair M, Anwar F, Ashraf M, Ashraf A, Chatha SAS (2012) Effect of green and farmyard manure on carbohydrates dynamics of salt-affected soil. J Soil Sci Plant Nutr 12(3):497–510

Heavy Metals Induced Physiological and Biochemical Changes in Fenugreek (*Trigonella foenum-graceum* L.)

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Abstract

The review presents the response of *Trigonella foenum-graceum L.* (Fenugreek) under stress induced by heavy metals on physiological and biological attributes with particular emphasis on plant growth and metal accumulation by plants. Pollution due to heavy metals is a global problem and a plenty of diseases are associated with metal pollution. These heavy metals cannot be degraded and stay in the environment for many years and are root of many other problems. The presence of heavy metals in soil, affects the plant morpho-physiological and biochemical attributes along with yeild. The availability of heavy metals in plant causes the reduction in nutrient uptake, increase in production of antioxidant enzymes and reactive oxygen species, reduction in chlorophyll content, inhibition in plant cell division, effect on electrical conductivity, and accumulation of heavy metal in the plant. The accumulation of metals in plant can cause biomagnification and affects the whole ecosystem.

Keywords

Fenugreek · Accumulation · Plant growth · Reactive oxygen species · Heavy metal toxicity

Abbreviations

APX	Ascorbate peroxidase
BCR	Bureau of reference
CAT	Catalase
DNA	Deoxyribonucleic acid
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
Eh	Redox potential
H_2O_2	Hydrogen per oxide
MDA	Malondialdehyde
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
USEPA	United States Environmental Protection Agency

1 Introduction

In current years, heavy metal contamination is a global problem. A group of metals having bulk more than 5 g/cm^3 is known as heavy metals (Madivoli et al. 2016). Heavy metal contamination is under consideration on global level, due to their potential to damage soil ecosystem function and human health through food consumption (Cui et al. 2004). Excessive amount of metals in soil ecosystem can transfer pollution in other ecosystem such as underground water and in food chain (Micó et al. 2006; Kumar Sharma et al. 2007; Zhuang et al. 2009). Some heavy metals are vital nutrients, which are required in low quantity, and are mandatory for standard growth and yield of plants. A few of them are nickel (Ni), zinc (Zn), cobalt (Co), iron (Fe), cadmium (Cd), manganese (Mn), molybdenum (Mo), copper (Cu), lead (Pb), arsenic (As), and chromium (Cr). These nutrients protect plant and microbes from stress against various environmental circumstances. But an accumulation of these vital nutrients in high concentration can affect plants and animals by damaging bacterial species, with inhibition of enzymatic activities, biochemical responses, and uncontrolled oxidation-reduction reactions in the cell. Heavy metals are persistent, as they cannot be degraded by natural procedures and hence are everlasting in the environment (Abd-Alla et al. 2014). Contamination of soil and water bodies with heavy metals is getting severe, almost in the entire globe. Numerous researchers have widely discussed the toxic effects of heavy metals. High concentration of metals in soil damages soil microbes, reduces soil productiveness, reduces the total yield and accumulation in the food chain, and causes multiple diseases in humans (McGrath et al. 1995; Dahmani-Muller et al. 2001; Khan 2001; Qiao and Luo 2001; McGrath et al. 2002). Contamination of soil ultimately decreased the quality of surface and groundwater which further damage the human health (Muchuweti et al. 2006; Guney et al. 2010). According to USEPA (1997) metals of prompt worry are Pb, Zn, Cd, Cu, Cr, and Fe on. *T. foenumgraecum* (fenugreek) is a yearly grown vegetable, nurtured all over the sphere, particularly Asia, Middle Eastern states, and extends in the Mediterranean sink. In count to healing possessions, it is identified to uptake huge amounts of particular heavy weight metals including Cd, Ni, Cu, Ar, Pb, Cr, etc. The high concentration of these metals reduces the growth of fenugreek (Zayneb et al. 2015). Research shows that vegetables can also uptake heavy metals, but relatively in low concentration by other plants. Foliar uptake of heavy metals is also a way of metals uptake by vegetables. Study suggests that high metal concentration is found in leaf of vegetables. Cr and Cu can accumulate in high concentration. Cd and Pb pose highest risk of cancer in children. The bioaccumulation of metals through non-dietary intake of soil is an important exposure way (Li et al. 2015).

1.1 Occurrence of Heavy Metals

Natural and human activities both are the reason of environmental degradation. Weathering of rocks is a natural source of these toxic metals (Gupta et al. 2009; Ignatius et al. 2014). Anthropogenic activities like release of metal and metalloids from industrial activities, mining tails, disposal of solid and liquid waste of industries, paints and gasoline products, use of chemical nourishments and pesticides, sewage sludge, burning of carbon-based raw material including fossil fuels, petrochemicals, irrigation by wastewater, and depositions from atmospheric overthrows are the reason of heavy metal contamination in soil and water (Wuana and Okieimen 2011). Heavy metals potentially are known to accumulate in the food web, and stay in the environment for numerous years. Heavy metals are toxic in nature and therefore these are concern of environmental pollution. Heavy metals take account of Fe, Cr, Ag, Pb, Cd, Zn, Ni, As, Co, and the platinum assembly components (Nagajyoti et al. 2010). Plants can uptake metals in high concentration, if there is great concentration of these metals in soil. As these metals are toxic in nature, acute and chronic disease can arise if heavy metals accumulate in the food chain (Geldmacher 1984; Alloway 2012; Farid et al. 2020a, b). Gastrointestinal, breathing problem, acute problems of heart, damage to brain, and kidney failure are acute disease due to contact with Cd and Zn. Exposure to heavy metals for a very long period causes long-term illness such as skin cancer, damage to lubricate membranes, and various general effects on the innards (Friberg et al. 1986; Mudgal et al. 2010; Mohod and Dhote, 2013). Lead is in highest quantity in the environment and poisonous among heavy metals all around the globe (Gupta et al. 2009; Ignatius et al. 2014). Toxicity of any metal hinges upon nature and concentration of metal. Metals are distributed in heterogeneous group according to their chemical and biological properties. Some metals like Pb, Cr, Co, Zn, Hg, Cd, Ni, Cu are extremely harmful in complex form as well as in soluble form. Occurrence of heavy metals in any media of environment, i.e., air, water, and soil, even in trace amount is harmful for organisms. Mostly heavy metals come into contact with an organism through food and water consumption (Pickering and Owen 1997; Jadia and Fulekar 2009).

1.2 Sources of Heavy Metals

Heavy metals in soil and water bodies are resulting from natural sources, including metals present in earth core and human activities which mostly contribute in metalloids contamination (Farid et al. 2018a). There are numerous natural and anthropogenic sources which are affecting agrarian soil and water bodies. The soil and water bodies near industrial units that are contaminated with different heavy metals depend upon raw material and activities in the industrial unit. The urban soil is contaminated mostly with Pb, Zn, Cd, and Cu (Wuana and Okieimen 2011). The sources of these metals are industries, paint, traffic, and numerous non-point sources. Heavy metals in agricultural land are due to the natural composition of land or by atmospheric depositions. The application of industrial contaminated wastewater to agricultural land is the reason of heavy metal contamination (Alloway 2013). Initially, spread of urban areas, expand in industrialization, use of hazardous compounds in industries, solid waste disposal, spillage of chemicals, use of fertilizers to improve crop yield, and the use of toxic herbicides and pesticides were considered the major reason of heavy metal contamination. But current research shows that reasons of heavy metal contamination are mineral phosphate use for the manufacturing of chemical fertilizers, municipal solid waste, untreated effluent from industries, sewage sludge and industrial sludge use in the fields, incineration of hospital, and other waste (Rahman et al. 2007; Jamali et al. 2007;

Javed et al. 2009; Malik and Zeb 2009). The occurrence of heavy metals is important to know, for the health of environment, food, water, and the biodiversity. The quantity of metals varies with spatial variations. Geochemical properties vary from place to place and variation in intensity of land use process. The contamination from numerous sources can result in high metal concentration and high toxicity on plants and microbial community (Alloway 2012; Farid et al. 2018b).

Contamination of environment in emerging states, comprising Pakistan, due to heavy metals is studied by many scientists (Hardoy et al. 1992; Qadir et al. 2001; Jamali et al. 2007). Aquatic ecosystems, including major rivers of Pakistan, have been reported as highly contaminated by heavy metals pollution like Hg (Tariq et al. 1994; Tehseen et al. 1994; Mubeen et al. 2010). Several studies reported different heavy metal polluted zones of Pakistan, that included the land contaminated by industrial activities and river ecosystems (Qadir et al. 2001; Javed et al. 2009; Malik et al. 2010; Mirza et al. 2010; Muhammad et al. 2011). In Pakistan constant wastewater application to the agrarian land has caused a gathering of poisonous metals in wastewater flooded land and also in underground water. Mahmood and Malik (2014) show that the heavy metals contaminated water was used for irrigation purposes in Lahore, Pakistan. Fan industry is releasing dust and high quantity of Cd, Cu, Pb, Fe, and Ni. Dust and emissions from fan industry could be related to human health hazard (Bashir et al. 2017; Azam et al. 2015).

1.3 Effects of Heavy Metals on Environment

There are numerous specific and possible causes of heavy metal release in the environment as it is extensively used in many processes. Metals are used in the metal industry, aircraft production and finishing, jewelry, as a preservation agent of wood, pigments, production of vehicle, paints, and in petroleum refinery industry. Food may be contaminated by metals, particularly mushrooms, nuts, shellfishes, and chocolates, which can also accumulate in the liver (Antunes et al. 2003). Copper is a micronutrient and many biological processes depend upon Cu. But like other heavy metal it is toxic at high concentration and it can generate Reactive oxygen species (Girotti 1985; Vos and Schat 1991). Copper exists in two forms, i.e., Cu^{2+} and Cu^{+}. Cu participates in the process of photosynthesis, respiration of mitochondria, response to oxidative stress, hormonal signaling, and for protein regulations (Marschner 1995; Raven et al. 1999). The potential sources of Cu are mining, pesticide production, chemical industry, and metal piping. The permissible limit is 0.1 mg\L. Its higher amount can affect fitness such as anemia, damage to liver and kidney, stomach, brain damage, skin, pancreas, myocardium, and intestinal irrigation (Antunes et al. 2003; Singh et al. 2011). If the Cu concentration increases from requiring level the plant growth is inhibited due to interference in the process of respiration and photosynthesis. Plants which grow under the access of Cu concentration usually produce low biomass and low chlorophyll content (Quartacci et al. 2000; Yruela 2005) (Fig. 1).

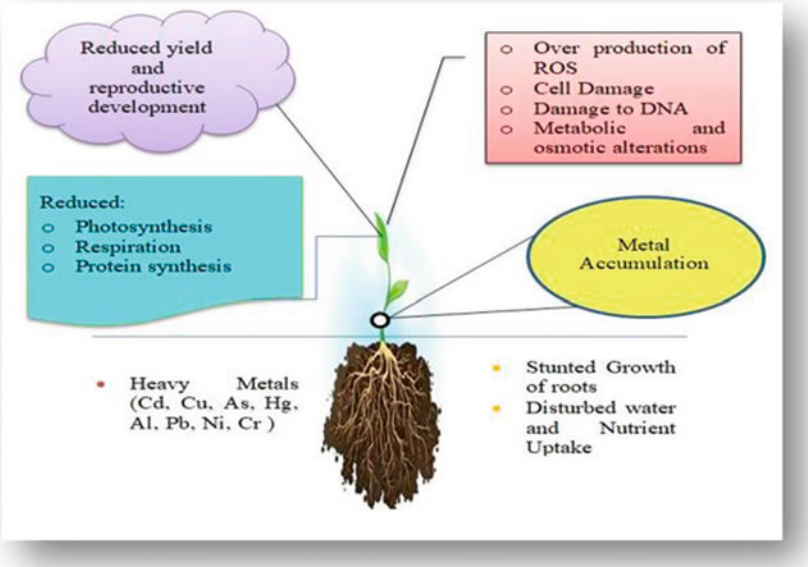

Fig. 1 Heavy metals uptake and possible toxic effects

1.4 Availability and Transportation of Metals

The availability of metals depends upon concentration of metals and different environmental circumstances. The plant species that are grown on metallic soils have established capability to store a large amount of metals and salts in higher portions of plants without displaying marks of harmful effects on plants (Baker and Brooks 1989; Entry et al. 1999). Therefore the plants are valued for the phytoremediation of environment, but also a vital character in biological and chemical prospecting, and thus having interferences on the health of humans through biomagnification in the food chain. The metals can reduce growth of neighbor plant species through leaching and will resist in contradiction of the fungal pathogens. Concentration of metal and its bioavailability define its toxicity (Farid et al. 2017a). To enter in biological systems, metals must be in solution form. Some heavy metals are unavailable due to their low concentration and solubility but some are in abundance on the earth surface and is readily soluble someway (Ahmad et al. 2020). The bioavailability of metal depends upon soil related dynamics such as the pH of the medium, redox potential (Eh) of soil, type of soil with texture, salts in water and soil, and carbon-based content. Toxicity or deficiency of metals depends upon these factors. Application of water to polluted soil gives rise to increased solubility of heavy metals in the environment (Farid et al. 2017b). Some metals concentration lower down due to complex formation and precipitation with sediments at the bottom layer in water (Forstner and Wittmann 1979; Flemming and Trevors 1989). Sungur et al. (2014) describes that Mn, Cd, and Pb in soil are more available and there is low fraction of immobile metal because of high acid and reducible fraction of metal. Availability of metals is related to soil characteristics and plant can uptake high fraction of mobile metals. BCR technique provides information about binding of metal in environment, uptake potential of plant, and rate of biomagnification in food chain. (Min et al. 2013) evaluated the availability of heavy metals in various environmental circumstances and trend of effects on ecosystem. According to this research heavy metals toxicity on ecosystem declines in following order: Cd > Zn > Cu > As > Pb. Cd has highest potential to damage ecological system.

1.5 Accumulation and Toxicity of Metals on Plants

Numerous mechanisms are adopted by plants to bear the toxic effects of metals. Excess metal concentration in soil is the reason of higher accumulation in plants and pose risk to food chain and stress on the plant. Usually the heavy metal uptake by plants is in small quantity due to limited availability, but accumulation of some heavy metals is high because they are readily available due to their solubility such as Cu, and also some other metals are investigated by many researchers (Mantovi et al. 2003). Cu mostly accumulates in plant roots in high concentration in comparison to other parts of plants amongst diverse types (Ernst 2000; Llugany et al. 2003; Lou et al. 2004).

Each metal damages different activity of plant. Mn damages the process of photosynthesis. Excess concentration of Ni causes chlorosis and necrosis in plants. The accumulation of Co restricts Fe uptake, growth inhibition of plant. In presence of Pb early seed growth is reduced. Cr metal has potential to inhibit seed germination (Farid et al. 2017c). Hg causes physical injuries and stomata closure in plants. In presence of Cu physical injuries and cytotoxic damages to plants are common. Cd has potential to damage physical attributes of plant and can injure plant cells along low growth, chlorosis, browning of plant roots, and ultimately plant death. Zn and Cd can reduce plant growth, metabolic disturbance, and production of ROS (reactive oxygen species) in plants (Nagajyoti et al. 2010). Metal toxicity in plants is determined by the amount of metal, time of contact, and on physiological plant development age (Ahsan et al. 2007). Symptoms of toxicity of metals are normally related to low growth, leaf damage due to inhibition of photosynthesis (Jabeen et al. 2016; Cuypers et al. 2002). Harmful effects of these metals led to low chlorophyll concentration in the leaves, changes the quantity of chloroplasts, and damage to cell membranes (Quartacci et al. 2000; Bernel et al. 2004; Yruela 2005). The development of genes is also poor under metal toxicity (Ahsan et al. 2007). Accumulation of some metals in seeds causes a reduction in root development as they do not have a strong mechanism of defense against the toxicity (Garrido et al. 2010). Nitrogen metabolism is affected under metal toxicity. Enzymes are changed which are involved in the amino acid and nitrate reduction under a higher quantity of metals and reduce the amount of total nitrogen (Llorens et al. 2000). Metal related stress causes damage in the cell membrane, and production of ROS in *H. vulgare* plant and damage to genes under the higher amount of heavy metals according to Tamas et al. (2006) and Gonzalez-Mendozaa et al. (2009). Numerous researches were accompanied to measure the toxic effects of heavy metals on the growth of plant, metabolism, and mineral uptake. Excess of metals causes reduction in plant biomass production and growth, reduction in nutrient uptake, and inhibition in photosynthetic process (Yang et al. 2002a, b). Metals have the capacity to harm cells and protein. Heavy metals generate ROS including H_2O_2, which are harmful to plant cells (Sahi et al. 2007). Chlorosis, wilting, reduction in nutrient uptake, carbohydrates metabolic disturbance, genotoxic and cytotoxic effects on plants are due to Cd accumulation in plans. Cd mostly accumulates in roots of lettuce. The accumulation of Cd generally reduces yield of all plants. In presence of Cd, Fe content is reduced and cause inhibition in biological processes (Santos et al. 2010; Dias et al. 2013).

2 Physiological Effects of Heavy Metals

2.1 Effects on Plant Morphology and Growth

High concentration of heavy metal affects plant physiology and reduces plant growth in fenugreek. Various studies show that accumulation or presence of heavy metals in soil or water reduces yield in fenugreek at different concentrations. Kaur (2016) studied the remediation and response of fenugreek (*T. foenum-graceum*) for Pb. The biomass of the plant was decreased as the quantity of metal increased in soil media. Pb was tolerated by the plant at concentration of 100 mg/L and plant progression was also good while at 800 mg/L of Pb usage, the plant growth was at the lowest point. At high metal concentrations the seed germination was also particularly inhibited. (Sengar et al. 2008; Bhardwaj et al. 2009; Azmat and Samaratunge 2009) also report the same mechanism of plant dry mass reduction when the Pb concentration was increased in soil, which causes water stress on plant due to reduced uptake of water. The potential of fenugreek to accumulate Pb in soil was also studied (Tanwar et al. 2013) by using Ethylenediaminetetraacetic acid (EDTA) with amended *Glomus mosseae*. Pb was provided to plant in five different concentrations, i.e., 0, 50, 100, 400, and 800 mg/kg. In result of increased metal concentration the growth of *G. mosseae* was reduced, but in the presence of EDTA G. mosseae provides more phosphorus (P) to plant roots and shoots.

To access the uptake of Cd and Pb a study was conducted by Singh and Chhibba (2010) in a screen house as test crops by using fenugreek and mustard. Pb was provided at 160 mg to mustard and at 80 mg/kg to fenugreek in soil, while Cd provided to mustard and fenugreek was at the rate of 20 mg/kg. EDTA was also provided in three different concentrations, i.e., 0.5, 1, and 1.5 g/kg to increase metals uptake, but the dry mass proportion was reduced by increasing the amount of EDTA. There were about 23% reduction in the roots of mustard, but fenugreek yield was reduced by 70% in presence of Cd at the rate of 20 mg/kg. Similarly for Pb, there was a decrease in fenugreek growth in comparison to mustard.

Dheri et al. (2007) have also conducted a research in order to measure natural ability for remediation of Cr contaminated silt and sandy soils by spinach, raya, and fenugreek. Five concentrations of Cr were provided (0, 1.25, 2.5, 5.0, and 10.0 mg/kg of Cr) for 21 days for all plants and after seedlings they were grown for 60 days. Diethylenetriaminepentaacetic acid (DTPA) extractable was also measured to evaluate the potential to uptake Cr. When the metal quantity was enhanced in both soils the main shoot was reduced in sandy soil as compared to silty soil in the presence of Cr. At high metal concentrations the decrease in Dry mass was significantly reduced irrespective of soil type. The decrease was from 3.91 to 1.73 g pot^{-1} in fenugreek plant under Cr stress.

Zayneb et al. (2015) measured the capability of fenugreek to extract the Cdcl2 in various concentrations in soil. At an initial stage plant growth was very sensitive even at a very low quantity of metal. At 0.1 mM of Cd no particular influence on the growth of the plant was observed, but as the metal concentration was increased from 0.5, 1, and 10 mM of Cd, there was a reduction in amylase activity. The Cd also exaggerated many other plant progression factors. At high metal concentration upper parts of the plant did not grow at all. By the application of Cd there was a reduction in plant dry mass growth by 50%.

Plants under natural conditions perform their functions at best. But Cu induced stress does not allow plants to work properly and disturb the plant natural process greatly, according to Walters (2003) and Mazen (2004). Inhibition of plant growth under Cu induced stress is reported by Groppa et al. (2008) along a damage to color of leaves, damage to root growth. The growth of roots under Cu stress is inhibited after 7–15 days of application in sunflower plant. Similarly, inhibition in plant root growth and reduction in dry biomass under Cu treatments were observed. In various plants along with reduction of root growth, polyamine production is also observed, which provide a mechanism against toxicity in pants under Cu induced stress according to Groppa et al. (2007).

3 Biochemical Effects of Metals

3.1 Reduction in Nutrient Uptake

Various studies show that under metal induced stress mineral uptake, such as potassium and magnesium by the plant, is also reduced. The deficiency in Fe content is high under metal accumulation and damage to biological activities. It has been also observed that under metal stress mineral uptake by root is also disturb which cause reduction in photosynthesis process and reduces plant yield (Sallah-ud-Din et al. 2017; Farid et al. 2020b) Thus, reduction in magnesium uptake is considered the main reason of low growth of plant under metal accumulation in fenugreek plant (Harrison et al. 1994; Ouzounidou et al. 1998; Clarkson and Lüttge 1989; Chen et al. 2000; Vinit-Dunand et al. 2002). Under even a low concentration of metals, metabolic variation and plant growth is reduced. In soil media metals are less mobile but in hydroponic media metals are readily available to plants and cause toxic effect on the plant. For vegetables, metals are more toxic and inhibit plant growth and show symptoms of toxicity by reducing plant growth (Fernande and Henriques 1991). At high metal concentration plant growth is inhibited and photosynthesis is reduced. The toxicity of metal on plant depends upon the growth stage of the plant as well (Xiong et al. 2006).

3.2 Production of Reactive Oxygen Species

All the heavy metals damage plant growth by prohibition of photosynthetic process and protein synthesis. Different studies reported that after the application of metals to various plant species cause a reduction in glutathione content. The production of lipid peroxides in plant roots is the indication of production of reactive oxygen species (ROS) under metal induced stress. The production of ROS causes damage to plasma membranes and blindness of cell membrane (Mittler 2002; Wang et al. 2004). Greater availability of metals to plant cause oxidative stress and cause reduction in carotenoid content, which play the role of the guard for chlorophyll (Han 1999). Production of MDA and H_2O_2 increases due to production of ROS.

3.3 Effects of Heavy Metals on Cell Division of Plant

Reduction in plant growth due to induction of metals is reported by many researchers (Lin and Parker 1999; Groppa et al. 2001, 2003; Xiong et al. 2006). Growth inhibition occurs due to cell death because metals bind themselves to proteins, which led alterations in protein structures. Because of alterations in protein structures enzymatic activities are also altered and plant growth is inhibited (Ranieri et al. 2005; Groppa et al. 2008). When the plant undergoes through metal stress it causes the mutation in tissues and mesophyll cells of the plant. Genotoxicity is most common effect of heavy metals on plants especially under Cd stress where it damage the DNA, proteins strand breaks and ultimately mutation through binding with nucleous. (Thomas and Thomas 2001; Pourakbar et al. 2007; Singh et al. 2007; Aly and Mohamed 2012; Azooz et al. 2012) reported that under increasing concentration of Cu to wheat species reduced seed germination, seedling growth, and reduction in root growth, moisture content, fresh and dry weight was reduced by higher concentration because of reduction in photosynthetic activity. Plant roots firstly come into contact with toxic metals, so by measuring potential to affect roots, we estimate damage to whole plant (Jiang et al. 2001; Gaperumal et al. 2011).

3.4 Damage to Chlorophyll Pigments and Reduction in Chlorophyll Content

Studies show that, metals affect the plant natural mechanism in many ways. Along damage to cells production of phenol and poly phenol is observed under Cd induced stress, which can protect plants from metal induced stress (Ehsan et al. 2014; Farid et al. 2015). Under application of metals reduction in chlorophyll content is the major factor that in return causes the leaf color damage and changes green color into yellowish shade. The reduction in content of chlorophyll is the reason of disappearance of the green natural color of leaves. Reduction in uptake of micronutrients by roots is the main reason of low chlorophyll content. The photosynthetic function is of major concern under high metals concentration. Sufficient chlorophyll content is required for efficient process of photosynthesis, which is the main factor in plant growth. Under stress of metals, the epidermal cells of root are damaged which cause low growth and low biomass production of root (Ouzounidou 1994; Ouzounidou et al. 1995). Reduction in pigments, amylase activity, and chlorophyll content were obvious with high metals concentration. The reduction in amylase activity was the main reason of reduction in plant growth. The low amylase causes low breakdown of starch under high concentration of metals. Reduction in protein synthesis was also observed under metal application (Samantary 2000; Tandon and Gupta 2002).

3.5 Damage to Enzymes

Plants have a natural defense mechanism against metal induced oxidative stress. Plants can naturally overcome the damage induced by the production of ROS. Enzymes play key role in the mitigation of damage induced by mental stress (Fediuc and Erdei 2002; Erdei 2002; Rios-Gonzalez et al. 2002; Mittler et al. 2004).

POD, SOD, CAT, and APX are antioxidant enzymes that protect plants from damage under harsh conditions. The activities of such enzymes increased with increasing concentration of metals however tend to decrease at elevated stress levels. POD plays a vital role in plant growth and development under metal stress however at higher metal concentration the activities of POD decreased (Asada 1992). CAT controls the excessive production of H_2O_2 in plants in the presence of metals and considered as a source of production of oxygen species. It acts on H_2O_2 and produces water and oxygen for cells (Mates 2000). Similarly SOD is essential for diminishing oxidative stress to the plant. High concentration of metals can reduce SOD production (Verma and Dubey 2003). This system includes both enzymatic and non-enzymatic antioxidants. The abovementioned antioxidant enzymes are responsible for conversion of H_2O_2 into oxygen species, i.e., water and oxygen. (Verma and Dubey 2003; Guecheva et al. 2003; Hou et al. 2007; Zayneb et al. 2015) show that in fenugreek SOD production was increased under Cd application which protects plant by adaptation of stress while other enzyme activity was low which can damage plant growth.

3.6 Production of H_2O_2 and Malondialdehyde Content

Production of MDA and H_2O_2 increases due to production of ROS. A high level of H_2O_2 that is produced in fenugreek by enhancement of MDA was reported under application of Cd by Zayneb et al. (2015). The high level of MDA is representing high stress to plant under metal toxicity. The membrane stability is reduced due to high MDA and H_2O_2 content. In metals treated plants the production of MDA and H_2O_2 was high, but the addition of ascorbic acid along with metals cause reduction in MDA and H_2O_2 production (Premachandra et al. 1991; Sudhakar et al. 2001). This shows that ascorbic acid is playing defense mechanism against induction of oxidative stress in fenugreek. It is clear from various studies that all the parameters like fresh weight, dry weight, stem length, and shoot length, leaf area increase under application of ascorbic acid as it is a growth regulator while in presence of metals all these parameters are reduced. So, the production of MDA and H_2O_2 increased under metal induced stress (Podmore et al. 1998; Rizwan et al. 2017a).

3.7 Accumulation of Heavy Metals by *Trigonella foenum-graceum*

The potential of *T. Foenumgraceum* against Pb was measured by Kaur (2016) by providing different concentrations of Pb in a soil based experiment. The study shows that fenugreek had accumulated the Pb in plant aerial parts. With increasing the dose of metal, accumulation in the plant was increased. The translocation factor of metal was increased from T1 To T5, respectively. Tanwar et al. (2013) suggest that EDTA enhances the metal uptake by fenugreek but decreases the plant dry mass, but when EDTA and *G. mosseae* were applied simultaneously it promotes the metal and mineral uptake as well as plant growth. The study suggests that for effective remediation of Pb from contaminated soils EDTA and G. mosseae should be used with fenugreek. A study conducted by Singh and Chhibba (2010) shows that the addition of EDTA in fenugreek and mustard increased the metal uptake in roots as well as in aerial parts of plants. At 1.5 g/kg of EDTA increased the metal accumulation of Cd by 1.5 folds while Pb was increased in plants by 3 folds in mustard.

Abd-Alla et al. (2014) accompanied a research to measure the potential of *T. foenum-graceum* for uptake of heavy metals from tannery sludge. The plant was analyzed for total Cr, Fe, Zn, and Pb at the initial stage of a plant blooming and soil was tested initially for Cr concentration in soil. In control conditions the metal uptake by the plant was within permissible limits and more fractions were residual but in the amended tannery sludge with oxalic acid/ammonium oxalate extract Cr was reduced in soil. The Pb uptake was hardly 1 mg/kg but Cr concentration was reported in high amount in fenugreek shoots in comparison to other leafy vegetables. This study suggested that fenugreek can be used to treat highrt Cr and low Pb contaminated soil, so that fenugreek plant can be protected from harmful effects of Pb and better Cr uptake. Dheri et al. (2007) show the Cr metal concentration in different plants. As a result of severe reduction in dry mass of fenugreek the metal concentration was up to 5 mg/kg on the soil wider application of high Cr concentration.

Zayneb et al. (2015) show that under the application of Cd, the maximum accumulation of metal occurs in the roots of plants and only a limited concentration was stored in shoots of the plant. By the application of Cd there was a reduction in plant dry mass by 50%, reduction in chlorophyll content, oxidative stress, and increased levels of hydrogen peroxide which leads to increase in antioxidant enzymes in different portions of the plant. At a concentration of 0.5 mm card there was a rise in the total amount of phenolic and flavonoid which cause reduction in lower reactive oxygen species (ROS). The study suggests that the balance in oxidant and antioxidant species can lead toward hyperaccumulation of Cd in fenugreek.

4 Conclusion

The present research shows the major physiological and biological variations in response to heavy metal stress to fenugreek (*T. foenum-graceum*). The analysis shows that plants exposed to heavy metals produce low biomass due to growth inhibition under various metal concentrations. At low metal concentration there is no significant damage to plant growth and other physiological attributes, but with increasing metal concentration and bioavailability the growth of fenugreek is significantly reduced due to reduction in low nutrient availability to plants. The plant cannot uptake required nitrogen and magnesium and the process of photosynthesis is inhibited. Production of reactive oxygen species and antioxidant enzymes occur. Production of H_2O_2 is also increased due to high production of MDA which reduces plant growth. The present study gave evidence that increasing metal concentration inhibited plant growth and affects plant morphology (Habiba et al. 2015; Rizwan et al. 2017b). Further details are required to obtain authentic data on the large-scale effect of heavy metal and tolerance on fenugreek. For the removal of heavy metals, trees should be used which can tolerate stress induced by heavy metals. In order to increase plant growth natural organic acids can be used for the protection of plant from metal induced stress. Eatable vegetables and plants should not be grown in highly contaminated land to avoid accumulation in a food chain or plants grown in these areas should not be used as food.

References

Abd-Alla HM, Bagy MK, El-enany AWES, Bashandy SR (2014) Activation of rhizobium tibeticum with flavonoids enhances nodulation, nitrogen fixation, and growth of fenugreek (*Trigonella foenum-graecum* L.) grown in cobalt-polluted soil. Arch Environ Contam Toxicol 66(2):303–315

Ahmad R, Ali S, Rizwan M, Dawood M, Farid M, Hussain A, Wijayae L, Alyemenie MN, Ahmad P (2020) Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. Physiol Plant 168(2):289–3000

Ahsan N, Lee DG, Lee SH, Kang KY, Lee JJ, Kim PJ, Yoon HS, Kim JS, Lee BH (2007) Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere 67:1182–1193

Alloway BJ (ed) (2012) Heavy metals in soils: trace metals and metalloids in soils and their bioavailability, vol 22. Springer, New York

Alloway BJ (2013) Sources of heavy metals and metalloids in soils. In: Heavy metals in soils. Springer, Dordrecht, pp 11–50

Aly AA, Mohamed AA (2012) The impact of copper ion on growth, thiol compounds and lipid peroxidation in two maize cultivars (*Zea mays* L.) grown in vitro. Austr J Crop Sci 6(3):541

Antunes WM, Luna AS, Henriques CA, da Costa ACA (2003) An evaluation of copper biosorption by a brown seaweed under optimized conditions. Electron J Biotechnol 6(3):174–184

Asada K (1992) Ascorbate peroxidase–a hydrogen peroxide-scavenging enzyme in plants. Physiol Plant 85(2):235–241

Azam I, Afsheen S, Zia A, Javed M, Saeed R, Sarwar MK, Munir B (2015) Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. Biomed Res Int 2015:942751. https://doi.org/10.1155/2015/942751

Azmat F, Samaratunge R (2009) Responsible entrepreneurship in developing countries: understanding the realities and complexities. J Business Ethics 90(3):437–452

Azooz MM, Abou-Elhamd MF, Al-Fredan MA (2012) Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. Aust J Crop Sci 6(4):688

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metal elements: a review of their distribution, ecology, and phytochemistry. Biorecov 1:81–126

Bashir N, Farid M, Saeed R, Tauqeer HM, Ali S, Rizwa M, Sallah-Ud-Din R (2017) Measurement of different heavy metals concentration in roadside dust in the vicinity of Gujrat, Pakistan. Science 5(4):51–57

Bernel M, Ronel M, Ortega JM, Picorel R, Yruela I (2004) Copper effect on cytochrome b555 of photosystem II under photo inhibitory conditions. Physiol Plant 120:686–694

Bhardwaj P, Chaturvedi AK, Prasad P (2009) Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. Nature Sci 7:63–75

Chen LM, Lin CC, Kao CH (2000) Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. Bot Bull of Acad Sin 41

Clarkson DT, Lüttge U (1989) Mineral nutrition: divalent cations, transport and compartmentation. In: Progress in botany. Springer, Berlin, Heidelberg, pp 93–112

Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qiu Y, Liang JZ (2004) Transfer of metals from soil to vegetables in an area near a smelter in Nanning. China Environ Int 30:785–791

Cuypers C, Grotenhuis T, Nierop KG, Franco EM, de Jager A, Rulkens W (2002) Amorphous and condensed organic matter domains: the effect of persulfate oxidation on the composition of soil/sediment organic matter. Chemosphere 48(9):919–931

Dahmani-Muller H, Van Oort F, Balabane M (2001) Metal extraction by *Arabidopsis halleri* grown on an unpolluted soil amended with various metal-bearing solids: a pot experiment. Environ Pollut 114:77–84

De Vos CHR, Schat H (1991) Free radicals and heavy metal tolerance. In: Rozema J, Verkleij JAC (eds) Ecological responses to environmental stress. Kluwer Academic Publishers, Dordrecht, pp 22–30

Dheri GS, Brar MS, Malhi SS (2007) Comparative phytoremediation of chromium-contaminated soils by fenugreek, spinach, and raya. Commun Soil Sci Plant Anal 38:1655–1672

Dias MC, Monteiro C, Moutinho-Pereira J, Correia C, Gonçalves B, Santos C (2013) Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiol Plant 35 (4):1281–1289

Ehsan S, Ali S, Noureen S, Mehmood K, Farid M, Ishaque W, Shakoor MB, Rizwan M (2014) Citric acid assisted phytoremediation of cd by *Brassica napus* L. Ecotoxicol Environ Saf 106:164–172

Entry JA, Watrud LS, Reeves M (1999) Accumulation of 137Cs and 90Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi. Environ Pollut 104:449–457

Erdei S (2002) Heavy metal induced physiological changes in the antioxidative response system. Acta Biolog Szegediensis 46(3–4):89–90

Ernst WHO (2000) Evolution and ecophysiology of metallophytes in Africa and Europe. In: Breckle SW, Schweizer B, Arndt U (eds) Results of worldwide ecological studies. G. Heimbach, Stuttgart, pp 23–35

Farid M, Ali S, Ishaque W, Shakoor MB, Niazi NK, Bibi I, Dawood M, Gill RA, Abbas F (2015) Exogenous application of EDTA enhanced phytoremediation of cadmium by *Brassica napus* L. Int J Environ Sci Technol 12(12):3981–3992

Farid M, Ali S, Rizwan M, Ali Q, Abbas F, Bukhari SAH, Saeed R, Wu L (2017a) Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. Ecotoxicol Environ Saf 145:90–102

Farid M, Ali S, Rizwan M, Saeed R, Tauqeer HM, Sallah-Ud-Din R, Azam A, Raza N (2017b) Microwave irradiation and citric acid assisted seed germination and phytoextraction of nickel (Ni) by *Brassica napus* L.; morpho-physiological and biochemical alterations under Ni stress. Environ Sci Pollut Res 24(25):2150–2164

Farid M, Ali S, Akram NA, Rizwan M, Abbas F, Bukhari SAH, Saeed R (2017c) Phyto-management of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. Environ Sci Pollut Res 24(20):16845–16859

Farid M, Ali S, Zubair M, Saeed R, Rizwan M, Sallah-Ud-Din R, Azam A, Ashraf R, Ashraf W (2018a) Glutamic acid assisted phyto-management of silver contaminated soils through sunflower; physiological and biochemical response. Environ Sci Pollut Res 25(25):25390–25400

Farid M, Ali S, Rizwan M, Ali Q, Saeed R, Nasir T, Abbasi GH, Rehmani MIA, Ata-Ul-Karim ST, Bukhari SAH (2018b) Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. Ecotoxicol Environ Saf 151(30):255–265

Farid M, Ali S, Rizwan M, Yasmeen T, Arif MS, Riaz M, Saqib M, Zia ur Rehman M, Ayub MA (2020a) Combined effects of citric acid and 5-aminolevulinic acid in mitigating chromium toxicity in sunflower (*Helianthus annuus* l.) grown in Cr spiked soil. Pak J Agri Sci 57(3):1–11

Farid M, Farid S, Zubair M, Rizwan M, Ishaq HK, Ali S, Ashraf U, Alhaithloul HAS, Gowayed S, Soliman MH (2020b) Efficacy of *Zea mays* L. for the management of marble effluent contaminated soil under citric acid amendment; morpho-physiological and biochemical response. Chemosphere 240:124930

Fediuc E, Erdei L (2002) Physiological and biochemical aspects of cadmium toxicity and protective mechanisms induced in Phragmites australis and *Typha latifolia*. J Plant Physiol 159 (3):265–271

Fernande JC, Henriques FS (1991) Biochemical, physiological, and structural effects of excess copper in plants. Bot Rev 57(3):246–273

Flemming CA, Trevors JT (1989) Copper toxicity and chemistry in the environment: a review. Water Air Soil Pollut 44(1–2):143–158

Forstner U, Wittmann GTW (1979) Metal pollution in the aquatic environment. Springer, Berlin, p 386

Friberg L, Nordberg GF, Vouk VB (1986) Handbook on the toxicology of metals, 2nd edn. Elsevier, Amsterdam

Gaperumal M, Vijayarengan P, Balamurugan S, Thiyagarajan G (2011) Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* (L.) Wilczek. J Phytology 3(3):53–62

Garrido T, Mendoza J, Riveros R, Sáez L (2010) Acute and chronic effect of copper on levels of reduced and oxidized glutathione and nutrient uptake of tomato plants. J Plant Nutr Soil Sci 173 (6):920–926

Geldmacher VM (1984) Meaning of the heavy metals in the toxicology. Fresenius' Z Anal Chem 317:427–432

Girotti AW (1985) Mechanisms of lipid peroxidation. J Free Radic Biol Med 1:87–95

Gonzalez-Mendozaa D, Quiroz-Moreno A, Medrano REG, Grimaldo-Juareza O, Zapata-Perez O (2009) Cell viability and leakage of electrolytes in *Avicennia germinans* exposed to heavy metals. Zeitschrift für Naturforschung 64(5–6):391–394

Groppa MD, Tomaro ML, Benavides MP (2001) Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. Plant Sci 161(3):481–488

Groppa MD, Benavides MP, Tomaro ML (2003) Polyamine metabolism in sunflower and wheat leaf discs under cadmium or copper stress. Plant Sci 164(2):293–299

Groppa MD, Ianuzzo MP, Tomaro ML, Benavides MP (2007) Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. Amino Acids 32(2):265–275

Groppa MD, Zawoznik MS, Tomaro ML, Benavides MP (2008) Inhibition of root growth and polyamine metabolism in sunflower (*Helianthus annuus*) seedlings under cadmium and copper stress. Biol Trace Elem Res 126(1–3):246

Guecheva TN, Erdtmann B, Benfato MS, Henriques JA (2003) Stress protein response and catalase activity in freshwater *Planarian Dugesia* (Girardia) schubarti exposed to copper. Ecotoxicol Environ Saf 56(3):351–357

Guney M, Zagury GJ, Dogan N, Onay TT (2010) Exposure assessment and risk characterization from trace elements following soil ingestion by children exposed to playgrounds, parks and picnic areas. J Hazard Mater 182(1–3):656–664

Gupta DK, Nicoloso FT, Schetinger MRC, Rossato LV, Pereira LB, Castro GY, Srivastava S, Tripathi RD (2009) Antioxidant defence mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. J Hazard Mater 172(1):479–484

Habiba U, Ali S, Farid M, Shakoor MB, Rizwan M, Ibrahim M, Abbasi GH, Hayat T, Ali B (2015) EDTA enhanced plant growth, antioxidant defense system and phytoextraction of copper by *Brassica napus* L. Environ Sci Pollut Res 22(2):1534–1544

Han Y (1999) Advances of the function of Beta-carotene and carotenoid. J China Agric Uni 4:5–10

Hardoy JE, Mitlin D, Satterthwaite D (1992) Environmental problems in third world cities. Earthscan, London

Harrison RB, Henry CL, Xue D (1994) Magnesium deficiency in Douglas-fir and grand fir growing on a sandy outwash soil amended with sewage sludge. Water Air Soil Pollut 75(1–2):37–50

Hou W, Chen X, Song G, Wang Q, Chang CC (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). Plant Physiol Biochem 45 (1):62–69

Ignatius A, Arunbabu V, Neethu J, Ramasamy EV (2014) Rhizofiltration of lead using an aromatic medicinal plant Plectranthus amboinicus cultured in a hydroponic nutrient film technique (NFT) system. Environ Sci Pollut Res 21(22):13007–13016

Jabeen N, Abbas Z, Iqbal M, Rizwan M, Jabbar A, Farid M, Ali S, Ibrahim M, Abbas F (2016) Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. Arch Agr Soil Sci 62(5):648–652

Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8:6

Jamali MK, Kazi TG, Arain MB, Afridi HI, Jalbani N, Memon RA, Shah A (2007) Heavy metals from soil and domestic sewage sludge and their transfer to sorghum plants. Environ Chem Lett 5:209–218

Javed I, Jan I, Muhammad F, Khan MZ, Aslam B, Sultan JI (2009) Heavy metal residues in the milk of cattle and goats during winter season. Bull Environ Contam Toxicol 82(5):616–620

Jiang W, Liu D, Liu X (2001) Effects of copper on root growth, cell division, and nucleolus of *Zea mays*. Biol Planta 44(1):105–109

Kaur L (2016) Tolerance and accumulation of lead by fenugreek. J Agric Ecol 1:22–34

Khan AG (2001) Relationships between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. Environ Int 26:417–423

Kumar Sharma R, Agrawal M, Marshall F (2007) Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicol Environ Saf 66:258–266

Li N, Kang Y, Pan W, Zeng L, Zhang Q, Luo J (2015) Concentration and transportation of heavy metals in vegetables and risk assessment of human exposure to bioaccessible heavy metals in soil near a waste-incinerator site, South China. Sci Total Environ 521:144–151

Lin J, Parker RG (1999) Analytical characterization of the unique properties of planetary gear free vibration. J Vibrat Acoust 121(3):316–321

Llorens N, Arola L, Blade C, Mas A (2000) Effects of copper exposure upon nitrogen metabolism in tissue culture. Plant Sci 7:159–163

Llugany M, Lombini A, Poschenrieder C, Dinelli E, Barceló J (2003) Different mechanisms account for enhanced copper resistance in Silene armeria ecotypes from mine spoil and serpentine sites. Plant and Soil 251:55–63

Lou LQ, Shen ZG, Li XD (2004) The copper tolerance mechanisms of Elsholtzia haichowensis, a plant from copper-enriched soils. Environ Exp Bot 51:111–120

Madivoli ES, Kareru PG, Gachanja AN, Mugo S, Murigi MK, Kairigo PK, Njonge FK (2016) Adsorption of selected heavy metals on modified nano cellulose. Int Res J Pure Appl Chem 12:1–9

Mahmood A, Malik RN (2014) Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. Arab J Chem 7(1):91–99

Malik RN, Zeb N (2009) Assessment of environmental contamination using feathers of *Bubulcus ibis* L., as a biomonitor of heavy metal pollution, Pakistan. Ecotoxicology 18(5):522–536

Malik RN, Husain SZ, Nazir I (2010) Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. Pak J Bot 42(1):291–301

Mantovi P, Bonazzi G, Maestri E, Marmiroli N (2003) Accumulation of copper and zinc from liquid manure in agricultural soils and crop plants. Plant and Soil 250(2):249–257

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London

Mates JM (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 153(1–3):83–104

Mazen AMA (2004) Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance. Biol Planta 48(2):267–272

McGrath SP, Chaudri AM, Giller KE (1995) Long-term effects of metals in sewage sludge on soils, microorganisms and plants. J Ind Microbiol 14:94–104

McGrath SP, Zhao FJ, Lombi E (2002) Phytoremediation of metals, metalloids, and radionuclides. Adv Agron 75:1–56

Micó C, Recatalá L, Peris M, Sánchez J (2006) Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. Chemosphere 65:863–872

Min XB, Xie XD, Chai LY, Liang YJ, Mi LI, Yong KE (2013) Environmental availability and ecological risk assessment of heavy metals in zinc leaching residue. Trans Nonfer Metal Soc China 23(1):208–218

Mirza N, Mahmood Q, Pervez A, Ahmad R, Farooq R, Shah MM, Azim MR (2010) Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater. Bioresour Technol 101(15):5815–5819

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9):405–410

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9(10):490–498

Mohod C, Dhote J (2013) Review of heavy metals in drinking water and their effect on human health. Int J Innovat Res Sci Eng Technol 2(7):2992–2996

Mubeen H, Naeem I, Taskeen A (2010) Phyto remediation of cu (II) by *Calotropis procera* roots. New York Sci J 3(3):1–5

Muchuweti M, Birkett JM, Chinyanga E, Zvauya R, Scrimshaw MD, Lester JN (2006) Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implications for human health. Agric Ecosyst Environ 112(1):41–48

Mudgal V, Madaan N, Mudgal A, Singh RB, Mishra S (2010) Effect of toxic metals on human health. Open Nutraceut J 3(1):94–99

Muhammad S, Shah MT, Khan S (2011) Heavy metal concentrations in soil and wild plants growing around Pb–Zn sulfide terrain in the Kohistan region, northern Pakistan. Microchem J 99(1):67–75

Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8(3):199–216

Ouzounidou G (1994) Copper-induced changes on growth, metal content and photosynthetic function of *Alyssum montanum* L. plants. Environ Exp Bot 34(2):165–172

Ouzounidou G, Čiamporová M, Moustakas M, Karataglis S (1995) Responses of maize (*Zea mays* L.) plants to copper stress—I. Growth, mineral content and ultrastructure of roots. Environ Exp Bot 35(2):167–176

Ouzounidou G, Ilias I, Tranopoulou H, Karataglis S (1998) Amelioration of copper toxicity by iron on spinach physiology. J Plant Nutr 21(10):2089–2101

Pickering KT, Owen LA (1997) Water resources and pollution. In: An introduction to global environmental issues, 2nd edn. Routledge, London, pp 187–207

Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, Lunec J (1998) Vitamin C exhibits pro-oxidant properties. Nature 392(6676):559

Pourakbar L, Khayami M, Khara J, Farbodnia T (2007) Physiological effects of copper on some biochemical parameters in *Zea mays* L. seedlings. Pak J Biol Sci 10(22):4092–4096

Premachandra GS, Saneoka H, Kanaya M, Ogata S (1991) Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. Environ Exp Bot 42(2):167–171

Qadir M, Schubert S, Ghafoor A, Murtaza G (2001) Amelioration strategies for sodic soils: a review. Land Degrad Dev 4:357–386

Qiao XL, Luo YM (2001) Land application of sewage sludge. Sewage sludge with high copper content. Soils 33:222–224. (in Chinese)

Quartacci MF, Pinzino C, Sgherr CL, Dalla Vecchia F, Navari-Izzo F (2000) Growth in excess copper induces changes in the lipid composition and fluidity of PSII-enriched membranes in wheat. Physiol Plant 108(1):87–93

Rahman MA, Hiroshi H, Kazumasa U, Teruya M, Chikako O, Muhammad MR (2007) Arsenic contamination in duckweed (*Spirodela polyrhiza* L.): a good option for phytoremediation. Chemosphere 69:493–499

Ranieri A, Castagna A, Scebba F, Careri M, Zagnoni I, Predieri G et al (2005) Oxidative stress and phytochelatin characterisation in bread wheat exposed to cadmium excess. Plant Physiol Biochem 43(1):45–54

Raven JA, Evans MCW, Korb RE (1999) The role of trace metals in photosynthetic electron transport in O2-evolving organisms. Photosynth Res 60:111–149

Rios-Gonzalez K, Erdei L, Lips SH (2002) The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. Plant Sci 162 (6):923–930

Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017a) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere 187:35–42

Rizwan M, Ali S, Qayyum MF, Ok YS, Adress M, Ibrahim M, Zia-ur-Reham M, Farid M, Abbas F (2017b) Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: a critical review. J Hazard Mater 322:2–16. https://doi.org/10.1016/j.jhazmat.2016.05.061

Sahi SV, Israr M, Srivastava AK, Gardea-Torresdey JL, Parsons JG (2007) Accumulation, speciation and cellular localization of copper in *Sesbania drummondii*. Chemosphere 67 (11):2257–2266

Sallah-Ud-Din R, Farid M, Saeed R, Ali S, Rizwan M, Tauqeer HM, Bukhari SAH (2017) Citric acid enhanced the antioxidant defense system and chromium uptake by Lemna minor L. grown in hydroponics under Cr stress. Environ Sci Pollut Res 24(21):17669–17678. https://doi.org/10.1007/s11356-017-9290-0

Samantary S (2000) Biological response of chromium tolerant and chromium sensitive Mung bean, cultivars growth on varying level of chromium. Chemosphere 47:1065–1072

Santos C, Monteiro M, Dias MC (2010) Cadmium toxicity in crops: a review. Environmental science, engineering and technology. Nova Publishers, Novinka

Sengar RS, Gautam M, Garg SK, Chaudhary R, Sengar K (2008) Effect of lead on seed germination, seedling growth, chlorophyll content and nitrate reductase activity in mung bean (*Vigna radiata*). Res J Phytochem 2:61–68

Singh R, Chhibba IM (2010) Chelate-assisted phytoextraction of cadmium and lead using mustard and fenugreek. Commun Soil Sci Plant Anal 41(18):2131–2142

Singh D, Nath K, Sharma YK (2007) Response of wheat seed germination and seedling growth under copper stress. J Environ Biol 28(2):409

Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharm 43(3):246

Sudhakar C, Lakshmi A, Giridarakumar S (2001) Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci 161 (3):613–619

Sungur A, Soylak M, Ozcan H (2014) Investigation of heavy metal mobility and availability by the BCR sequential extraction procedure: relationship between soil properties and heavy metals availability. Chem Speciat Bioavail 26(4):219–230

Tamas L, Budikova S, Simonovicova M, Huttova J, Siroka B, Mistrik I (2006) Rapid and simple method for Al-toxicity analysis in emerging barley roots during germination. Biol Planta 50 (1):87–93

Tandon PK, Gupta S (2002) Effect of cobalt and lead on the growth and metabolism of gram (*Cicer arietinum*) seeds. Indian J Agric Biochem 15(1):2

Tanwar A, Aggarwal A, Charaya MU, Kumar P (2013) Enhancement of lead uptake by fenugreek using EDTA and Glomus mosseae. Commun Soil Sci Plant Anal 44(22):3431–3443

Tariq J, Jaffar M, Ashraf M (1994) Trace metal concentration, distribution and correlation in water, sediment and fish from the Ravi River, Pakistan. Fish Res 19(1):131–139

Tehseen W, Hansen L, Wood S, Hanif M (1994) Assessment of chemical contaminants in water and sediment samples from Degh Nala in the province of Punjab, Pakistan. Arch Environ Contam Toxicol 26(1):79–89

Thomas T, Thomas TJ (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 58(2):244–258

USEPA (1997) Cleaning up the Nation's waste sites: markets and technology trends, United States Environmental Protection Agency EPA/542/R-96/005, Office of Solid Waste and Emergency Response, Washington, DC

Verma S, Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci 164(4):645–655

Vinit-Dunand F, Epron D, Alaoui-Sossé B, Badot PM (2002) Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. Plant Sci 163(1):53–58

Walters D (2003) Resistance to plant pathogens: possible roles for free polyamines and polyamine catabolism. New Phytol 159(1):109–115

Wang SH, Yang ZM, Yang H, Lu B, Li SQ, Lu YP (2004) Copper-induced stress and antioxidative responses in roots of *Brassica juncea* L. Bot Bull Acad Sin 45:203–212

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:402647. https://doi.org/10.5402/2011/402647

Xiong ZT, Liu C, Geng B (2006) Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotoxicol Environ Saf 64(3):273–280

Yang L, Luo C, Liu Y, Quan L, Chen Y, Shen Z (2002a) Residual effects of EDDS leachates on plants during EDDS-assisted phytoremediation of copper contaminated soil. Sci Total Environ 444:263–270

Yang P, Yan H, Mao S, Russo R, Johnson J, Saykally R, Choi HJ (2002b) Controlled growth of ZnO nanowires and their optical properties. Adv Funct Mater 12(5):323–331

Yruela I (2005) Copper in plants. Brazil J Plant Physiol 17(1):145–156

Zayneb C, Bassem K, Zeineb K, Grubb CD, Noureddine D, Hafedh M, Amine E (2015) Physiological responses of fenugreek seedlings and plants treated with cadmium. Environ Sci Pollut Res 22(14):10679–10689

Zhuang P, McBride MB, Xia HP, Li NY, Li ZA (2009) Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Sci Total Environ 407:1551–1561

Copper-Induced Responses in Different Plant Species

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Abstract

Copper (Cu)-induced stress caused adverse effects to plant growth and productivity thus considered as a severe threat for sustainable crop production. This article presents an overview of copper stress in plants. Copper participates in many physiological processes as a co-factor for catalysis of many metalloproteins; however, problem occurs when excess amount of copper is present in cells. The high concentration of copper suppresses biomass accumulation and linear plant growth. Copper affected root growth stronger than shoot growth. The reduced mobility of Cu in soil is due to its strong binding to organic and inorganic colloids, where it acts as a barrier to Cu toxicity in terrestrial plants. Excess of Cu inhibits a large number of enzymes and interferes with several aspects of plant biochemistry, including photosynthesis, pigment synthesis, and membrane integrity. So, the most important effect of copper toxicity is associated with the blocking of photosynthetic electron transport, leading to the production of radicals which start peroxidative chain reactions. Copper induces oxidative stress that involves induction of lipid peroxidation in the plant which further cause a severe damage to the cell membrane. High copper concentration can disturb the chloroplast ultrastructure by disturbing the photosynthetic process. Like chromium and iron, copper is also a redox metal that can have direct involvement in inducing oxidative stress in plants. In addition, Cu stress induced -production of reactive oxygen species is well recognized and controlled at both the production and consumption levels, through increased antioxidative systems.

Keywords

Copper · Growth · Yield · Antioxidant · Photosynthesis

Abbreviations

As	Arsenic
CAT	Catalase
Cd	Cadmium
CO^2	Carbon dioxide
Cr	Chromium
Cu	Copper
CuO	Copper oxide
$CuSO^4$	Copper sulfate
Cyt	Cytochrome
DNA	Deoxyribonucleic acid
DW	Dry weight

H_2O_2	Hydrogen peroxide
Hg	Mercury
HR	Hypersensitive response
LPO	Lipid peroxidation
MDA	Malondialdehyde
NPs	Nanoparticles
O^2	Oxygen
$O_2^{\bullet -}$	Superoxide radical
OH•	Hydroxyl radical
Pb	Lead
PSI	Photosystem I
PSII	Photosystem II
ROS	Reactive oxygen species

1 Introduction

Over the last few decades, sustainability in agriculture has emerged as a major goal in changing environmental conditions to fulfill the requirements of enough food to feed an intensively increasing overall population in the world (Sallah-ud-Din et al. 2017). Many biotic and abiotic stress factors affect several aspects of plant development, growth, and productivity (Anjum et al. 2010). In terrestrial ecosystem metals are important for rising and development of plant species (Hall and Williams 2003; Ali et al. 2013; Farid et al. 2019). “Heavy metal” is toxic metals; exist in the form of distinct metals or as metallic complexes that show negative impact on the health of people (Farid et al. 2018a). Minimal amount of some metals is essential for sustainability of life. But the high concentration of metals has potential to accumulate in ecosystem at toxic level and poses a major health hazard (Habiba et al. 2014; Jabeen et al. 2016).

Heavy metal contamination of soil ecosystem is due to the pesticide formulations, contamination of chemical fertilizers, atmospheric fall-out, and irrigation with factory effluent and sewage sludge (Farid et al. 2015; Mishra et al. 2019). Heavy metals may accumulate to toxic levels in the food chain when growing cereal crops with wastewater (Farid et al. 2018b). The use of polluted water in agriculture soil may cause failure of crops and food security problem (Munzuroglu and Geckil 2002; Farid et al. 2013). In addition, these metals are considered as a major group of contaminants as they persist in the environment for extended time period and their concentration is actually dangerous for humans, fauna, and flora (Benavides et al. 2005; Gratão et al. 2005; Panda 2008; Ehsan et al. 2014; Kumar et al. 2019). Cu nanoparticles (NPs) are used for multiple purposes in industries including gas sensing, optoelectronics, catalysis, solar cells, semi-conductors, and pigments and as fungicides (Zhu et al. 2004; Meshram et al. 2012; Gawande et al. 2016). Cu is a vital micronutrient for proper growth of plant. Cu is an essential part for protein synthesis, transportation of photosynthetic electron, mitochondrial respiration, response to oxidative stress, metabolism, and signaling (Das 2014; Printz et al.

2016). When the amount of Cu in the soil is elevated then it becomes very toxic. It disturbs fundamental physical procedures including photosynthetic and mitochondrial electron transport activity, nitrogen integration, metabolic rate, and several others (Bhakuni et al. 2009; Habiba et al. 2014; Cota-Ruiz et al. 2018). The liable portion of Cu in soil depends upon the soil type and characteristics including content of organic matter, pH, texture, cation exchange capacity, and redox potential. For satisfactory nutrition of plant, the average concentration of Cu is enough between 1 and 50 $\mu g\ g^{-1}$ (Thornton 1979). The bioavailable metal to plants is typically minor and is merely a limited proportion of the total Cu present in the soil (Lange et al. 2017). Cu occurs both in ionic (Cu^{+2}) and complex form where it bound to organic or inorganic substances. Resemblance of Cu with organic matter is high in comparison to other metals and organic complex of Cu are abundant in soil (Araújo et al. 2019). According to Khan et al. (2015), the Cu concentration for vigorous growth of plant is usually between 5 and 20 $\mu g\ g^{-1}$ on base of dry mass (DM) and above this concentration Cu becomes toxic and symptoms start to appear.

Numerous researches have been conducted to measure the influence of Cu on the growth, inorganic nutrition uptake, and metabolic rate of plants. Cu in high concentration decreases growth, uptake of mineral nutrient (Gardea-Torresdey et al. 2004), inhibits the process of photosynthetic activity (Ali et al. 2011; Da Costa et al. 2016) and nitrogen metabolism in plants (Xiong et al. 2006). There is very limited attention in Pakistan on the adverse effects of heavy metals on cereal crops in response to widespread disposal of untreated solid and liquid waste over agricultural soil (Mahmood and Islam 2005).

2 Copper in the Environment

Cu is a vital component for plant growth. However, its concentration in the soil more or less than the optimal amount could adversely affect the plant growth. The presence of Cu in the soil is in organically bound form, therefore retains strong in the uppermost layers (Manceau and Matynia 2010). The normal concentration range of total Cu in the soil is 2–250 $\mu g\ g^{-1}$ (Khan et al. 2015). The normal growth, development, and enzymatic catalysis are regulated by Cu (Rajput et al. 2018). About 98% of Cu in soil exist in the form of low molecular organic compound complex and concentration of Cu^{+2} is between 10^{-4} and 10^{-9} M (Fox and Guerinot 1998; Yruela 2009; Manceau and Matynia 2010). Most recent focus is given to the contamination of environment and soil due to the nanoparticles (NPs) of Cu (Dimkpa et al. 2014). Cu is one of the most essential micronutrients and involves in many physical processes as a part of numerous enzymes, generally with least requirement, ranges 1–5 mg kg^{-1} in tissue dependent on plant type (Ryan et al. 2013).

Nanoparticles (NPs) have elevated global environmental worry. Shaw et al. (2014) reported the effect of nano-CuO induced stress in Syrian barley (*Hordeum vulgare* L.) on sprout performances in relation to antioxidant resistance and chlorophyll fluorescence. Results showed significant reduction in growth and antioxidant defense system of plant.

3 Copper as Contaminant in the Soil

Soil with presence of heavy metals is amongst the main contaminants which can strictly hamper growth of the plant (Chibuike and Obiora 2014; Farid et al. 2020b). Currently, ecological pollution and contact to heavy metal are an emergent problem around the globe. 235 million hectares of land is contaminated with heavy metals (Giordani et al. 2005). Cu performs numerous functions in all kinds of life forms and important for health of biological systems. Contamination of metals appears from natural sources such as in situ weathering of rocks (Fig. 1). The main reason for metal accumulation in soil is agricultural activities including the use of fertilizers, pesticides, disposal of contaminated solid and liquid waste with nutrients and minerals from industries (Maanan et al. 2015). However, the similar process that marks Cu vital also shows its cytotoxic influence when Cu homeostatic control fails (Georgopoulus et al. 2002). Fertilizers, pesticides, fungicides, and the use of sludge from sewage are the major sources of Cu^{2+} pollution in the environment (Yu et al. 2013), whereas Cu^{2+} is mostly released into the environment from industrialized waste (Maanan et al. 2015). The Cu concentration that is above 20 mg kg^{-1} s as dry weight becomes poisonous and changes membrane permeability, structure of chromatin, enzyme, photosynthesis activities, and respiratory procedures along with persuade senescence (Srivastava et al. 2006). The deficiency of Cu usually arises when its concentration is smaller than 5 mg kg^{-1} of DW (Khan et al. 2015). Due to strong adsorption of Cu in the soil particles, the level of Cu decreased with an increase of pH in soil (Soja et al. 2018). Hence, high pH of soil may pose problem for low Cu availability. Excess of Cu in leaf tissue, corresponding to 20–30 μg mg^{-1} as dry weight, is harmful to most of the plants and may alter the membrane permeability (Sergiev et al. 2016), structure of the nucleus, leading to decreased DNA and protein synthesis (Ngozi 2011) and photosynthetic activity (Connan and Stengel 2011).

4 Copper Deficiency

Plants require mineral contents for growth and to ensure the completion of life cycle, which is predominantly obtained from the soil and foliar applications. There is a need to maintain the concentration of Cu in cells at low level for plant productivity and because of its high redox properties, this element is extremely toxic for plants. The normal component of Cu in plant matter is about 10 μg g^{-1} of DW (Baker and Senef 1995; Yruela 2005).

Cu-deficient plants showed changes in succession of genes expression and morphological variations in the structure of plant root and leaf. A typical indication of Cu insufficiency chiefly appeared at the tips of new greeneries and then moves down across the leaf boundaries. The leaf could be warped or deformed and express

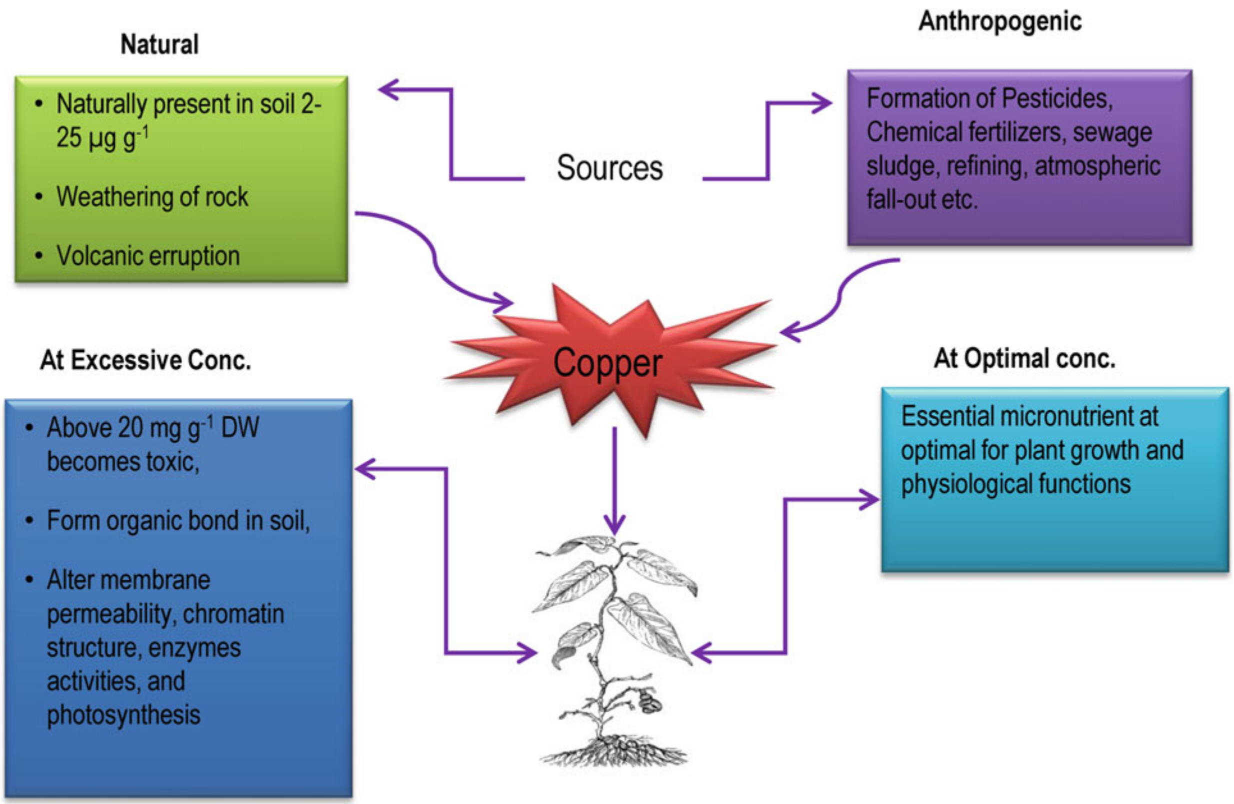

Fig. 1 Natural and anthropogenic sources of copper and its effects at excessive and optimal level

chlorosis or sometimes necrosis and overall reduction in the biomass of affected plants (Küpper and Kroneck 2005; Habiba et al. 2014). The key mark spot of Cu shortage in photosynthetic activity was found to reduce photosystem I (PSI) electron carriage because of reduction in production of plastocyanin (Shikanai et al. 2003).

It is also observed that reduction in the activity of photosystem II (PSII) occurs in Cu-deficient chloroplast (Gonzalez-Mendoza et al. 2013; Deng et al. 2014). It is confirmed that severe Cu scarcity modifies thylakoid membranes by changing the surrounding of PSII acceptor site (Yruela 2005). In Cu-deficient plant the thylakoid membranes of chloroplast disintegrate that cause reduction in the concentration of chlorophyll and carotenoid, reduction in the synthesis of plastocyanin, and lowered the contents of saturated fatty acid (Peng et al. 2013). The molecular procedure that initiates through the plant "recognizing" the scarcity by the up- and down- regulation of genes and through plant vascular system transmits the signal alongside transduction paths. Plants transmit the indications amongst the above-ground parts involving the apical meristem, and the roots cause the triggering or inactivation of transcription aspects that effect the manifestation of definite genetic factor (Sağlam et al. 2016).

5 Toxic Effects of Copper on Plants

Environmental stresses bring about a comprehensive variation of plant reactions, fluctuating from changed DNA expression and cellular metabolism modifications in progression and production of plant (Fig. 2).

5.1 Growth

Heavy metals present in soil can successively move in the food chain and cause hazardous effect on human health. The use of Cu as a mineral pesticide in agriculture becomes a widespread contaminant (e.g., $CuSO_4$ in Bordeaux mixture) (Ahmad et al. 2008; Rizwan et al. 2017a). Metals pose a strong influence on the growing and developmental factors of plant. In plant, Cu is a necessary and important nutrient component for normal growth whereas several physiological processes performed by this metal to confirm the proper transfer of a component (co-factor) to level metalloproteins (Yruela 2005). Phytotoxicity due to high Cu level alters the physiological activities. Plant affected more or less seriously is associated with an acclimation according to new environmental condition (Tausz et al. 2004; Liu et al. 2018).

High dose of Cu to plant may cause the oxidative stress that might be cytotoxic and can harm significant compound of the cell components (Posmyk et al. 2009). Heavy metal taken up in an excessive amount by the plants will adversely affect the seed germination, plant normal growth, and influence the various genetic factors which are part of several cellular stress reactions (Ahmad et al. 2020). This Cu toxicity does not depend on only metal amount but also on the development stages and physical stage of the plant. The most sensitive physiological process in the plant

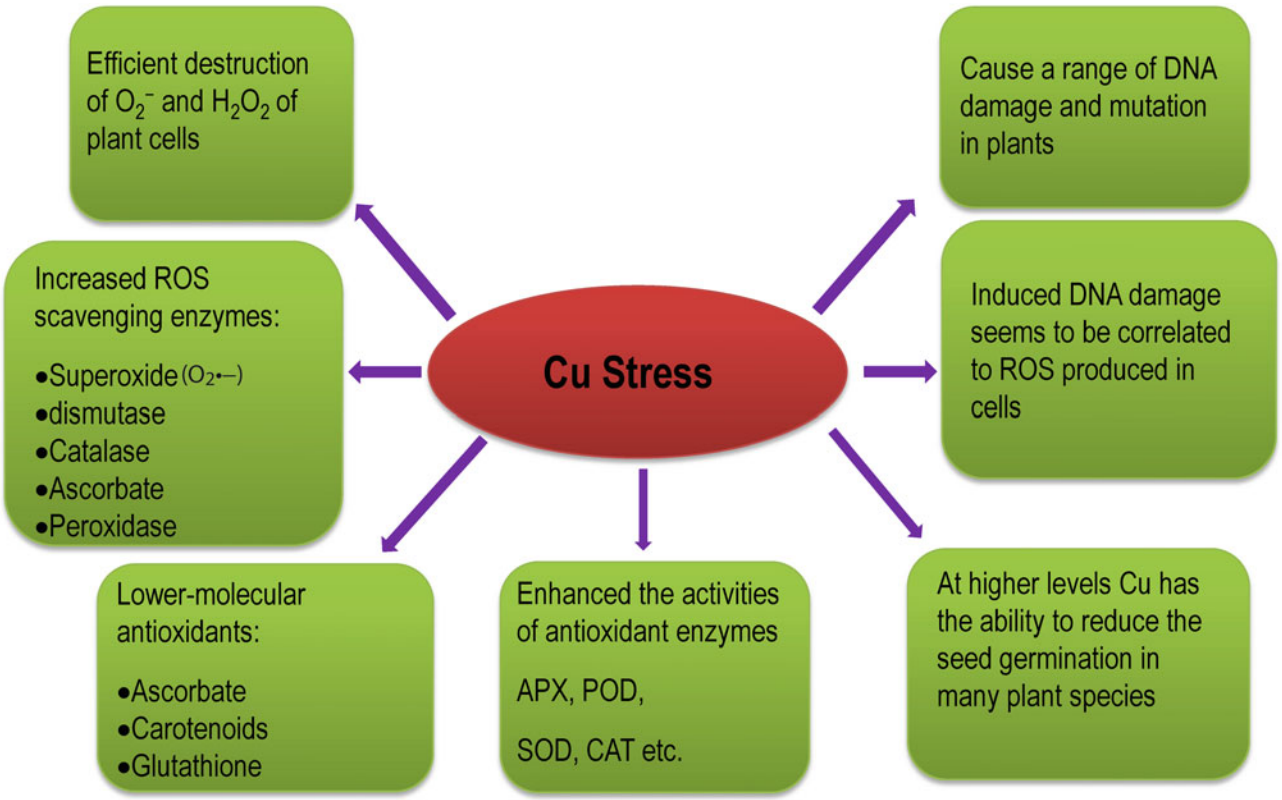

Fig. 2 Physiological and biochemical effects of copper toxicity on plants

is seed germination where new developing sprouts and roots have poor resistance mechanism (Ahsan et al. 2007). Inside the plant, Cu shows low mobility, so root accumulates higher content of metals than the shoot followed by root which is the first organ to which Cu makes contact (Panou-Filotheou and Bosabalidis 2004; Lange et al. 2017). However, visible indications of poisonousness comprise leaves chlorosis and hinderers of growth disturbing equally of shoot and root (Küpper and Andresen 2016). Uptake of Cu in plant affects different aspects such as growth parameter, mineral chlorophyll contents, and several enzyme interactions (Brun et al. 2003; Shaw et al. 2014). As the plant growth and productivity are affected; the relation between the heavy metal and plants needs to be investigated (Adrees et al. 2015).

Reduction in root progression owed by heavy metals in numerous trees and crops has been reported on a large scale (Tang et al. 2001; Ivanov et al. 2003; Shahid et al. 2017). A widespread root arrangement is beneficial for up keeping plant growth throughout the initial crop growth phase (Rizwan et al. 2017b). The root structure shows a significant character in the uptake of water, nutrient and provides manual sustenance for growing plants (Habiba et al. 2015). Vigorous root arrangement can affect the amount of minerals and water with respect to its weight and length which consequently influences the yields. Atmospheric elements significantly affect the growth and progress of the root structure of yearly crops (Rogers and Benfey 2015; Vergani and Graf 2016).

Cu is a necessary content for plants, but while its concentration in soil is high and becomes poisonous, florae carry out prohibiting tools that cause an upsurge of the metal in roots that rapidly bounds its movement to the above-ground portions. Thus, Cu in excess concentration is injurious chiefly to the roots. Symptoms of Cu toxicity are leaf chlorosis, root discoloration, inhibition of root growth and elongation (Kalaivanan and Ganeshamurthy 2016). The high concentration of Cu decreased the ability of plant root for uptake of water and nutrients from soil (Manasa et al. 2015). In particular, Cu reduced transport potential and harms the plasma membrane, producing ion efflux and thus hindering cell elongation (Sánchez-Viveros et al. 2010). It has been shown that environmental stresses can induce damage in the cell walls of root tissues through binding to the sulfhydryl group of membrane protein and by bringing lipid peroxidation (Sánchez-Viveros et al. 2010; Chen et al. 2015).

The storage of Cu is greater in the root than shoot, so the root length and change in chloroplast structure were decreased as observed in *Arabidopsis thaliana* cultivated in a medium of excess Cu (Wojcik and Tukiendorf 2003). In many non-tolerant plants, the elevated concentration of Cu has been reported, which inhibits the root elongation, harms epidermal cells and cell membranes of roots (Küpper and Andresen 2016). In numerous plant types a huge amount of Cu engaged is retained in the root, though its carriage to shoot might differ significantly amongst diverse types or genotypes (Llugany et al. 2003; Lou et al. 2004; Monni et al. 2000). Bipasha et al. (2000) has also reported that high Cu content affected root growth than shoot growth, while similar trend was found in *Brassica napus* by Habiba et al. (2014).

5.2 Yield

Environmental stresses affect numerous yield-determining procedures in plants and several of these yield-determining methods integrate in a complex manner to one another (Farid et al. 2017a). It is challenging to clarify how it accumulates, conglomerates, and exhibits the ever-changing and undefined procedures terminated over the complete lifespan of the crop. Crop yield depends on the expression association of several plant growth components (Farid et al. 2017b).

The decrease in yield biomass by excess Cu is due to low protein synthesis, reducing photosynthetic activity along hindered carbohydrate translocation (Wani et al. 2007). Plants treated with excess Cu reduced the biomass in *Vigna radiata* (L.) *Wilczek* have been reported by Manivasagaperumal et al. (2011). A similar decrease in dry mass yields of *Vigna radiata* (L.) *Wilczek* at greater amount of heavy metal cadmium was detected by Wahid et al. (2007) and Ahmad et al. (2007) under Ni induced stress.

The grain yield decreased significantly with raising soil Cu level. The researchers have showed that the grain yield decreased significantly with the increase of soil Cu content. The limited data on the reasons of rice yield reductions by Cu stress were derived from rice plants, which showed that yield of rice grain was reduced by 10, 50, and 90% at concentration of 100, 300–500, and 1000 mg kg^{-1}, respectively. The higher Cu concentration in soil hinders the growing procedure, declines the grain yield, and also poses healthiness risks to human and faunae by accumulating in the food chain (Xu et al. 2006).

5.3 Physiological Responses

5.3.1 Photosynthesis

The capacity of plants response to the environmental stress is related to their capability to acclimatize at photosynthetic activity, which disturbs biochemical, physical procedures, result in the decreased growth of the plant (Chandra 2003; Farid et al. 2017c). Cu toxicity severely hampered the metabolic processes and growth inhibition at tissue level when Cu contents are slightly higher than the normal level (Habiba et al. 2014; Shaw et al. 2014). The most important effects of Cu on physiological activities are photosynthesis, synthesis of chlorophyll, respiration, carbon dioxide (CO_2) fixation, gas exchanges, and other nutrient transportation inside the plants (Adrees et al. 2015). It is found that photosystem II (PSII) is a more sensitive site to Cu toxicity than photosystem I (PSI) (Husak 2015). The micro molar concentration of Cu has been referred to alter the lipid composition in thylakoid (Anjum et al. 2015), to bring about lipid peroxidation (Husak 2015), to ease photo-inhibition (Patsikka et al. 2002), to inhibit photophosphorylation (Gupta et al. 2018), electron transport (Kalaivanan and Ganeshamurthy 2016). Although Cu can disturb all vital practices of plant metabolic activity directly or indirectly, it is the photosystem II (PSII) level where maximum possessions have been stated. The supreme obvious consequence of Cu toxicity on PSII is the hinderers of oxygen and the changes go together with the quenching of variable fluorescence (Husak 2015). It was suggested that both the acceptor and the donor side of PSII are the key objects of Cu toxicity. It has been reported that at the donor side Cu affects the Mn-cluster and the external proteins of the oxygen-evolving-complex and the acceptor side interrelating with the non-heminic Fe^{2+} and cytochrome (Cyt) b559 (Sersen et al. 1997; Yruela et al. 2000; Burda et al. 2003; Bernal et al. 2004). After affecting the photosynthetic apparatus, Cu is absorbed by the plant, transport to the leaves and enter the green cell, crossing to envelope of the chloroplast and hit the thylakoid photosystem (Pilon et al. 2006). When oxygenic creatures interact to prolong high light concentrations the photosynthesis decrease, including to destruction of PSII electron carriage and the mechanical impairment of the PSII reaction edge is known as photo-inhibition (Możdżeń et al. 2017). Cu is an effective catalytic agent in production of reactive oxygen species (ROS). It is recommended that accumulation of Cu and poisonousness of light through photo-inhibition is owed to making of hydroxyl radicals (Mohiseni et al. 2017).

5.3.2 Chlorophyll

Chlorophyll is the main chloroplast constituents for photosynthesis process, and the chlorophyll elements have a progressive association with photosynthetic rate (Farid et al. 2020a). Several authors reported the adverse effects of Cu on photosynthetic processes, and they found lower chlorophyll concentration and modification in the assembly of chloroplast and thylakoid tissue in leaves (Bernal et al. 2004; Yruela 2005).

After exposure to Cu stress, decrease in chlorophyll contents in the plant is often reported (Singh et al. 2007; Asati et al. 2016). Due to the higher accumulation of Cu in the plant tissues, the leaves showed chlorotic symptoms that cause reduction in chlorophyll. It is obvious that heavy metals can carry out changes in chlorophyll, constituted by the alteration in the chlorophyll a/b percentages (Dazy et al. 2008; Mysliwa-Kurdziel et al. 2004). The changes appear to be associated with the biological class and the type of the specific metal stress. Through intervention with enzymes such as aminolevulinic dehydratase (an enzyme used in the porphyric path) Cu catalyzes chlorosis, which might be the consequence of chlorophyll production (Scarponil and Perucci 1984). Concentration of chlorophyll reduced, plastocyanin production compact, and the content of saturated fatty acid is low in a Cu-deficient plant (Hippler, et al. 2016). In Cu-deficient chickpea leaf the absorption of both chlorophyll a and b reduced owing to change in the chloroplast ultra-arrangement and disarray of thylakoid membrane (Yruela 2005). Due to the excessive Cu action, the reduction in chlorophyll of leaf occur (Chatterjee et al. 2006), might be because of the direct metal effect on the creation of chlorophyll or because of reduction in iron uptake by plant. Cu can also substitute for Mg in chlorophyll in both antenna complex and reaction center, thus have a deleterious effect on the structure and action of chlorophyll (Nakajima et al. 2015). In first step of the chlorophyll synthesis, iron is a co-factor and Cu can block the concentration and uptake of iron in plant (Ke et al. 2007). The low chlorophyll amount detected in plants grown up in the existence of high Cu concentration which affect leaves severely as a consequence of a Cu-induced iron (Fe) scarcity.

5.4 Mineral Nutrient Uptake

Nutrient deficiency has become a major constraint for crop productivity. The deficiency either may be primary or secondary. Primary is due to the low total contents and secondary is due to soil aspects that decrease their accessibility to plants (Sharma and Chaudhary 2007). Mineral inequity could also arise from the occurrence of an unnecessary quantity of a mineral content that obstructs other nutrients in performance of regular metabolic tasks (Malevar 2005; Zengin and Kirbag 2007). Optimum arrangement of nutrients is crucial for metabolic activity of plant. Mineral nutrients are adversely affected in presence of heavy metals. Reduction in mineral uptake was detected in numerous Cu-treated plant species (Ali et al. 2002; Michaud et al. 2008) and a number of studies are also available on the persuaded deficiencies of the several mineral contents due to Cu toxicity (Bouazizi et al. 2010; Lequeux et al. 2010; Manivasagaperumal et al. 2011).

Cu as a necessary micronutrient for ordinary growing and metabolic activity of plants, is well documented (Singh et al. 2007). However, it is stated in many studies that excess Cu disturbed mineral uptake and enzymatic activity negatively (Kopittke and Menzies 2006; Jain et al. 2010). The symptoms of heavy metals toxicity can be appeared in the form of nutrient imbalance. The disturbance of mineral element uptake could appear in the form of low growth by inhibition of leaves growth (Rouphael et al. 2008; Dimkpa et al. 2014). Reduced iron uptake disturbs the general mineral element homeostasis responsible for the chlorosis in the leaves. Iron is an essential component in the process of photosynthesis and synthesis of chlorophyll but in the presence of excess Cu, iron uptake and translocation is reduced (Ke et al. 2007). The indirect impacts of variations in Cu concentration are due to mainly imbalance of vital nutrients required to plants.

5.5 Biochemical Responses

5.5.1 Generation of Reactive Oxygen Species (ROS)

The earlier biochemical response of eukaryotic cells is the production of reactive oxygen species (ROS) under biotic and abiotic strains. Some metabolic procedures could utilize ROS in an effective way through participating in an oxidative burst, the early plant defense response. The ROS directly protect plant from attacking substances but also give activating signals (HR-hypersensitive response) for further response (Gupta et al. 2015). The significant amount of ROS is crucial for functioning of cellular procedures including signal carriage (Smeets et al. 2008). The molecular oxygen is very less reactive in comparison of ROS. The plants try to keep the least level of ROS as they made effort to react with each organic part of living cell. The specific electronic structure of ROS is motive of great reactivity with cells. The ROS has potential to harm membrane of cells by steady peroxidation of lipid structures (Baryla et al. 2000), antioxidant enzyme inactivation (Teisseire and Guy 2000), oxidative DNA damage (Kasprzak 2002), and eventually death of cell. The ROS generation in plants induced oxidative stress and damage because of free Cu translocated in plants (Okamoto et al. 2001; Wang et al. 2004; Zhang et al. 2008). The generation of ROS in plant takes place via the Haber–Weiss and Fenton reactions (Elstner et al. 1988) as it is a redox-active. Cu-induced oxidative stress and lipid peroxidation by producing extremely harmless hydroxyl radical ($OH^{\bullet}$) through the formation of superoxide radical ($O_2^{\bullet -}$) or by producing hydrogen peroxide (H_2O_2) (Schützendübel and Polle 2002). In excess Cu, the oxidative stress is exerted in plant cells because of the production of hydrogen peroxide, superoxide radical, and hydroxyl radical (Panda 2003) and the content of malondialdehyde (MDA) could also be increased because of the oxidation of the protein and membrane lipid (Cho and Seo 2005). The production of MDA results in lipid peroxidation due to heavy metals accumulation on a large scale (Zhang et al. 2005; Tripathi et al. 2006; Naser et al. 2008). The level of oxidative damage is measured by the content of produced MDA (Moller et al. 2007) and indication for membrane lipid peroxidation.

5.5.2 Antioxidant Defense

Plants developed numerous defense tactics when exposed to high concentration of heavy metals. However, plants have fine and complicated internal protective enzyme-catalyzed sweep up system to survive from damages of ROS and gives regular functioning of cellular procedures (Horváth et al. 2007). The equilibrium among ROS production and the role of antioxidative enzyme activities determined the process of oxidative signaling or injury that will took place in the plant tissues (Moller et al. 2007). Plants have developed antioxidant mechanism of enzymatic and non-enzymatic complexes to reduce the impact of oxidative stress. Antioxidants such as ascorbate, carotenoids, glutathione are low in molecular weight and are most significant (Tausz et al. 2003; Thounaojam et al. 2012; Shazia et al. 2012). Superoxide dismutase, catalase, ascorbate, and peroxidase are the ROS hunting enzymes (Apel and Hirt 2004; Shaw et al. 2014). The ascorbate–glutathione cycle (Halliwell-Asada cycle) is a highly established purification method and at least four antioxidant enzymes participate in this cycle (Morabito and Guerri 2000; Singh et al. 2012; Mostofa et al. 2015). In addition, antioxidants (glutathione, ascorbate, and phenolic metabolites) synthesized by the plant (Mittler 2002; Wang et al. 2008) are actual hunters of ROS (Sgherri et al. 2003). Though, oxidative stress in plant could be increased if the reductions in antioxidation capacity occur due to over production of ROS. The imbalance between the production and elimination of ROS degrade protein, injury to DNA, lipid peroxidation (LPO) and finally the death of plant cells (Atha et al. 2012; Barata et al. 2005). The type of plant and particular metal involved in stress define the response of antioxidant enzymes and response varies from each plant species to individual metal (Bhaduri and Fulekar 2012). Efficient destruction of $O_2^{\bullet -}$ and H_2O_2 in cells involves the determined response of antioxidants. At the initial stage of ascorbate–glutathione cycle, ascorbic acid is used to reduce the stress. Peroxidase ascorbic acid is the most crucial in cytosol and chloroplast for detoxification of H_2O_2 (Smirnoff 2000). The elimination of H_2O_2 in the mitochondrion micro body is mainly accomplished by catalase (CAT) enzyme (Shigeoka et al. 2002) and ultimately improves the condition of cells after oxidative stress. It is reported that ascorbate–glutathione cycle is the utmost essential for decreasing the harmful effects of Cu in plants but the reaction mechanism varies in various plant species for antioxidative response (Drazkiewicz et al. 2003).

6 Conclusion

The current study reviewed the general mechanism for toxicity of Cu in plants. Presence of Cu in excess amount in soil is toxic to plants. The level of toxicity in plant depends on the growth and development stage of plant. The selective permeability of plant is lost as Cu has potential to alternate the arrangement of cell wall and plasma membrane. The damage to cell membrane permits the Cu for entering in cytoplasm, which starts a sequence of deterioration that might produce variations in essential metabolic process of plants including photosynthetic or oxidative activity. In addition, Cu-induced stress disturbs the growth, development, dry mass yield, and harvestable yield of plant.

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References

Adrees M, Ali S, Rizwan M, Ibrahim M, Abbas F, Farid M, Bharwana SA (2015) The effect of excess copper on growth and physiology of important food crops: a review. Environ Sci Pollut Res 22(11):8148–8162

Ahmad MSA, Hussain M, Saddiq R, Alvi AK (2007) Mungbean: a nickel indicator, accumulator or excluder? Bull Environ Contam Toxicol 78(5):319–324

Ahmad MSA, Hussain M, Ijaz S, Alvi AK (2008) Photosynthetic performance of two mung bean [*Vigna radiata* (L) wilczek] cultivars under lead and copper stress. Int J Biol 10:167–172

Ahmad R, Ali S, Rizwan M, Dawood M, Farid M, Hussain A, Wijayae L, Alyemenie MN, Ahmad P (2020) Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. Physiol Planta 168:289–300

Ahsan N, Lee DG, Lee SH, Kang KY, Lee JJ, Kim PJ, Yoon HS, Kim JS, Lee BH (2007) Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere 67:1182–1193

Ali NA, Bernal MP, Ater M (2002) Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. Plant Soil 239:103–111

Ali, Q., Ahsan, M., Khaliq, I., Elahi, M., Ali, S., Ali, F., & Naees, M. (2011). Role of rhizobacteria in phytoremediation of heavy metals: an overview. Int Res J Plant Sci, 2, 220–232.

Ali B, Wang B, Ali S, Ghani MA, Hayat MT, Yang C, Zhou WJ (2013) 5-Aminolevulinic acid ameliorates the growth, photosynthetic gas exchange capacity, and ultrastructural changes under cadmium stress in Brassica napus L. J Plant Growth Regul 32(3): 604–614.

Anjum NA, Umar S, Chan MT (2010) Ascorbate-glutathione pathway and stress tolerance in plants. Springer, Dordrecht, pp 1–428

Anjum NA, Sofo A, Scopa A, Roychoudhury A, Gill SS, Iqbal M, Ahmad I (2015) Lipids and proteins—major targets of oxidative modifications in abiotic stressed plants. Environ Sci Pollut Res 22(6):4099–4121

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. Annu Rev Plant Biol 55:373–399

Araújo E, Strawn DG, Morra M, Moore A, Alleoni LRF (2019) Association between extracted copper and dissolved organic matter in dairy-manure amended soils. Environ Pollut 246:1020–1026

Asati A, Pichhode M, Nikhil K (2016) Effect of heavy metals on plants: an overview. Int J Appl Innov Eng Manage 5:2319–4847

Atha DH, Wang H, Petersen EJ, Cleveland D, Holbrook RD, Jaruga P, Nelson BC (2012) Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. Environ Sci Technol 46 (3):1819–1827

Baker DE, Senef JP (1995) Copper. In: Alloway BJ (ed) Heavy metals in soils. Blackie Academic and Professional, London, pp 179–205

Barata C, Varo I, Navarro JC, Arun S, Porte C (2005) Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. Comput Biochem Physiol 140:175–186

Baryla A, Laborde CJ, Montillet L, Triantaphylides C, Chagvardieff P (2000) Evaluation of lipid peroxidation as a toxicity bioassay for plant exposed to copper. Environ Pollut 109:131–135

Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34

Bernal M, Roncel M, Ortega JM, Picorel R, Yruela I (2004) Copper effect on cytochrome b559 of photosystem II under photoinhibitory conditions. Physiol Plantarum 120:686–694

Bhaduri AM, Fulekar MH (2012) Antioxidant enzyme responses of plants to heavy metal stress. Rev Environ Sci Bio 11(1):55–69

Bhakuni G, Dube BK, Sinha P, Chatterjee C (2009) Copper stress affects metabolism and reproductive yield of chickpea. J Plant Nutr 32(4):703–711

Bipasha C, Goswami BC, Chakravarty B (2000) Effect of zinc and copper on growth and regenerating cultures of *Citrus reticulata* Blanco. Indian J Plant Physiol 5:52–58

Bouazizi H, Jouili H, Geitmann A, Ferjani EEI (2010) Copper toxicity in expanding leaves of *Phaseolus vulgaris* L: antioxidant enzyme response and nutrient element uptake. Ecotoxicol Environ Safe 73:1304–1308

Brun LA, LeCorff J, Maillet J (2003) Effects of elevated soil cu on phenology, growth and reproduction of five ruderal plant species. Environ Pollut 122:361–368

Burda K, Kruk J, Schmid GH, Strzalka K (2003) Inhibition of oxygen evolution in photosystem II by cu (II) ions is associated with oxidation of cytochrome b559. Biochem J 371:597–601

Chandra S (2003) Effects of leaf age on transpiration and energy exchange of *Ficus glomerata*, a multipurpose tree species of Central Himalayas. Physiol Mol Bio Plants 9:255–260

Chatterjee C, Sinha P, Dube BK, Gopal R (2006) Excess copper-induced oxidative damages and changes in radish physiology. Commun Soil Sci Plants 37:2069–2076

Chen C, Song Y, Zhuang K, Li L, Xia Y, Shen Z (2015) Proteomic analysis of copper-binding proteins in excess copper-stressed roots of two rice (*Oryza sativa* L.) varieties with different cu tolerances. PLoS One 10(4):e0125367

Chibuike GU, Obiora SC (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. Appl Environ Soil Sci 2014:752708

Cho UH, Seo NH (2005) Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. Plant Sci 168:113–120

Connan S, Stengel DB (2011) Impacts of ambient salinity and copper on brown algae: interactive effects on photosynthesis, growth, and copper accumulation. Aquat Toxicol 104(1–2):94–107

Cota-Ruiz K, Hernández-Viezcas JA, Varela-Ramírez A, Valdés C, Núñez-Gastélum JA, Martínez-Martínez A, Gardea-Torresdey JL (2018) Toxicity of copper hydroxide nanoparticles, bulk copper hydroxide, and ionic copper to alfalfa plants: a spectroscopic and gene expression study. Environ Pollut 243:703–712

Das SK (2014) Role of micronutrient in rice cultivation and management strategy in organic culture—a reappraisal. Agric Sci 5(09):765

Dazy M, Be'raud E, Cotelle S, Meux E, Masfaraud JF, Fe'rard JF (2008) Antioxidant enzyme activities as affected by trivalent and hexavalent chromium species in *Fontinalis antipyretica* Hedw. Chemosphere 73:281–290

da Costa, C. H., Perreault, F., Oukarroum, A., Melegari, S. P., Popovic, R., & Matias, W. G. (2016). Effect of chromium oxide (III) nanoparticles on the production of reactive oxygen species and photosystem II activity in the green alga Chlamydomonas reinhardtii. Science of the Total Environment, 565, 951–960.

Deng C, Pan X, Wang S, Zhang D (2014) Cu 2+ inhibits photosystem II activities but enhances photosystem I quantum yield of *Microcystis aeruginosa*. Biol Trace Elem Res 160(2):268–275

Dimkpa CO, McLean JE, Britt DW, Anderson AJ (2014) Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. Ecotoxicology 24(1):119–129

Drazkiewicz M, Skorzynska-Polit E, Krupa Z (2003) Responses of the ascorbate-glutathione cycle to excess copper in *Arabidopsis thaliana* (L). Plant Sci 164:195–202

Ehsan S, Ali S, Noureen S, Mehmood K, Farid M, Ishaque W, Shakoor MB, Rizwan M (2014) Citric acid assisted phytoremediation of cd by *Brassica napus* L. Ecotoxicol Environ Saf 106:164–172

Elstner EF, Wagner GA, Schutz W (1988) Activated oxygen in green plants in relation to stress situations current top. Plant Biochem Physiol 7:159–187

Farid M, Shakoor MB, Ehsan S, Ali S, Zubair M, Hanif MS (2013) Morphological, physiological and biochemical responses of different plant species to cd stress. Int J Chem Biochem Sci 3:53–60

Farid M, Ali S, Ishaque W, Shakoor MB, Niazi NK, Bibi I, Dawood M, Gill RA, Abbas F (2015) Exogenous application of EDTA enhanced phytoremediation of cadmium by *Brassica napus* L. Int J Environ Sci Technol 12(12):3981–3992

Farid M, Ali S, Rizwan M, Ali Q, Abbas F, Bukhari SAH, Saeed R, Wu L (2017a) Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. Ecotoxicol Environ Saf 145:90–102

Farid M, Ali S, Rizwan M, Saeed R, Tauqeer HM, Sallah-Ud-Din R, Azam A, Raza N (2017b) Microwave irradiation and citric acid assisted seed germination and phytoextraction of nickel (Ni) by *Brassica napus* L.; morpho-physiological and biochemical alterations under Ni stress. Environ Sci Pollut Res 24(25):2150–2164

Farid M, Ali S, Akram NA, Rizwan M, Abbas F, Bukhari SAH, Saeed R (2017c) Phyto-management of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. Environ Sci Pollut Res 24(20):16845–16859

Farid M, Ali S, Zubair M, Saeed R, Rizwan M, Sallah-Ud-Din R, Azam A, Ashraf R, Ashraf W (2018a) Glutamic acid assisted phyto-management of silver contaminated soils through sunflower; physiological and biochemical response. Environ Sci Pollut Res 25(25):25390–25400

Farid M, Ali S, Rizwan M, Ali Q, Saeed R, Nasir T, Abbasi GH, Rehmani MIA, Ata-Ul-Karim ST, Bukhari SAH (2018b) Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. Ecotoxicol Environ Saf 151(30):255–265

Farid M, Ali S, Saeed R, Rizwan M, Ali B, Azam A, Ashraf Hussain A, Ahmad I (2019) Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. Int J Phytorem 21:8

Farid M, Ali S, Rizwan M, Yasmeen T, Arif MS, Riaz M, Saqib M, Zia ur Rehman M, Ayub MA (2020a) Combined effects of citric acid and 5-aminolevulinic acid in mitigating chromium toxicity in sunflower (*Helianthus annuus* L.) grown in Cr spiked soil. Pak J Agri Sci 57(2):1–9

Farid M, Farid S, Zubair M, Rizwan M, Ishaq HK, Ali S, Ashraf U, Alhaithloul HAS, Gowayed S, Soliman MH (2020b) Efficacy of *Zea mays* L. for the management of marble effluent contaminated soil under citric acid amendment; morpho-physiological and biochemical response. Chemosphere 240:124930

Fox TC, Guerinot ML (1998) Molecular biology of cation transport in plants. Annu Rev Plant Physiol Plant Mol Biol 49:669–696

Gardea-Torresdey JL, Peralta-Videa JR, Montes M, De la Rosa G, Corral-Diaz B (2004) Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L: impact on plant growth and uptake of nutritional elements. Bioresour Technol 92(3):229–235

Gawande, M. B., Goswami, A., Felpin, F. X., Asefa, T., Huang, X., Silva, R., ... & Varma, R. S. (2016). Cu and Cu-based nanoparticles: synthesis and applications in catalysis. Chemical reviews, 116(6), 3722–3811

Georgopoulus PG, Roy A, Opiekun RE, Yonone-Lioy MJ, Lioy PJ (2002) Environmental dynamics and human exposure. International Copper Association, New York, pp 10–23

Giordani C, Cecchi S, Zanchi C (2005) Phytoremediation of soil polluted by nickel using cultural crops. Environ Manag 36:675–681

Gonzalez-Mendoza D, Escoboza-Garcia F, Santamaria JM, Zapata-Perez O (2013) Copper stress on photosynthesis of black mangle (*Avicennia germinans*). An Acad Bras Ciênc 85(2):665–670

Gratão PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Function Plant Biol 32:481–494

Gupta DK, Palma JM, Corpas FJ (2015) Reactive oxygen species and oxidative damage in plants under stress. Springer, Heidelberg, pp 1–22

Gupta SK, Sharma M, Deeba F, Pandey V (2018) Role of reactive oxygen species in photophosphorylation and damage to D1 protein: past and present. Function Plant Biol 9:165–186

Habiba U, Masood R, Shibli MA, Niazi MA (2014) Cloud identity management security issues & solutions: a taxonomy. Complex Adap Syst Model 2(1):5

Habiba U, Ali S, Farid M, Shakoor MB, Rizwan M, Ibrahim M, Abbasi GH, Hayat T, Ali B (2015) EDTA enhanced plant growth, antioxidant defense system and phytoextraction of copper by *Brassica napus* L. Environ Sci Pollut Res 22(2):1534–1544

Hall JL, Williams LE (2003) Transition metal transporters in plants. J Exp Bot 54:2601–2613

Hippler FW, Cipriano DO, Boaretto RM, Quaggio JA, Gaziola SA, Azevedo RA, Mattos-Jr D (2016) *Citrus rootstocks* regulate the nutritional status and antioxidant system of trees under copper stress. Environ Exp Bot 130:42–52

Horváth E, Pál M, Szalai G, Páldi E, Janda T (2007) Exogenous 4- hydroxybenzoic acid and salicylic acid modulate the effect of short term drought and freezing stress on wheat plants. Biol Plant 51:480–487

Husak VV (2015) Copper and copper-containing pesticides: metabolism, toxicity and oxidative stress. J Vasyl Stefanyk Precarpathian National Univ 2(1):39–51

Ivanov VB, Bystrova EI, Seregin IV (2003) Comparative impacts of heavy metals on root growth as related to their specificity and selectivity. Russ J Plant Physiol 50(3):398–406

Jabeen N, Abbas Z, Iqbal M, Rizwan M, Jabbar A, Farid M, Ali S, Ibrahim M, Abbas F (2016) Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. Arch Agr Soil Sci 62(5):648–652

Jain R, Srivastava S, Solomon S, Shrivastava AK, Chandrax AK (2010) Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (*Saccharum spp*). Acta Physiol Plant 32:979–986

Kalaivanan D, Ganeshamurthy AN (2016) Mechanisms of heavy metal toxicity in plants. In: abiotic stress physiology of horticultural crops. Springer, New Delhi, pp 85–102

Kasprzak KS (2002) Oxidative DNA and protein damage in metal-induced toxicity and carcinogenesis free radical. Biol Med 32:958–967

Ke W, Xiong Z, Chen S, Chen J (2007) Effect of copper and mineral nutrition on growth, copper accumulation and mineral element up take in two *Rumex japonicus* populations from a copper mine and an uncontaminated filed site. Environ Exp Bot 59:59–67

Khan A, Khan S, Khan MA, Qamar Z, Waqas M (2015) The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environ Sci Pollut Res 22(18):13772–13799

Kopittke PM, Menzies NW (2006) Effect of copper toxicity on growth of cowpea (*Vigna unguiculata*). Plant Soil 279:287–296

Kumar V, Sharma A, Kaur P, Sidhu GPS, Bali AS, Bhardwaj R, Cerda A (2019) Pollution assessment of heavy metals in soils of India and ecological risk assessment: a state-of-the-art. Chemosphere 216:449–462

Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. Metallomics 8(3):269–285

Küpper H, Kroneck PMH (2005) Heavy metal uptake by plants and cyanobacteria. Met Ions Biol Syst 44:97–144

Lange B, van der Ent A, Baker AJM, Echevarria G, Mahy G, Malaisse F, Faucon MP (2017) Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge. New Phytol 213(2):537–551

Lequeux H, Hermans C, Lutts S, Verbruggen N (2010) Response to copper excess in *Arabidopsis thaliana*: impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol Biochem 48:673–682

Liu J, Dhungana B, Cobb GP (2018) Environmental behavior, potential phytotoxicity, and accumulation of copper oxide nanoparticles and arsenic in rice plants. Environ Toxicol Chem 37 (1):11–20

Llugany M, Lombini A, Poschenrieder C, Dinelli E, Barcel'o J (2003) Different mechanisms account for enhanced copper resistance in *Silene armeria* ecotypes from mine spoil and serpentine sites. Plant Soil 251:55–63

Lou LQ, Shen ZG, Li XD (2004) The copper tolerance mechanisms of *Elsholtzia haichowensis*, a plant from copper-enriched soils. Environ Exp Bot 51:111–120

Maanan M, Saddik M, Maanan M, Chaibi M, Assobhei O, Zourarah B (2015) Environmental and ecological risk assessment of heavy metals in sediments of Nador lagoon, Morocco. Ecol Indic 48:616–626

Mahmood T, Islam KR (2005) Rice seedlings response to copper toxicity and acidity. J Plant Nutr 29(5):943–957

Malevar GU (2005) Micronutrient stresses in soils and crops: serious sickness and clinical approaches for sustainable culture. J Indian Soc Soil Sci 53:484–499

Manasa V, Hebsur NS, Malligawad LH, Shiva Kumar L, Ramakrishna B (2015) Effect of water-soluble fertilizers on uptake of major and micro nutrients by groundnut and post-harvest nutrient status in a vertisol of northern transition zone of Karnataka. Ecoscan 9(1–2):01–05

Manceau A, Matynia A (2010) The nature of cu bonding to natural organic matter. Geochim et Cosmochim Acta 74(9):2556–2580

Manivasagaperumal R, Vijayarengan P, Balamurugan S, Thiyagarajan G (2011) Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* (L.) Wilczek. J Phytology 3 (3):53–62

Meshram SP, Adhyapak PV, Mulik UP, Amalnerkar DP (2012) Facile synthesis of CuO nanomorphs and their morphology dependent sunlight driven photocatalytic properties. Chem Eng J 204:158–168

Michaud AM, Chappellaz C, Hinsinger P (2008) Copper phytotoxicity affects root elongation and iron nutrition in durum wheat (*Triticum turgidum durum* L). Plant Soil 310:151–165

Mishra S, Bharagava RN, More N, Yadav A, Zainith S, Mani S, Chowdhary P (2019) Heavy metal contamination: an alarming threat to environment and human health in environmental biotechnology: for sustainable future. Springer, Singapore, pp 103–125

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mohiseni M, Farhangi M, Agh N, Mirvaghefi A, Talebi K (2017) Toxicity and bioconcentration of cadmium and copper in Artemia Urmiana Nauplii Iranian. J Toxicol 11(1):33–41

Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481

Monni S, Salemaa M, White C, Tuittila E, Huopalainen M (2000) Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a cu-Ni smelter, in Southwest Finland. Environ Pollut 109:211–219

Morabito D, Guerri EG (2000) The free oxygen radical scavenging enzymes and redox status in roots and leaves of *Populus euramericana* in response to osmotic stress, desiccation and rehydration. J Plant Physiol 157:74–80

Mostofa MG, Hossain MA, Fujita M, Tran LSP (2015) Physiological and biochemical mechanisms associated with trehalose-induced copper-stress tolerance in rice. Sci Rep 5:11433

Możdżeń K, Wanic T, Rut G, Łaciak T, Rzepka A (2017) Toxic effects of high copper content on physiological processes in *Pinus sylvestris* L. Photosynthetica 55(1):193–200

Munzuroglu O, Geckil H (2002) Effects of metals on seed germination, root elongation, and coleoptiles and hypocotyls growth in *Triticum aestivum* and Cucumis sativus. Arch of Environ Con Toxicol 43:203–213

Mysliwa-Kurdziel B, Prasad MNV, Strzalka K (2004) Photosynthesis in heavy metal stressed plants in: Prasad, MNV (Ed), heavy metal stress in plants: from biomolecules to ecosystems. University of Hyderabad, Hyderabad, pp 146–181

Nakajima H, Hara K, Yamamoto Y, Itoh K (2015) Effects of cu on the content of chlorophylls and secondary metabolites in the cu-hyperaccumulator lichen *Stereocaulon japonicum*. Ecotoxicol Environ Safe 113:477–482

Naser A, Anjum NA, Umar S, Ahmad A, Iqbal M, Khan NA (2008) Sulphur protects mustard (*Brassica campestris* L) from cadmium toxicity by improving leaf ascorbate and glutathione Sulphur protects mustard from cadmium toxicity. Plant Growth Regul 54:271–279

Ngozi E (2011) Mutagenecity testing of phamaceutical effluents on *Allium cepa* root tip meristems. J Toxicol Environ Health Sci 3(2):44–51

Okamoto OK, Pinto E, Latorre LR, Bechara EJH, Colepicolo P (2001) Antioxidant modulation in response to metal-induced oxidative stress in algal chloroplasts. Arch Environ Cont Toxicol 40:18–24

Panda SK (2003) Heavy metal phytotoxicity induces oxidative stress in *moss taxithelium* sp. Curr Sci 84:631–633

Panda SK (2008) Impact of copper on reactive oxygen species, lipid peroxidation and antioxidants in *Lemna minor*. Biol Plant 52:561–564

Panou-Filotheou AM, Bosabalidis A (2004) Root structural aspects associated with copper toxicity in oregano (*Origanum vulgare* subsp *hirtum*). Plant Sci 166:1497–1504

Patsikka E, Kairavuo M, Sersen F, Aro EM, Tyystjarvi E (2002) Excess copper predisposes PSII to photo inhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll. Plant Physiol 129:1359–1367

Peng H, Kroneck PM, Küpper H (2013) Toxicity and deficiency of copper in *Elsholtzia splendens* affect photosynthesis biophysics, pigments and metal accumulation. Environ Sci Technol 47 (12):6120–6128

Pilon M, Abdel-Ghany S, Cohu C, Gogolin K, Ye H (2006) Copper cofactor delivery in plant cells. Curr Opin Plant Bio 9:256–263

Posmyk MM, Kontek R, Janas KM (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper ions. Ecotoxicol Environ Saf 72:596–602

Printz B, Lutts S, Hausman JF, Sergeant K (2016) Copper trafficking in plants and its implication on cell wall dynamics. Front Plant Sci 7:601

Rajput VD, Minkina T, Suskova S, Mandzhieva S, Tsitsuashvili V, Chapligin V, Fedorenko A (2018) Effects of copper nanoparticles (CuO NPs) on crop plants: a mini review. BioNanoSci 8 (1):36–42

Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017a) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere 187:35–42

Rizwan M, Ali S, Qayyum MF, Ok YS, Adress M, Ibrahim M, Zia-ur-Reham M, Farid M, Abbas F (2017b) Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: a critical review. J Hazard Mater 322:2–16. https://doi.org/10.1016/j.jhazmat.2016.05.061

Rogers ED, Benfey PN (2015) Regulation of plant root system architecture: implications for crop advancement. Curr Opin Biotech 32:93–98

Rouphael Y, Cardarelli M, Reab E, Colla G (2008) Grafting of cucumber as a means to minimize copper toxicity. Environ Exp Bot 63:49–58

Ryan BM, Kirby JK, Degryse F, Harris H, McLaughlin MJ, Scheiderich K (2013) Copper speciation and isotopic fractionation in plants: uptake and translocation mechanisms. New Phytol 199 (2):367–378

Sağlam A, Yetişsin F, Demiralay M, Terzi R (2016) Copper stress and responses in plants. In: Plant metal interact. Elsevier, Amsterdam, pp 21–40

Sallah-Ud-Din R, Farid M, Saeed R, Ali S, Rizwan M, Tauqeer HM, Bukhari SAH (2017) Citric acid enhanced the antioxidant defense system and chromium uptake by *Lemna minor* L. grown in hydroponics under Cr stress. Environ Sci Pollut Res 24(21):17669–17678. https://doi.org/10.1007/s11356-017-9290-0

Sánchez-Viveros G, Gonzalez-Mendoza D, Alarcon A, Ferrera-Cerrato R (2010) Copper effects on photosynthetic activity and membrane leakage of *Azolla filiculoides* and *Anemone caroliniana*. Inter J cult Biol 12(3):365–368

Scarponil L, Perucci P (1984) Effects of some metals and related metal organic compounds on ALA-dehydratase activity of corn. Plant Soil 79:69–75

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Sergiev I, Todorova D, Brankova L, Alexieva V (2016) Prohexadione-Ca and Coper effect on growth and accumulation of endogenous polyamines in pea plants. Bot Lith 22(1):65–71

Sersen K, Králová K, Bumbálová A, Svajlenova O (1997) The effect of cu (II) ions bound with tridentate Schiff base ligands upon the photosynthetic apparatus. J Plant Physiol 151:299–305

Sgherri C, Cosi E, Navari-Izzo F (2003) Phenols and antioxidative status of *Raphanus sativus* grown in copper excess. Physiol Plant 118:21–28

Shahid M, Dumat C, Khalid S, Schreck E, Xiong T, Niazi NK (2017) Foliar heavy metal uptake, toxicity and detoxification in plants: a comparison of foliar and root metal uptake. J Hazard Mater 325:36–58

Sharma JC, Chaudhary SK (2007) Vertical distribution of micronutrient cations in relation to soil characteristics in lower shivaliks of Solan district in north-West Himalayas. J India Soc Soil Sci 55:40–44

Shaw AK, Ghosh S, Kalaji HM, Bosa K, Brestic M, Zivcak M, Hossain Z (2014) Nano-CuO stress induced modulation of antioxidative defense and photosynthetic performance of Syrian barley (*Hordeum vulgare* L). Environ Exp Bot 102:37–47

Shazia Q, Mohammad ZH, Rahman T, Shekhar HU (2012) Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in Beta thalassemia major patients: a review of the literature. Anemia 2012:270923

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot 53:1305–1319

Shikanai T, Müller-Moulé P, Munekage Y, Niyogi KK, Pilon M (2003) PPA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. Plant Cell 15:1333–1346

Singh D, Nath K, Sharma YK (2007) Response of wheat seed germination and seedling growth under copper stress. J Environ Bio 28:409–414

Singh VP, Srivastava PK, Prasad SM (2012) Differential effect of UV-B radiation on growth, oxidative stress and ascorbate–glutathione cycle in two cyanobacteria under copper toxicity. Plant Physiol Biochem 61:61–70

Smeets K, Ruytinx J, Semane B, Van Belleghem F, Remans T, Van Sanden S, Vangronsveld J, Cuypers A (2008) Cadmium-induced transcriptional and enzymatic alterations related to oxidative stress. Environ Exp Bot 63:1–8

Smirnoff N (2000) Ascorbic acid: metabolism and functions of multi-facetted molecule. Curr Opin Plant Biol 3:229–235

Soja G, Wimmer B, Rosner F, Faber F, Dersch G, von Chamier J, Zehetner F (2018) Compost and biochar interactions with copper immobilisation in copper-enriched vineyard soils. Appl Geochem 88:40–48

Srivastava S, Mishra S, Tripathi RD, Dwivedi S, Gupta DK (2006) Copper induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (lf) Royle. Aquat Toxicol 80:405–415

Tang SR, Wilke BM, Brooks RR, Tang SR (2001) Heavy-metal uptake by metal tolerant *Elsholtzia haichowensis* and *Commelina communis* from China. Commun Soil Sci Plant 32:895–905

Tausz M, Wonisch A, Grill D, Moral Es D, Jimenez MS (2003) Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. J Exp Bot 54:1505–1510

Tausz M, Sircelj H, Grill D (2004) The glutathione system as a stress marker in plant ecophysiology: is a stress response concept valid? J Exp Bot 55:1955–1962

Teisseire H, Guy V (2000) Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna minor*). Plant Sci 153:65–72

Thornton I (1979) In: Nriagu JO (ed) Copper in soils and sediments part I: ecological cycling in: copper in the environment. Wiley, New York, pp 171–216

Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma GD, Sahoo L, Sanjib P (2012) Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol Biochem 53:33–39

Tripathi BN, Mehta SK, Amar A, Gaur JP (2006) Oxidative stress in *Scenedesmus* sp during short- and long-term exposure to Cu2+? And Zn2+? Chemosphere 62:538–544

Vergani C, Graf F (2016) Soil permeability, aggregate stability and root growth: a pot experiment from a soil bioengineering perspective. Ecohydrology 9(5):830–842

Wahid A, Ghani A, Ali I, Ashraf MY (2007) Effects of cadmium on carbon and nitrogen assimilation in shoots of mungbean [*Vigna radiata* (L) Wilczek] seedlings. J Agron Crop Sci 193 (5):357–365

Wang SH, Yang ZM, Lu B, Li SQ, Lu YP (2004) Copper-induced stress and antioxidative responses in roots of *Brassica juncea* L. Bot Bull Acad Sinica 45:203–212

Wang Z, Zhang YX, Huang ZB, Huang L (2008) Antioxidative response of metal-accumulator and non-accumulator plants under cadmium stress. Plant Soil 310:137–149

Wani PA, Khan MS, Zaidi A (2007) Cadmium, chromium and copper in greengram plants. Agron Sustain Dev 27:145–153

Wojcik M, Tukiendorf A (2003) Response of wild type of *Arabidopsis thaliana* to copper stress. Biol Planta 46:79–84

Xiong ZT, Liu C, Geng B (2006) Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotoxicol Environ Safe 64(3):273–280

Xu J, Yang L, Wang Z, Dong G, Huang J, Wang Y (2006) Toxicity of copper on rice growth and accumulation of copper in rice grain in copper contaminated soil. Chemosphere 62(4):602–607

Yruela I (2005) Copper in plants. Braz J Plant Physiol 17:145–156

Yruela I (2009) Copper in plants: acquisition, transport and interactions functional. Plant Biol 36 (5):409–430

Yruela I, Alfonso M, Barón M, Picorel R (2000) Copper effect on the protein composition of photosystem II. Physiol Plantarum 110:551–557

Yu B, Xu J, Liu JH, Yang ST, Luo J, Zhou Q, Liu Y (2013) Adsorption behavior of copper ions on graphene oxide–chitosan aerogel. J Environ Chem Eng 1(4):1044–1050

Zengin FK, Kirbag S (2007) Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. J Environ Biol 28:561–566

Zhang H, Jiang Y, He Z, Ma M (2005) Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). J Plant Physiol 162:977–984

Zhang LP, Mehta SK, Liu ZP, Yang ZM (2008) Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. Plant Cell Physiol 49:411–419

Zhu J, Li D, Chen H, Yang X, Lu L, Wang X (2004) Highly dispersed CuO nanoparticles prepared by a novel quick-precipitation method. Mater Lett 58:3324–3327

Concept and Types of Phytoremediation

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Abstract

Phytoremediation is a form of bioremediation which deals with the enormous potential of plants for degrading or immobilizing contaminants in soil and groundwater. Anthropogenic activities release huge amounts of chemicals, wastes, and noxious gases into the environment, which alter the physical, chemical, and biological properties of ecosystem, worldwide. Biological agents utilize these contaminants as part of their metabolism and turn them into less toxic or harmless by-products. It reflects the natural ability of certain plants called hyperaccumulators to clean up a variety of contaminants, viz. polyaromatic hydrocarbons, pesticides, heavy metals, etc., in soil, water, or air. The choice of process; biotransformation, phytodegradation, phytostimulation, phytovolatilization, etc., to be opted for clean-up depends upon the habitats, supply of nutrients, contaminants type, and environmental conditions. Phytoremediation involves life forms, and ensures complete degradation of various toxicants without releasing harmful products, hence is an effective and cheaper solution for waste management.

Keywords

Phytoremediation · Phytodegradation · Phytostimulation · Phytovolatilization · Phytoextraction

1 Introduction

“Phytoremediation” is a combination of two words: the Greek prefix “phyto” meaning plant and the Latin root “remedium” meaning to remove the contaminant. Phytoremediation is an old concept which is used since many decades to clean the toxicity of different contaminants present in soil, groundwater, and other contaminated areas. The theory of phytoremediation depends on the usage of plant species to clean up the environmental pollution, making it an ecologically friendly and cost-effective technology, which can be applied both for in situ and ex situ treatments (Schwitzguébel et al. 2011). The phytoremediation method does not require any expensive instruments or skilled person, but is often based on the selection of appropriate plant which is able to survive in the perilous milieu and can degrade hazardous materials in a wide range of environment. The method can serve as an alternative tool against physical and chemical treatment methods which demand high capital inputs and are labour and energy intensive. Phytoremediation in combination with other remediation processes, viz. microbial remediation can be effectively employed in the treatment of wetlands, establishment of crops in stressed soils, and promoting sustainable agriculture (Garbisu et al. 2002; Gerhardt et al. 2017).

Plants can grow in almost all climatic conditions and can be employed to clean up variety of pollutants, viz. sewage and industrial effluents, pesticides, heavy metals, hydrocarbons, explosives, surfactants, nutrients, mineral oil, etc. (Van Aken 2009; Huang et al. 2011; Sarwar et al. 2017). The different mechanisms of phytoremediation differ on the basis of the type of pollutants which is addressed. The optimization of growth conditions can selectively enhance the process of remediation under suitable climatic conditions which not only improve performance of plant species but also can actively participate in treating varied environmental pollutants of different ecosystems.

Plant-assisted bioremediation involves the interaction of plant roots and the soil microorganisms in the biological treatment of large number of contaminated soils. The possible use of genetically modified plants is also gaining recognition as a potential remediation technique in many parts of the world (Abhilash et al. 2009). Some of the plants are also used as bioindicators to determine water quality, the level and nature of toxicants present in different aquifers, in the evaluation of municipal and industrial waste, etc. Plants called as hyperaccumulators are much studied these days which have the ability to store large amount of metals, without disturbing the normal plant functioning (Dhankher et al. 2002; Chen et al. 2013; Raj et al. 2020). Such metabolically active plant species can be used to inhabit soils contaminated with heavy metals for the enhanced uptake and remediation. It satisfies the concept of green revolution in the field of innovative clean-up technology which can be used for in situ or ex situ applications with minimum distribution of soil and reduced spread of contaminants (Bert et al. 2009).

Phytoremediation technologies are in the early stages of development, involving only laboratory research and limited field trials. Although the beneficial properties of plant species for the treatment of contaminated ecosystems are known and studied, their inherent abilities to remove pollutants from the environment have yet to be explored commercially (Gerhardt et al. 2017). Extensive research including genetic engineering is required to investigate and develop plant species with potential phytoremediation applications, which can be applied for the selective degradation or removal of pollutants at specific sites (Dhankher et al. 2002). Furthermore, the total cost of operation, time required, method of waste disposal, and growth requirements to remediate per acre of land or per gallon water should be worked out and optimized for the industrial and commercial usages of this technology.

2 Environmental Contaminants

Extensive human activities are continuously introducing hazardous compounds into the environment at an alarming rate. The soil, air, and water have been contaminated with toxic materials which are altering their physical and chemical properties. The effluents coming out from different industries and also the industrial processes had become a major concern worldwide as the foremost source of environmental contamination. Environmental contaminants are broadly defined as those chemicals that accidentally or deliberately enter the environment, and impair its properties. These contaminants are very stable and they do not break down easily, as a result become persistent and circulate in the environment for indefinite time period (Novak and Trapp 2005). If released to the environment, these contaminants may enter the food chain and disturb all forms of life.

The other naturally occurring chemicals like heavy metals (locked in soils, rocks, and sediments) are introduced into the environment through intensive industrial activity which increase their mobility and availability, allowing them to enter the food chain up to higher levels. Heavy metal accumulation is of primary concern because they cannot be destroyed or degraded. Apart from this, wide variety of environmental contaminants have been detected in food, water, and soil which could be either inorganic or the so-called persistent organic pollutants (POPs). A broad classification of contaminants that can be remediated using plant-assisted bioremediation includes:

1. Heavy metals (As, Hg, Cd, Cr, Pb, Co, Cu, Ni, Se, Zn)
2. Petroleum hydrocarbons
3. Polyaromatic hydrocarbons (PAHs)
4. Dioxins and furans
5. Chlorinated solvents (trichloroethylene)
6. Polychlorinated biphenyls (PCBs)
7. Chlorinated naphthalenes
8. Perfluorinated chemicals in food
9. Chlorinated pesticides
10. Organophosphate insecticides
11. Radionuclides
12. Explosives (TNT, DNT, TNB, RDX)
13. Nutrients (nitrate, ammonium, phosphate)
14. Surfactants

3 Principle of Phytoremediation

Phytoremediation relies on the basic principle of using plants such as algae, fungi, grasses, shrubs, trees, etc., for the stabilization, volatilization, degradation, extraction, or precipitation of pollutants present in soil, water, or air. Plants might promote the removal of pollutants from their surroundings by varied mechanisms, in which

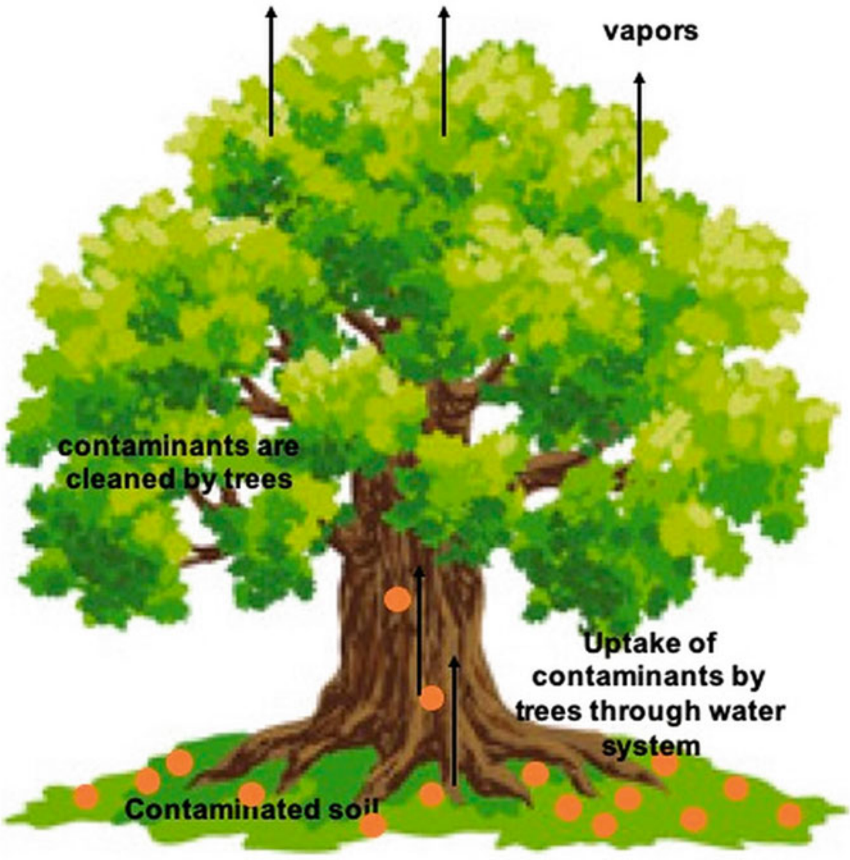

Fig. 1 Principle of phytoremediation

they not only help in breaking down of the waste products but also promote the growth of bacteria in the root zone that in turn break down pollutants (Garbisu et al. 2002; Schwitzguébel et al. 2011). Plants can also uptake and trap the pollutants from soil by acting as a filter. The uptake of contaminants in plants occurs primarily through the root system, which provides an enormous surface area to absorb and accumulate the non-essential contaminants along with water and nutrients to undertake the process of remediation (Fig. 1).

4 How Does Phytoremediation Works?

Phytoremediation is solely dependent upon the metabolic activities of certain plant species and their affinity towards various contaminants. It is a natural process accomplished by plants, which can clean up pollutants as deep as their roots can reach taking the following steps:

1. Plants get adapted and try to establish themselves in the polluted ecosystems (Schwitzguébel et al. 2011). Certain plants are able to remove or break down harmful chemicals from their vicinity by absorbing water and nutrients from the contaminated soil through their extensive root system (Novak and Trapp 2005). The roots of plants play an important role not only in the absorption of contaminants (moderately water soluble) but also locking them to some extent in the soil to limit their uptake and further translocation (Wang et al. 2018). Other plant species tend to remove the pollutants from the soil and accumulate them inside the tissues, without disturbing the vital cell functioning.
2. Uptake of the pollutants is facilitated by specific ion channels and transporter proteins which are present on the cell surface (LeBlanc et al. 2013). These protein channels allow a vast variety of pollutants to enter inside the cell.
3. Entry of the pollutants in the cell is followed by their processing via lignification, volatilization, metabolization, mineralization, transformation, etc.
4. The processes involve the use of enzymes to breakdown complex organic molecules into simpler ones (probably CO_2 and water). This step is very significant, as it determines the fate of contaminants in plant tissues (Feng et al. 2017). Further, there are unique enzyme systems present in cells which are specific for a type of chemical/pollutants.
5. The by-products of the remediation processes are either used up by the cells as building blocks, or they are released in the soil. The release of chemicals (exudates) or by-products increases the carbon and oxygen content of soil around roots which promotes microbial activity. The microbes in turn enhance plant growth and establish them in contaminated soils.

5 Mechanism of Phytoremediation

Depending on the type of contaminant, its bioavailability and soil properties, distinct plant species have different efficiencies towards noxious waste and show varied mechanisms of phytoremediation. The generously used mechanisms by the plant species to clean up and/or remediate contaminated sites are briefly discussed below:

5.1 Phytoextraction

Phytoextraction, also called phytoaccumulation, is the ability of plant roots to absorb, uptake, concentrate, and precipitate metals and other compounds from soil and accumulate it into above-ground portions (shoots and leaves). Once allowed to grow for some time, plants can be harvested and either incinerated or composted to recycle the metals. Plants varieties that can absorb and accumulate heavy metals and metalloids at an extraordinary rate from the contaminated soils have long been studied and identified (Sarwar et al. 2017). These can be grouped into metal accumulating and non-accumulating plants. Studies prove that metals such as As, Pb, Ni, Zn, and Cu can be effectively removed by phytoextraction. Such species have high affinity for the metals, which facilitates permanent removal of contaminants from the surrounding, especially soil (Afonso et al. 2020). They also have mechanism to detoxify and/or tolerate high metal concentration. Phytoextraction of heavy metals represents one of the promising opportunities for phytoremediation because of the significance of environmental problems associated with metals. This approach is highly influenced by rate of metal uptake by roots, soil properties like pH, presence of other components in the soil, cellular tolerance to toxic metals, etc. (do Nascimento et al. 2020).

5.2 Phytovolatilization

Phytovolatilization is the uptake, transport, and transpiration of a volatile contaminant by a plant (Doty et al. 2007). The method is generally executed through the plant leaves, from where the modified contaminants or wastes are evaporated and released to the atmosphere. The process of phytovolatilization occurs as growing plants take up the organic contaminants from the soil, convert them into volatile forms and finally release them into the atmosphere through transpiration as detoxified vapour. Many different contaminants such as metals, hydrocarbons, etc., can be treated by this method; however, the released components are recycled through different means which then re-enters into biological system (Feng et al. 2017). Fast growing trees are particularly useful in the process for the rapid uptake of contaminants. Further, experimental success has been achieved for the use of genetically modified variety of plant species having enormous transpiration pull as a useful choice to clean up pollutants.

5.3 Phytodegradation

Phytodegradation, also called phyto-transformation, is the breakdown of contaminants through metabolic processes within the plants. Complex organic pollutants are degraded into simpler molecules through the enzyme systems, which are further used as building blocks in the plant tissues to help the plant grow faster (Feng et al. 2017). The process can take place either internally or externally using specific secretory enzymes which catalyze and accelerate chemical reactions. The approach is useful for biodegradation of pollutants like chlorinated hydrocarbons, herbicides, and explosives (Van Aken 2009). In the process, plants modify the surrounding soil by producing root exudates which attracts many rhizosphere-inhabiting microbial species like bacteria and fungi. These microbial species stimulate an array of positive interactions with plants and accelerate the process of biodegradation of xenobiotics.

5.4 Rhizofiltration

Rhizofiltration is the methodology which is primarily executed by the intensive root system of plants involving absorption, concentration, and precipitation of contaminants present at lower concentrations for the immediate remediation of the polluted aqueous and land sources. The approach largely involves the locking and retaining of chemicals in the soil by the roots to limit their further transportation. Many different terrestrial plants, viz. Indian mustard, sunflower, rye, spinach, and corn have been investigated for their ability to remove contaminants from the surrounding. The fibrous and extended root system of the plants presents an increased surface area for the pollutants, making them the promising candidates to be used both for in situ or ex situ applications (Salt et al. 1995).

5.5 Rhizodegradation

Rhizodegradation, also called as phytostimulation or plant-assisted degradation, is the breakdown of contaminants in the rhizosphere through microbial activity (Abdullah et al. 2020). The plant roots produce certain chemicals, viz. sugars, alcohols, and acids, which attract soil microorganisms like yeast, fungi, or bacteria to inhabit rhizosphere. The microbes then consume and digest organic substances into harmless products for their nutrition and energy. However, the process is much slower than phytodegradation; it is broadly employed in the remediation of contaminated soils.

5.6 Phytostabilization

Phytostabilization also known as place-inactivation is the use of certain plant species to immobilize contaminants in the soil and groundwater through absorption and accumulation by roots, or precipitation within the rhizosphere zone of plants. This process reduces movement of contaminants and prevents migration to the groundwater. It also reduces bioavailability of pollutants for entry into the food chain. This technique prevents soil erosion, distribution of toxic metal to other areas, and can be used to re-establish a vegetative cover at sites where natural vegetation is lacking due to high levels of contaminants (Ahsan et al. 2018). Recent studies prove that the use of metal-tolerant plant species can be used to restore vegetation by decreasing the potential migration and leaching of soil contaminants to groundwater. The process can also be effectively used for the treatment of contaminated land areas affected by mining activities and to overcome the problems associated with disposal of hazardous waste.

6 Types of Plants Used in Phytoremediation

Many different plant varieties are used since decades for the remediation of varied contaminants and pollutants from distinct ecosystems. Extensive research in this field had added to the knowledge of many existing plant species (both terrestrial and aquatic) that belong to distantly related families and have the ability to grow on stressed soils. These plants can easily prosper on the contaminated and metalliferous soils and accumulate extraordinarily high amounts of pollutants/heavy metals in their roots and aerial organs, without suffering phytotoxic effects (Sarwar et al. 2017). A list of such dynamic plant species used in the phytoremediation technology is presented in Table 1.

Some of the plants are known to uptake and accumulate remarkable amount of contaminants especially heavy metals from their surroundings. Such species are termed as “hyperaccumulators” to describe a number of plants with an enhanced rate of uptake, a faster root-to-shoot translocation, and a greater ability to detoxify the pollutants (Dhankher et al. 2002). Such determinant role is played by the constitutive overexpression of genes encoding transmembrane transporters, which acts as a defence mechanism against many environmental contaminants (Rascio and Navari-Izzo 2011; LeBlanc et al. 2013). Metal accumulating species can be used for phytoremediation to treat the heavy metal contaminated soils which otherwise pose many serious problems to human and animal health. The mechanisms adopted by higher plants in metal clean-up process include: uptake and translocation (Wang et al. 2018); metal removal through volatilization; conversion of metals to less toxic and volatile forms; detoxification and sequestration of metals; accumulation; metal complexation and immobilization, and metal–microbe interaction (Fig. 2). Most of the hyperaccumulating plants have the capability to absorb and accumulate two or more than two heavy metals (Meagher and Heaton 2005). Hyperaccumulators have been studied and identified in different families like Brassicaceae, Poaceae, Hydrangeaceae, Pteridaceae, Asteraceae, Pontederiaceae, and Fabaceae (Raj et al. 2020).

7 Factors Affecting Phytoremediation

There are several factors which can affect the phytoremediation process, as described in Fig. 3. Understanding these factors could play a vital role in understanding the fate of pollutants in plants and the mechanisms adopted by different plant species to combat their detrimental effects both in their own cell and in the outer surroundings including humans. Further, the performance of plants can be greatly improved. Some of the important factors are underlined below:

Table 1 Plant species used in remediation of various contaminants

S. No.	Contaminants/pollutants	Plant species involved in remediation	References
1	Heavy metals: Cd, Cu, Fe, Pb, Sb, uranium, Pb, Zn	*Cynara cardunculus*, poplar plants, *Setaria pumila*, *Pennisetum sinese, Sedum plumbizincicola*, *Elsholtzia splendens*, *Althaea rosea* Cavan, *Pisum sativum* L., *Coronopus didymus* L.	Sidhu et al. (2018), Gupta et al. (2019), Huang et al. (2019a, b), Sidhu et al. (2019), Capozzi et al. (2020), and Cui et al. (2020)
2	Sewage water	*Typha angustifolia*, *Eichhornia crassipes*	Pandey et al. (2019)
3	1-butyl-3-methylimidazolium bromide, Cd	*Lolium perenne* L.	Hu et al. (2019)
4	Polycyclic aromatic hydrocarbons (PAHs)	*Lolium perenne, Ricinus communis*	Wang et al. (2013)
5	Mineral oil, industrial waste	*Ricinus communis*	Boda et al. (2017) and Rehn et al. (2019)
6	Persistent organic pollutants (POPs)	Cucurbits (pumpkin, cucumber, and squash)	Iwabuchi et al. (2019)
7	Dieldrin	*Sorghum vulgare Moench*, *Helianthus annuus* L., *Glycine max*, *Brassica rapa var. perviridis*, *Lagenaria siceraria var. hispida*, *Cucumis sativus* L., *Cucurbita pepo* L.	Murano et al. (2010)
8	β-1,2,3,4,5,6-hexachlorocyclohexane (β-HCH), 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphthalene	*Hordeum vulgare*, *Glycine max*, *Solanum lycopersicum*, *Brassica oleracea*, *Cucurbita pepo*	Namiki et al. (2015)
9	[^{14}C]-1,2,4-triazole	Wheat and tomato	Lamshoeft et al. (2018)
10	Domestic and textile effluents	*Lemna minor*, *Daphnia magna*	de Alkimin et al. (2019)
11	Acidic mine waste	*Cymbopogon martini* (*Roxb.*) *Wats*	Jain et al. (2019)
12	Tetrabromobisphenol A	*A. marina, K. obovata*	Jiang et al. (2019)
13	Polybrominated diphenyl ethers (PBDEs)	*Avicennia marina* (*Am*), *Aegiceras corniculatum* (*Ac*)	Chen et al. (2017a)
14	Phenanthrene and pyrene	*Kandelia candel* (L.) *Druce*	Lu et al. (2011)

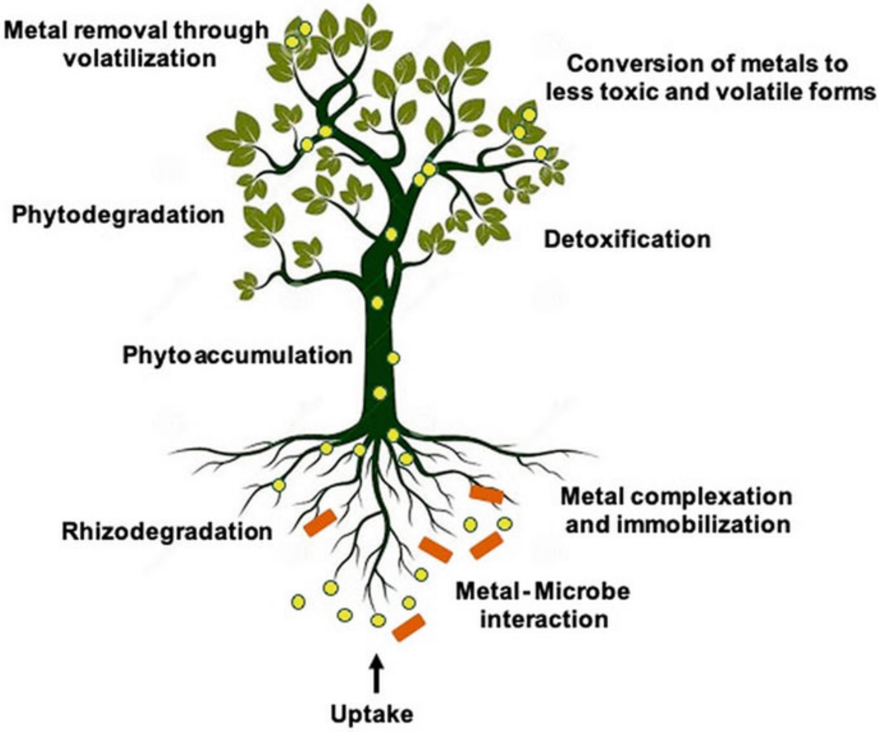

Fig. 2 Major mechanisms involved in metal remediation by plants

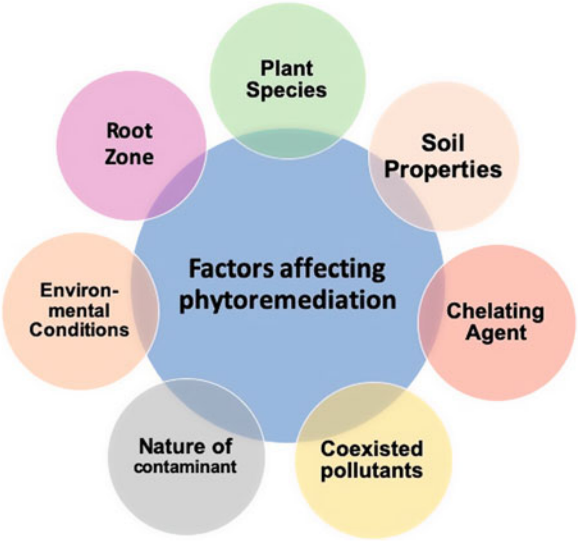

Fig. 3 Factors affecting the phytoremediation process

7.1 Soil Properties

Several soil physical and chemical properties and agronomical practices constitute one of the important factors to enhance remediation. The concentration of pollutants and/or contaminants absorbed by the plants is affected by the organic matter, pH, moisture content, sulphur and phosphorus content of the soil, clay content, cation exchange capacity, and total nitrogen content. Optimization of the soil conditions and evaluation of the effects of relevant soil properties on the distribution of total contaminants can appreciate the levels of phytoremediation and sustainability.

7.2 Plant Species

The uptake of any compound is largely affected by the type of plant species, its ability to inhabit polluted soil, and its potential in executing remediation process. Screening of the suitable plant species or varieties with those having superior remediation properties should be selected (Sarwar et al. 2017). The role of plant is to decrease the mobility of contaminants in soil by absorption, complexation, and precipitation in the rhizosphere by successive binding of the chemicals with root exudates. Success of the phytoremediation technique depends upon the increased metabolic rate of plants in the presence of pollutants. Identification of such plant species which can hyperaccumulate varieties of contaminants including heavy metals without generating noxious waste products by establishing itself in the stressed soils is the need of the time.

7.3 Environmental Conditions

The phytoremediation process is essentially affected by the environmental conditions which determines the success of the growth of the plants and their establishment in polluted soils. One such factor is temperature which influences plant growth, specifically root length and signifies the binding of contaminants with soil particles. It also affects the level of uptake of the contaminants by the plant roots. Optimal temperature should be maintained between 22 and 40 °C for the smooth accomplishment of phytoremediation. Other environmental factors which contribute towards remediation process are pH (6–8), nutrient concentrations, salinity, microbial population density (ideally should be between 10^4 and 10^7 CFU/g of soil), dissolved iron concentration, etc., which are the keys of the applicability of phytoremediation (Zhang et al. 2020).

7.4 Root Zone

The Root Zone is the most crucial area which is of special interest in phytoremediation. Root structure determines the applicability of plant species in contributing towards the environmental clean-up. Roots absorb contaminants and affect their mobility by locking the particulates in soil (Dhankher et al. 2002). Extensive root system helps the plants for the increased uptake of contaminants from the root zone which is also facilitated by the microbes inhabiting that area. Complexation and degradation of contaminants in the soil by plant enzymes exuded from the roots is another factor which supports the phytoremediation mechanism. A morphological adaptation in the root system, regardless of the plant used, is marked by an increase in root length and diameter for enhanced uptake and sequestration (Galal et al. 2018).

7.5 Nature of Contaminants

Metal uptake by plants depends on the bioavailability of the contaminants, solubility in the water phase, mobility, retention time, and interaction with root, soil particles, and other elements in vicinity. Furthermore, the contaminants remain bounded with the soil which is influenced by the soil characteristics like, pH, redox potential, and organic matter content (Novak and Trapp 2005).

7.6 Chelating Agent

The increased uptake of heavy metals by the plant roots is dependent on their availability which is achieved through the addition of biodegradable compounds called as chelating agents. These are chemical compounds that react with metal ions to form a stable and ring-like water-soluble complex known as chelators, or sequestering agents (Dhankher et al. 2002). They stimulate the heavy-metal-uptake capacity of the plant roots and define the microbial community in and around the rhizosphere. Exposing plants to the suitable chelating agent (e.g., EDTA) for a longer time could improve metal translocation in plant tissue as well as the overall plant performance. The process is also influenced with the presence of a ligand that affects the uptake mechanisms through the formation of metal–ligand complexes and changes the potential to leach metals below the root zone. Selection of chelating substances should be solely based on the properties like cost effectiveness and environmental friendly nature, and its ability to promote leaching of the contaminants into the soil.

7.7 Co-existed Pollutants

Soils co-contaminated by different levels of pollutants like organic and inorganic compounds, heavy metals, chlorinated mixtures, pesticides, aromatic hydrocarbons, etc., strongly effect the response of the plant species involved in remediation process (Huang et al. 2011; Feng et al. 2017). Extensively polluted ecosystems undeniably reduce the effectiveness of plants to deal with the contaminants. Further, the presence of one chemical could reduce the availability of other which may then be outstripped by the remediation method.

8 Applications of Phytoremediation Technique

8.1 Ground Water Remediation

Shallow and surface water may be treated well using the techniques of phytoremediation. One such mechanism is rhizofiltration, having vast application to be conducted in situ, with plants being grown directly in the contaminated water body. Rhizofiltration involves the pumping of contaminated water through root systems of appropriate plant species within the rhizosphere. It is facilitated through the efficient absorption of pollutants from the water into root tissues. The intensive root system of plants also assists in precipitation and concentration of the contaminants by releasing low-molecular-weight organic substances in the rhizosphere which accelerate the remediation process (Galal et al. 2018).

Phytotransportation is yet another remediation technique which can be accomplished under large-scale treatment strategy, specifically for ponds or wetlands. In this process, plants take up organic contaminants within the rhizosphere zone by their deep roots and degrade them to less toxic or non-toxic compounds. Roots are harvested, and treated to ensure constant operation results. Oxygen released during plant activity increases the rates of microbial activity and thus the rates of contaminant degradation.

8.2 Soil Remediation

This process involves the removal of contaminants and metals from soil, by direct uptake into plant tissue by the method of phytoextraction. The mechanism works in a satisfying way on the application of hyperaccumulators. These species show rapid growth rate, high biomass production, and can tolerate high concentrations of pollutants in harvestable tissue. The process can be boosted by using several plant species at the same time in a site, to remove more than one contaminant. After the remediation process, plant tissues are removed and carefully disposed of. The method of phytoextraction is being used for the treatment of sites contaminated with metals like arsenic and cadmium, radionuclides, hydrocarbons, etc.

Soil remediation also involves the use of certain plant species of high biomass to absorb, precipitate, and immobilize contaminants, reducing their bioavailability and mobility. This technique is called as phytostabilization which signifies the use of metal-tolerant plant species to be established in surface soils. It helps in restoring natural vegetation in the sites, by decreasing the potential migration of contaminants and limiting the transport of soil contamination to groundwater by locking the soil particles around rhizosphere. The contaminants are retained in the roots for a longer time period or are transferred along the upper plant parts as a strategic step towards soil remediation (Ye et al. 2017).

9 Technology Performance/Implementation

Use of phytoremediation is an emerging branch of science with unrestricted possibilities; however, it is currently limited to research activities with partial field testing. Different small-scale demonstrations have been tried and reported in lowering contaminant concentrations but full-scale applications are currently limited in number. Further, full-scale research proposals and remediation projects with regulatory approval are necessary to evaluate the applicability of the process (Salt et al. 1995). Optimization and standardization of the methodologies, applicability of the technology for specific pollutant, investigation of the pollutant chemistry, cost estimates, and determination of the regulations for field trials, are necessary to validate the remediation technique for in situ applications (Gerhardt et al. 2017). The general terms followed for the implementation of phytoremediation involve: (1) characterization of soil and water properties, nature of the contaminants, their concentrations and distribution, (2) determination and selection of appropriate plant species and elucidating the fate of contaminants in metabolic machinery of the cell, (3) understanding the environmental conditions and climatic parameters of the area to be treated, (4) illuminating the rates of remediation, treatment time, by-products produced, and density of the plantation after field testing, (5) consideration and monitoring the results obtained for the proper disposition of affected plant material for the future refine design parameters, and (6) estimation of the capital costs of phytoremediation per acre for planting, testing, maintenance, monitoring, verification, waste disposal and loss.

10 Genetic Engineering Based Improvements for Phytoremediation

The conventional method of phytoremediation has proved to significantly reduce contamination with limited environmental disturbances. However, the process is largely affected due to diverse reasons; one such is the viability of plants in the presence of extreme toxic compounds. This limits the feasibility of using remediation methods for extended time period and becomes restricted only to plant species which can withstand toxicity. Moreover, the tendency to absorb and withdraw pollutants by the plants from soil and water also possesses high risk of bioaccumulation and its successive transfer to different tropic levels of the food chain. These limitations had contributed towards extended research effort in developing new varieties of plant species using genetic engineering, capable of tolerating high levels of pollutants with its limited transportation to the edible parts (Abhilash et al. 2009; Chen et al. 2017b). Many of the naturally occurring plant species, viz. *Brassica juncea* and *Helianthus annuus* have been genetically engineered to develop transgenic plants for use in phytoremediation process (Raj et al. 2020).

Genetic engineering approach is to enhance and improve the ability of plants to uptake and sequester pollutants from their immediate environment for better phytoremediation results (Van Aken 2008). However, majority of genes have been transferred from other organisms in the progression of the process (James et al. 2008; Ali et al. 2012). Improvement in this approach will have an important role to play in commercial phytoremediation within next few years by adopting several conceivable approaches which have been summarized below:

1. Over expression of metallothionins and phytochelators.
2. Introduction and/or alteration in genes encoding transport proteins for the enhanced metal transport into roots.
3. Engineering of metabolic pathways by introducing genes encoding key biodegradation enzymes.
4. Introduction of genes encoding efficient metal chelators.
5. Genetic manipulation for the enhancement of root growth, branching, depth, biomass, and penetration.
6. Introduction of genes for the stimulation of rhizosphere microflora.

10.1 Metallothioneins, Phytochelatins, and Metal Chelators

Metallothioneins (MTs) are a family of cysteine-rich metal-binding proteins which are highly conserved in nature (Cobbett and Goldsbrough 2002). They help the plants to provide protection against oxidative stress and toxic heavy metals. Successful expression of metallothionein genes might support increased metal tolerance and accumulation in plants. Further, the genetic engineering process can be used to transform any suitable plant species to perform remediation. Tobacco and *Brassica* plants have been transformed for the constitutive uptake and Cd tolerance.

Phytochelatins are oligomers of glutathione, produced by the enzyme phytochelatin synthase. They are found in plants, and act as chelators for heavy metal detoxification. Over expression of this gene enhances metal tolerance in plants (Dhankher et al. 2002). Transgenic Indian mustard and cauliflower were developed by overexpression of glutathione synthase for the enhanced Cd tolerance and accumulation. Similarly, overproduction of iron chelator in rice has resulted in enhanced Al tolerance compared to control plants.

10.2 Metal Transporters

Many plant genes are involved to encode proteins involved in metal uptake, translocation, and sequestration (Sarwar et al. 2017; Wang et al. 2018). Transfer of these genes into candidate plants or genetic manipulation of metal transporters may result in many fold increase in metal accumulation (LeBlanc et al. 2013). Study of Zn transporter gene from *Thlaspi* to other plant species has been reported to result in two-fold higher Zn accumulation in roots of the transgenic plant. Transgenic *Pteris vittata* has been developed after heterologous expression of arsenite antiporter PvACR3 which reduces arsenic accumulation and transfer in plant shoots (Chen et al. 2017b).

10.3 Engineering of Metabolic Pathways

Plants exhibits diverse metabolic pathways for the breakdown of vast variety of compounds. Alteration in the genetic make-up of the genes and enzymes involved at different steps of metabolism can lead to the development of transgenic plants with novel pathways for increased resistance towards diverse types of pollutants (Dhankher et al. 2002; Abhilash et al. 2009). Researchers have reported that the modification in case of specific genes or introduction of new genes into plants resulted in increased uptake and tolerance to heavy metals (Hg, As) (Meagher and Heaton 2005), hydrocarbons, chlorinated compounds, and complex cyclic compounds.

10.4 Modification in Roots

Plants have extensive root system which ensures an effective uptake and absorption of the pollutants from their surroundings, especially soil. The large surface area provided by the roots make an efficient platform for the uptake of toxicants. Modifications involving increased branched root system and increase root biomass with large surface area are possible by inducing hairy roots in candidate plants through *Agrobacterium rhizogenes* infection (Ibañez et al. 2016). Transgenic plants developed by this method were shown to have high efficiency for rhizofiltration of radionuclides, pesticides, and heavy metals.

10.5 Enhanced Biomass Production

Understanding the biosynthetic pathways for phytohormone synthesis and over production of genes encoding hormones in plant species of interest is becoming an interesting tool which can be effectively employed in biomass production. Such plant species would show extensive growth and could promote higher levels of absorption of contaminants for the remediation of contaminated environment (Van Aken 2008; Wang et al. 2018). Enhanced production of gibberellin in transgenic plants has been shown to promote growth and biomass production for several cycles of decontamination.

11 Advantages and Limitations

Phytoremediation has proved to be the efficient mechanism for the management of varieties of contaminants and the technology is largely adopted in the current scenario for in situ and ex situ treatments. Although the process presents phenomenal improvement of the contaminated ecosystems, it does have some drawbacks associated with its use. Some of the merits and demerits of the method are described here:

11.1 Advantages

- The method is applicable to a broad range of contaminants and the remediation goals can be achieved without using toxic chemicals.
- The technology offers environmental friendly and cost-effective way of treating pollutants even at large scale.
- The process can be performed with minimal environmental disturbances with the possibility of no or less generation of secondary air, particulates, and water wastes.
- It limits the transfer of pollutants, thereby reduce environmental toxicity.
- Although surface soil and topsoil are moderately disturbed, it is not required to be isolated or disposed and may be reclaimed for agricultural use.
- Reduces volume of contaminated material to be landfilled or incinerated.
- Plant uptake of contaminants prevents off-site migration.
- The clean-up process reduces the risk of exposure by limiting direct contact with contaminated soils.

11.2 Limitations and Concern

- Phytoremediation requires a large surface area of land for remediation.
- The time period required for the remediation to get completed is relatively long.
- Treatment is generally limited to shallow soils at less than 3 ft from the surface and water streams within 10 ft of the surface.
- It is a surface phenomenon and does not find application for the treatment of ground water sources.
- The process is completely dependent upon the climatic or hydrologic conditions which may restrict the rate of remediation.
- Appropriate working conditions like optimum pH, temperature, water concentration, metal solubility, presence of chelators, etc., are required for the smooth conduct of the process.
- Plants involved in remediation might transfer the chemicals to the food chain through animals/insects that eat plant material containing contaminants.
- Disposal of harvested plants carrying contaminants can be a problem.
- Degraded by-products may be mobilized in groundwater or bio-accumulated in other living species.
- If contaminant concentrations are too high, plants may die.
- Phytoremediation is not effective for strongly sorbed contaminants such as polychlorinated biphenyls (PCBs).

The use of phytoremediation is limited to shallow soils, streams, and groundwater with lower contaminant concentrations. Additional research is needed to find the ways of using trees rather than smaller plants to treat deeper contamination zone because tree roots penetrate more deeply into the ground. Scientists need to establish the fate of various pollutants in the plant metabolic cycle to ensure that the treatment process does not contribute to the release of toxic or harmful chemicals/products into the food chain.

12 Conclusions and Future Prospects

Phytoremediation is a solar energy driven natural phenomenon which harnesses inherent plant processes to clean up the pollutants in the environment. Further, it is a low cost, and eco-friendly technique, which can be applied for the treatment of a wide variety of contaminants and to detoxify various compounds. It is easy to implement and maintain, decreases soil disturbance, reduces the amount of generated waste and the possibility of spreading of contaminants. The technology has been increasingly investigated and employed both for the in situ and ex situ treatments. Sites contaminated with heavy metals, explosives, poly-hydrocarbons, pesticides, solvents, oil, etc., can be effectively remediated. Though the technology has wide applications, it requires a long-term commitment and not all plant species can be used for the process. Intensive research is required to develop new plant varieties to grow and thrive in contaminated ecosystem for the careful remediation of broad spectrum of pollutants.

References

Abdullah SRS, Al-Baldawi IA, Almansoory AF, Purwanti IF, Al-Sbani NH, Sharuddin SSN (2020) Plant-assisted remediation of hydrocarbons in water and soil: application, mechanisms, challenges and opportunities. Chemosphere 247:125932

Abhilash PC, Jamil S, Singh N (2009) Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol Adv 27(4):474–488

Afonso TF, Demarco CF, Pieniz S, Quadro MS, Camargo FAO, Andreazza R (2020) Bioprospection of indigenous flora grown in copper mining tailing area for phytoremediation of metals. J Environ Manag 256:109953

Ahsan MT, Najam-Ul-Haq M, Saeed A, Mustafa T, Afzal M (2018) Augmentation with potential endophytes enhances phytostabilization of Cr in contaminated soil. Environ Sci Pollut Res Int 25(7):7021–7032

Ali W, Isner JC, Isayenkov SV, Liu W, Zhao FJ, Maathuis FJ (2012) Heterologous expression of the yeast arsenite efflux system ACR3 improves *Arabidopsis thaliana* tolerance to arsenic stress. New Phytol 194(3):716–723

de Alkimin GD, Paisio C, Agostini E, Nunes B (2019) Phytoremediation processes of domestic and textile effluents: evaluation of the efficacy and toxicological effects in *Lemna minor* and *Daphnia magna*. Environ Sci Pollut Res Int 27(4):4423–4441. https://doi.org/10.1007/s11356-019-07098-3

Bert V, Seuntjens P, Dejonghe W, Lacherez S, Thuy HT, Vandecasteele B (2009) Phytoremediation as a management option for contaminated sediments in tidal marshes, flood control areas and dredged sediment landfill sites. Environ Sci Pollut Res Int 16(7):745–764

Boda RK, Majeti NVP, Suthari S (2017) *Ricinus communis* L. (castor bean) as a potential candidate for revegetating industrial waste contaminated sites in peri-urban greater Hyderabad: remarks on seed oil. Environ Sci Pollut Res Int 24:19955–19964

Capozzi F, Sorrentino MC, Caporale AG, Fiorentino N, Giordano S, Spagnuolo V (2020) Exploring the phytoremediation potential of *Cynara cardunculus*: a trial on an industrial soil highly contaminated by heavy metals. Environ Sci Pollut Res Int 27(9):9075–9084. https://doi.org/10.1007/s11356-019-07575-9

Chen Y, Xu W, Shen H, Yan H, Xu W, He Z, Ma M (2013) Engineering arsenic tolerance and hyperaccumulation in plants for phytoremediation by a PvACR3 transgenic approach. Environ Sci Technol 47(16):9355–9362

Chen J, Wang C, Shen ZJ, Gao GF, Zheng HL (2017a) Insight into the long-term effect of mangrove species on removal of polybrominated diphenyl ethers (PBDEs) from BDE-47 contaminated sediments. Sci Total Environ 575:390–399

Chen Y, Hua CY, Jia MR, Fu JW, Liu X, Han YH, Liu Y, Rathinasabapathi B, Cao Y, Ma LQ (2017b) Heterologous expression of *Pteris vittata* arsenite antiporter PvACR3;1 reduces arsenic accumulation in plant shoots. Environ Sci Technol 51(18):10387–10395

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cui H, Li H, Zhang S, Yi Q, Zhou J, Fang G, Zhou J (2020) Bioavailability and mobility of copper and cadmium in polluted soil after phytostabilization using different plants aided by limestone. Chemosphere 242:125252

Dhankher OP, Li Y, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB (2002) Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamylcysteine synthetase expression. Nat Biotechnol 20 (11):1140–1145

do Nascimento CWA, Hesterberg D, Tappero R, Nicholas S, da Silva FBV (2020) Citric acid-assisted accumulation of Ni and other metals by *Odontarrhena muralis*: implications for phytoextraction and metal foliar distribution assessed by μ-SXRF. Environ Pollut 260:114025

Doty SL, James CA, Moore AL, Vajzovic A, Singleton GL, Ma C, Khan Z, Xin G, Kang JW, Park JY, Meilan R, Strauss SH, Wilkerson J, Farin F, Strand SE (2007) Enhanced phytoremediation of volatile environmental pollutants with transgenic trees. Proc Natl Acad Sci USA 104 (43):16816–16821

Feng NX, Yu J, Zhao HM, Cheng YT, Mo CH, Cai QY, Li YW, Li H, Wong MH (2017) Efficient phytoremediation of organic contaminants in soils using plant-endophyte partnerships. Sci Total Environ 583:352–368

Galal TM, Eid EM, Dakhil MA, Hassan LM (2018) Bioaccumulation and rhizofiltration potential of Pistia stratiotes L. for mitigating water pollution in the Egyptian wetlands. Int J Phytorem 20 (5):440–447

Garbisu C, Hernández-Allica J, Barrutia O, Alkorta I, Becerril JM (2002) Phytoremediation: a technology using green plants to remove contaminants from polluted areas. Rev Environ Health 17(3):173–188

Gerhardt KE, Gerwing PD, Greenberg BM (2017) Opinion: taking phytoremediation from proven technology to accepted practice. Plant Sci 256:170–185

Gupta DK, Vuković A, Semenishchev VS, Inouhe M, Walther C (2019) Uranium accumulation and its phytotoxicity symptoms in *Pisum sativum* L. Environ Sci Pollut Res Int 27(3):3513–3522. https://doi.org/10.1007/s11356-019-07068-9

Hu Y, Habibul N, Hu YY, Meng FL, Zhang X, Sheng GP (2019) Mixture toxicity and uptake of 1-butyl-3-methylimidazolium bromide and cadmium co-contaminants in water by perennial ryegrass (*Lolium perenne* L.). J Hazard Mater 386:121972

Huang H, Yu N, Wang L, Gupta DK, He Z, Wang K, Zhu Z, Yan X, Li T, Yang XE (2011) The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil. Bioresour Technol 102(23):11034–11038

Huang J, Wu X, Tian F, Chen Q, Luo P, Zhang F, Wan X, Zhong Y, Liu Q, Lin T (2019a) Changes in proteome and protein phosphorylation reveal the protective roles of exogenous nitrogen in alleviating cadmium toxicity in poplar plants. Int J Mol Sci 21:E278

Huang Y, Zu L, Zhang M, Yang T, Zhou M, Shi C, Shi F, Zhang W (2019b) Tolerance and distribution of cadmium in an ornamental species *Althaea rosea* Cavan. Int J Phytoremediation 22(7):713–724. https://doi.org/10.1080/15226514.2019.1707771

Ibañez S, Talano M, Ontañon O, Suman J, Medina MI, Macek T, Agostini E (2016) Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. N Biotechnol 33:625–635

Iwabuchi A, Katte N, Suwa M, Goto J, Inui H (2019) Factors regulating the differential uptake of persistent organic pollutants in cucurbits and non-cucurbits. J Plant Physiol 245:153094

Jain S, Khare P, Mishra D, Shanker K, Singh P, Singh RP, Das P, Yadav R, Saikia BK, Baruah BP (2019) Biochar aided aromatic grass [*Cymbopogon martini (Roxb.) Wats*.] vegetation: a sustainable method for stabilization of highly acidic mine waste. J Hazard Mater 390:121799. https://doi.org/10.1016/j.jhazmat.2019.121799

James CA, Xin G, Doty SL, Strand SE (2008) Degradation of low molecular weight volatile organic compounds by plants genetically modified with mammalian cytochrome P450 2E1. Environ Sci Technol 42(1):289–293

Jiang Y, Lu H, Xia K, Wang Q, Yang J, Hong H, Liu J, Yan C (2019) Effect of mangrove species on removal of tetrabromobisphenol a from contaminated sediments. Chemosphere 244:125385

Lamshoeft M, Gao Z, Resseler H, Schriever C, Sur R, Sweeney P, Webb S, Zillgens B, Reitz MU (2018) Evaluation of a novel test design to determine uptake of chemicals by plant roots. Sci Total Environ 613:10–19

LeBlanc MS, McKinney EC, Meagher RB, Smith AP (2013) Hijacking membrane transporters for arsenic phytoextraction. J Biotechnol 163(1):1–9

Lu H, Zhang Y, Liu B, Liu J, Ye J, Yan C (2011) Rhizodegradation gradients of phenanthrene and pyrene in sediment of mangrove (*Kandelia candel* (L.) *Druce*). J Hazard Mater 196:263–269

Meagher RB, Heaton AC (2005) Strategies for the engineered phytoremediation of toxic element pollution: mercury and arsenic. J Ind Microbiol Biotechnol 32(11–12):502–513

Murano H, Otani T, Seike N, Sakai M (2010) Dieldrin uptake and translocation in plants growing in hydroponic medium. Environ Toxicol Chem 29:142–148

Namiki S, Otani T, Seike N, Satoh S (2015) Differential uptake and translocation of β-HCH and dieldrin by several plant species from hydroponic medium. Environ Toxicol Chem 34:536–544

Novak J, Trapp S (2005) Growth of plants on TBT-contaminated harbour sludge and effect on TBT removal. Environ Sci Pollut Res Int 12(6):332–341

Pandey SK, Upadhyay RK, Gupta VK, Worku K, Lamba D (2019) Phytoremediation potential of macrophytes of urban waterbodies in Central India. J Health Pollut 9:191206

Raj D, Kumar A, Maiti SK (2020) Mercury remediation potential of *Brassica juncea* (L.) Czern. For clean-up of flyash contaminated sites. Chemosphere 248:125857

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181

Rehn LS, Rodrigues AA, Vasconcelos-Filho SC, Rodrigues DA, de Freitas Moura LM, Costa AC, Carlos L, de Fátima SJ, Zuchi J, Angelini LP, de Lima Silva FH, Müller C (2019) *Ricinus communis* as a phytoremediator of soil mineral oil: morphoanatomical and physiological traits. Ecotoxicology 29(2):129–139. https://doi.org/10.1007/s10646-019-02147-6

Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13(5):468–474

Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, Rehim A, Hussain S (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721

Schwitzguébel JP, Comino E, Plata N, Khalvati M (2011) Is phytoremediation a sustainable and reliable approach to clean-up contaminated water and soil in alpine areas? Environ Sci Pollut Res Int 18(6):842–856

Sidhu GPS, Bali AS, Singh HP, Batish DR, Kohli RK (2018) Phytoremediation of lead by a wild, non-edible Pb accumulator *Coronopus didymus* (L.) Brassicaceae. Int J Phytorem 20:483–489

Sidhu GPS, Bali AS, Singh HP, Batish DR, Kohli RK (2019) Insights into the tolerance and phytoremediation potential of *Coronopus didymus* L. (Sm) grown under zinc stress. Chemosphere 244:125350

Van Aken B (2008) Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. Trends Biotechnol 26(5):225–227

Van Aken B (2009) Transgenic plants for enhanced phytoremediation of toxic explosives. Curr Opin Biotechnol 20(2):231–236

Wang K, Huang H, Zhu Z, Li T, He Z, Yang X, Alva A (2013) Phytoextraction of metals and rhizoremediation of PAHs in co-contaminated soil by co-planting of sedum alfredii with ryegrass (*Lolium perenne*) or castor (*Ricinus communis*). Int J Phytorem 15:283–298

Wang C, Na G, Bermejo ES, Chen Y, Banks JA, Salt DE, Zhao FJ (2018) Dissecting the components controlling root-to-shoot arsenic translocation in *Arabidopsis thaliana*. New Phytol 217(1):206–218

Ye S, Zeng G, Wu H, Zhang C, Dai J, Liang J, Yu J, Ren X, Yi H, Cheng M, Zhang C (2017) Biological technologies for the remediation of co-contaminated soil. Crit Rev Biotechnol 37 (8):1062–1076

Zhang S, Ni X, Arif M, Yuan Z, Li L, Li C (2020) Salinity influences cd accumulation and distribution characteristics in two contrasting halophytes, *Suaeda glauca* and *Limonium aureum*. Ecotoxicol Environ Saf 191:110230

Bioremediation of Heavy Metals Using the Symbiosis Between Leguminous Plants and Genetically Engineered Rhizobia

Yasser A. El-Tahlawy and Osama A. M. Ali

Contents

Abstract

The contamination of agricultural soils with heavy metals is resulted from anthropogenic and/or natural sources which lead to adversely affect the environment and in turn human health. Rhizoremediation is a bioremediation form that harnesses the plant-microbes interaction in the rhizosphere for pollutant remediation. The microbiologists and ecologists have exploited the symbiosis between plants and microbes in the rhizosphere to remediate heavy metal contamination and alleviate such stress. High levels of heavy metals could inhibit the symbiosis between legumes and rhizobia. Research efforts are turned to harness genetic

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engineering techniques for producing genetically manipulated rhizobia to stimulate the symbiotic relationship under such a harsh situation with cleaning the polluted environment along with improving soil fertility. Genetic engineering includes introducing new genes conferring high heavy metals tolerance into the wild rhizobia or make rhizobia act as PGPR by stimulate phytohormone and/or siderophores production for reducing heavy metals stress.

Keywords

Heavy metal bioremediation · Legumes · Genetically engineered rhizobia · Rhizoremediation

Abbreviations

AMF	Arbuscular mycorrhiza fungi
EPS	Extracellular polymeric substance
GEMs	Genetically engineered microorganisms
MT	Metallothionein
NADPH	Nicotinamide adenine dinucleotide phosphate
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
ROS	Reactive oxygen species

1 Introduction

The contamination or pollution of our environment has recently become a worldwide concern, especially in parallel with the expanded evolution of urbanization and industrialization, which in turn a plethora of different contaminants types have released. The contamination usually refers to the presence of unwanted one or more substance where it would not normally occur or at concentrations above natural background, but they do not provide information on pollution which will happen when such contamination causes adverse biological effects in the natural environment. Accordingly, all pollutants are contaminants, but not all contaminants are pollutants because the substances introduced into the environment may be more or less bioavailable to organisms depending on their chemical form, modifying factors in the environment, the environmental compartment they occupy, and the reactions of exposed biota (Chapman et al. 2003). One of the most widespread contamination and has polluted impact are heavy metals (Nriagu 1990). Heavy metals have been widely assigned as a group name for metals and metalloids, with densities above 5 g cm^{-3}, as well as their compounds that have been associated with contamination and potential toxicity or ecotoxicity (Hawkes 1997; Duffus 2002). Such groups are being widespread naturally in our environment components including soils, rocks, sediments, waters, and microorganisms with natural background concentrations. However, certain activities could release overdoses of them into the environment which cannot be degraded or destroyed to persist in the environment contamination of agriculture soils with heavy metals.

The healthy soils are characterized by a state of dynamic equilibrium between various organisms and its surrounding environment in which all the metabolic activities of inhabitant organisms optimally proceed without any hindrance, stress, or impedance from the latter (Goswami and Rattan 1992). The enhancement along with the maintenance of cultivated soils quality, against various contaminates that could pollute it, is an important issue that potentially contributes toward the sustainability of agricultural production (Saha et al. 2017). Heavy metals represent one of the soil pollutants with special concern because their persistence leads to toxicity and public health threats (Facchinelli et al. 2001; Mico et al. 2006).

The geological characteristics of soils contribute to some extent in the heavy metals rates which also naturally occurred in point sources in limited areas (Singh et al. 2015). Agrochemicals that have been augmented in the soil during agronomical practice in order to obtain maximum yields including overdoses of inorganic fertilizers, biosolids from sewage sludge and manures, wastewater and pesticides could be source of heavy metals into cultivated soils. Synthetic fertilizers overuse or misuse act as carrier of many metals such as Cadmium (Cd), Lead (Pb), Arsenic (As), and Copper (Cu) (Atafar et al. 2010; Sakizadeh et al. 2015). In Swedish land, where wheat plants are fertilized by nitrate of lime, each 10 kg increase in nitrogen application gave an increased Cd concentration in grain of approximately 0.001–-0.003 mg kg^{-1} due to ion exchange reactions in the soil solution, where an increased concentration of the fertilizer cation Ca^{2+} may have caused an increased concentration of Cd^{2+} in soil solution and eventually in wheat grain (Wångstrand et al. 2007; Roberts 2014). Phosphate fertilizers also aired a considerable concentration of Cd, Fluorine (F), Mercury (Hg), As, and Pb into soils (Jayasumana et al. 2015). Naturally materials like rock phosphate could be a source of several metals like Cd, Cu, Cr, Nickel (Ni), Pb, Manganese (Mn), and Zinc (Zn) (Sabiha et al. 2009; Aoun et al. 2010). The usages of biosolids as organic fertilizers release the heavy metals into manured soils such as Cd, As, Chromium (Cr), Cu, Pb, Hg, Ni, Thallium (Tl) and Antimony (Sb) (Basta et al. 2005). The soils irrigated with wastewater are being polluted with Cd, Cr, Pb, Ni, Zn and Mn (Ahmad and Goni 2010; Shaheen et al. 2015). Application of pesticides for long time accumulates concentrations of metals such Cu, As, Hg, Mn, Pb, Bromine (Br), Strontium (Sr), Titanium (Ti), and Zn that greatly exceed background (Chen et al. 2008; Wuana and Okieimen 2011; Kelepertzis 2014).

2 Rhizoremediation of Heavy Metals

The clean-up of heavy metals from the contaminated sites is announced as remediation of it for reducing the harmful effects at heavy metal-contaminated sites. The remediation is conventionally carried through three main strategies (Fig. 1) including physical, chemical, and biological technologies (Lambert et al. 2000). Comparing with biological methods, many disadvantages due to application of physiochemical

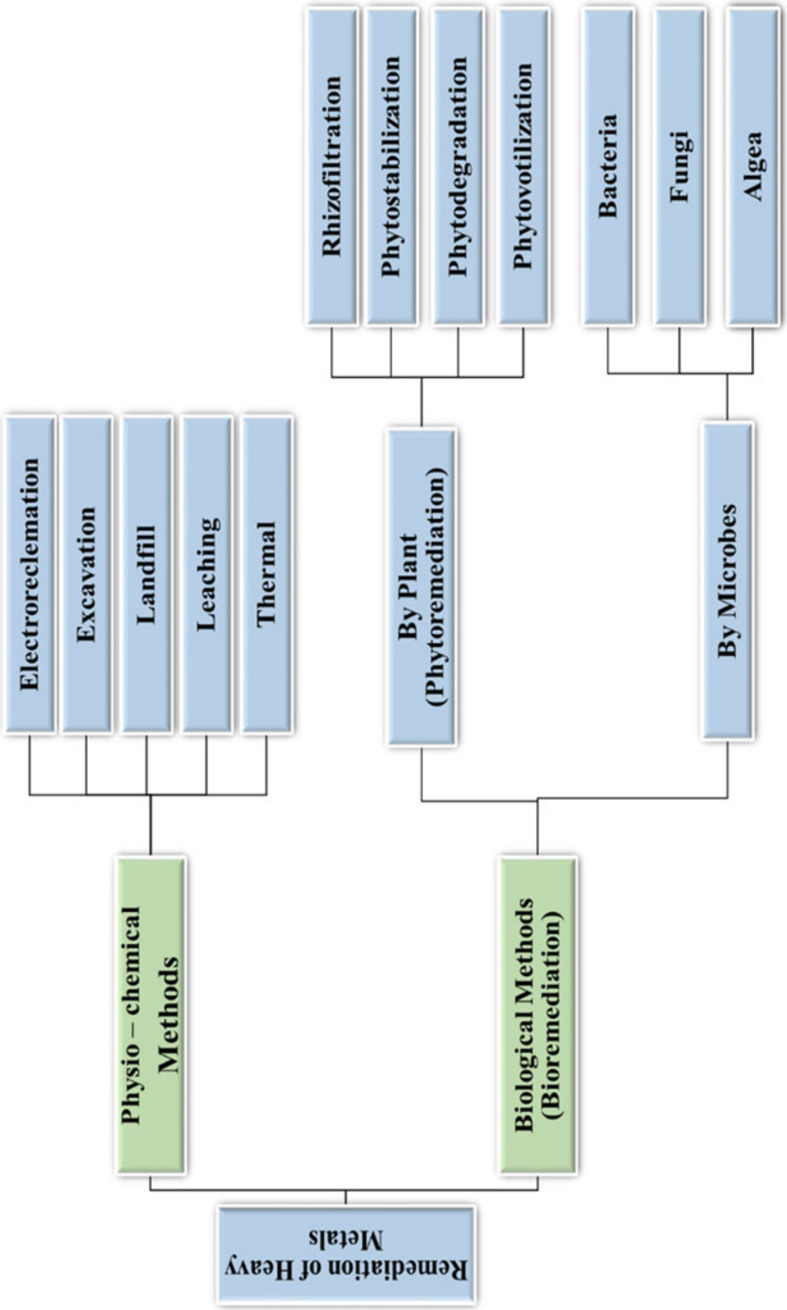


Fig. 1 Various methods used for heavy metal remediation

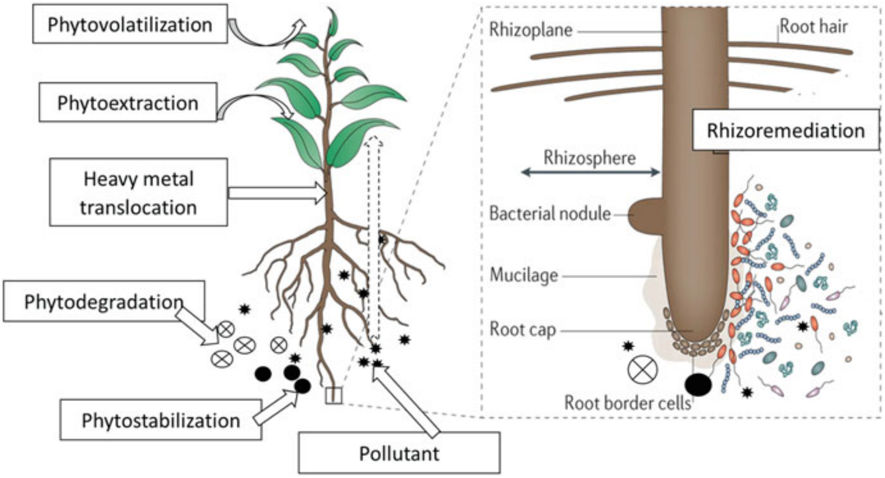


Fig. 2 Phytoremediation techniques and rhizoremediation of heavy metals

methods have been raised because such later techniques transfer the contaminants from contaminated sites to some other material, which needs to be transported and recycled. Such advantages could be leading to several setbacks including the less efficient, high cost, safety problems, recycling, transfer, need to analyze the nature of the contaminants and type of soil, possibilities of spread of pollutants during transport (Ganesan 2012; Ali et al. 2013; Wang et al. 2015). Furthermore, the nonbiological methods disrupt the soil characteristics and ecology that make the land unsuitable for agriculture and other purposes (Mulligan et al. 2001). Bioremediation is an inventive and optimistic technology which is applicable for the retrieval and reduction of heavy metals in the polluted lands (Verma and Kuila 2019). Phytoremediation concept was first introduced by Chaney (1983) as a biological method by which harnessing the innate capabilities of plants for abatement or/and rehabilitation of adverse effects at heavy metal-stressed soils. Many techniques have been involved in phytoremediation (Fig. 2) including phytoextraction (or phytoaccumulation) (Rafati et al. 2011), phytostabilization (Wuana and Okieimen 2011), phytodegradation (Wild et al. 2005), phytovolatilization (Sarwar et al. 2017), and rhizoremediation (Kuiper et al. 2004).

Extraordinarily microorganisms could be able to colonize the rhizosphere of plants which may arise up to huge numbers surpass those number of hosted plant cells and construct the plant microbiome (Mendes et al. 2013). The rhizosphere concept was elaborated by Lorenz Hiltner in 1904 and progressively reported as a hot spot of microbial activities in response to the plant's roots exudates or rhizodeposits (Reinhold-Hurek et al. 2015). Ever since then, the potential interactions between the roots and microorganisms have attention by various disciplines resulted in elaboration many terms of the beneficial microbes' action in the plant root zone. The root is the part of plant that first and directly interacts with heavy metal contaminants in the surrounding soil (rhizosphere) and therefore, it would be highly beneficial if such contaminants could be degraded in the rhizosphere zone as there are microbes that interact with plant roots and degrade contaminants in the rhizoremediation process (Schwab and Banks 1994).

Rhizoremediation process involves the mutual relationship between the rhizosphere, the root part of the plant, and the soil microbes present. There are several advantages of rhizoremediation like being eco-friendly, cost effective, as we are not adding any chemicals for its removal, and the side effect is little or negligible, but in case of chemical degradation processes like volatilization, chemical oxidation, it affects the functioning of our environment (Saravanan et al. 2019). The importance of rhizoremediation compared to absolute phytoremediation lies in its rate of degrading contaminants. In rhizoremediation, the rate of degradation of contaminants is higher due to the assistance provided by microbes (Euliss et al. 2008; Sathishkumar et al. 2008).

The microorganisms could be able to remediate the toxicity of heavy metals through several ways, including transformation into nontoxic forms as done by *Pseudomonas metafolia*, which reduces toxic Cr, Hg, Pb, and Cd into nontoxic forms (Blake II et al. 1993); increase of metal bioavailability and enhance their uptake in metal ions such as Cr, Pb, Zn, As, and Ni by *Diplachne fusca*, *Alyssum murale,* and *Pteris vittata* which correlated with activities of various rhizobacteria (Al Agely et al. 2005; Gonzaga et al. 2006); as well as prevent translocation into plant by stabilizing metals in the rhizosphere (Praveen et al. 2019).

The enhancement of rhizoremediation of soil contaminants, especially heavy metals, could be achieved by engineering soil, plants, and microorganisms (Praveen et al. 2019). The microbial ability could be enhanced through the use of genetically modified microbes in order to increase their capability to remediate the contaminants using selective gene addition strategy (Lorito et al. 1998).

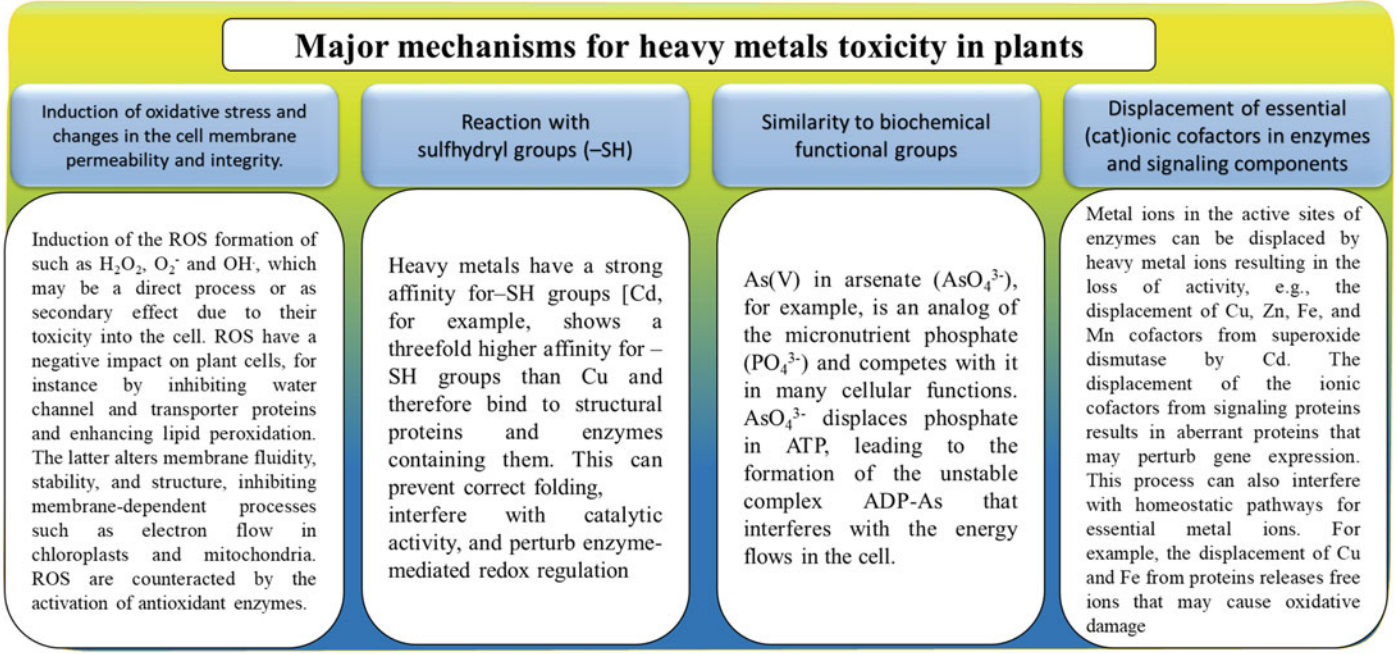
3 Legume–Rhizobium Symbiotic Relationship Under the Influence of Metal Polluted Soils

Plants primarily capture the nutrients from their surrounding spheres as inorganic ions form mineral elements by means of their extended root apparatus which have the ability to absorb it even at low concentrations with high absorption efficiency (DalCorso 2012). Such mineral elements can be classified into two groups: essential nutrient elements and toxic non-nutrient elements. The essential minerals are acquired by plants for their metabolism and structure and the absence or deficiency reduces fitness and inhibits growth and reproduction. The essential minerals include the macronutrients nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorous (P), sulfur (S), and silicon (Si) as well as the micronutrients chlorine (Cl), iron (Fe), boron (B), manganese (Mn), sodium (Na), zinc (Zn), copper (Cu), nickel (Ni), and molybdenum (Mo). The micronutrient elements are required in only small quantities but on the other hand it becomes detrimental to majority of plant species when their abundance in the soil is excessive (such as Cu, Ni, and Zn) either by natural occurrence or from anthropogenic activities. Other minerals with no known function in plant metabolism including Cd, Hg, Pb, Cr, As, silver (Ag), and Sb appeared as more or less toxic agents to both eukaryotic and prokaryotic organisms including plants even at low concentration (Sanità di Toppi and Gabbrielli 1999; Cárdenas et al. 2008).

Legumes, like any plants, have the ability to uptake heavy metals/metalloids from their surrounding environment, which leads to have a negative impact on their physiological and biochemical processes. Four major mechanisms have been suggested for heavy metals/metalloids toxicity impacts in plants (Fig. 3). Such impacts depend on the dose of heavy metal that the plants are exposure, comparing to essential metals which responses have several dose-dependent phases–from deficiency-sufficiency at low doses of the metal, tolerance at moderate doses and to toxicity at high doses, while nonessential metals only tolerance and toxicity stages take place (Maheswari et al. 2012).

The heavy metals directly have the ability to modulate several proteins and DNA as well as to negatively affect chlorophylls and protein biosynthesis (Bibi and Hussain 2005; Xue et al. 2014; Ma et al. 2016). Those actions of heavy metal could result in adverse impacts that have several features including hindrance of seed germination, retardation of seedling development, disturbance in root and shoot growth, mutagenic impacts, early senescence, and death of grown plants including progressive chlorosis and necrosis as well as reduction in quality of harvested crop yield with low protein content (Maheswari et al. 2012; Maiti et al. 2012; Ahmed 2015). Some heavy metals indirectly oblige oxidative stress via several mechanisms such as glutathione depletion, binding to sulfhydryl groups of proteins, inhibiting antioxidative enzymes, or inducing reactive oxygen species (ROS) producing enzymes like NADPH oxidases (Valko et al. 2005; Bielen et al. 2013)

Effect of soil contaminated with heavy metals on the legume–Rhizobium symbiotic interaction has to be outlined in order to explore the use of legumes and rhizobia for the bioremediation considering the nodulation process and legume plant growth. During various stages of legume plant growth in heavy metal contaminated soils, the overall nodulation process appeared to be more sensitive to such contaminates than other vegetative growth features such as roots, shoots, and/or photosynthetic chlorophyll pigments which have to be taken as parameters commonly used to assay the toxicity degree of metals onto grown legumes (Gupta et al. 2007). According to Pajuelo et al. (2008) nodule numbers decreased as a result of various factors including atrophy of root hairs, diminution in number of root hairs, less extended root zone susceptible to nodulation, and low number of infections events. Also, Hecht-Buchholz et al. (1990) and Kopittke et al. (2007) stated that decrease of nodule number under increasing activity of Aluminum (Al) or Cu metals was corelated to an inhibition of root hair formation of soybean or cowpea, respectively, rather than to a reduction in the size of the Rhizobium population. When *Medicago sativa* inoculated with *Sinorhizobium medicae* under treatments of the presence or absence of 25 mM sodium arsenite, the root length and chlorophyll content recorded decreased by 33% and 15%, respectively, as compared to grown legumes under control treatment of As absence (Pajuelo et al. 2008). They add, the microscopic



Major mechanisms for heavy metals toxicity in plants

Induction of oxidative stress and changes in the cell membrane permeability and integrity.

Induction of the ROS formation of such as H_2O_2, $O_2^{\cdot-}$ and $OH^{\cdot}$, which may be a direct process or as secondary effect due to their toxicity into the cell. ROS have a negative impact on plant cells, for instance by inhibiting water channel and transporter proteins and enhancing lipid peroxidation. The latter alters membrane fluidity, stability, and structure, inhibiting membrane-dependent processes such as electron flow in chloroplasts and mitochondria. ROS are counteracted by the activation of antioxidant enzymes.

Reaction with sulfhydryl groups (–SH)

Heavy metals have a strong affinity for–SH groups [Cd, for example, shows a threefold higher affinity for –SH groups than Cu and therefore bind to structural proteins and enzymes containing them. This can prevent correct folding, interfere with catalytic activity, and perturb enzyme-mediated redox regulation

Similarity to biochemical functional groups

As(V) in arsenate (AsO_4^{3-}), for example, is an analog of the micronutrient phosphate (PO_4^{3-}) and competes with it in many cellular functions. AsO_4^{3-} displaces phosphate in ATP, leading to the formation of the unstable complex ADP-As that interferes with the energy flows in the cell.

Displacement of essential (cat)ionic cofactors in enzymes and signaling components

Metal ions in the active sites of enzymes can be displaced by heavy metal ions resulting in the loss of activity, e.g., the displacement of Cu, Zn, Fe, and Mn cofactors from superoxide dismutase by Cd. The displacement of the ionic cofactors from signaling proteins results in aberrant proteins that may perturb gene expression. This process can also interfere with homeostatic pathways for essential metal ions. For example, the displacement of Cu and Fe from proteins releases free ions that may cause oxidative damage

Fig. 3 Toxicity mechanisms of heavy metals (reproduced from DalCorso 2012)

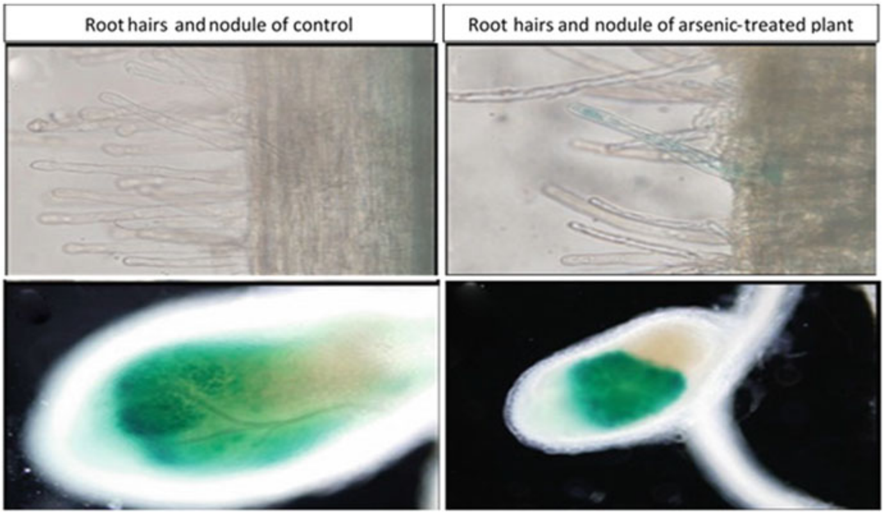


Fig. 4 *Medicago sativa* inoculated with *Sinorhizobium medicae* under treatments of the presence or absence of 25 mM sodium arsenite (Pajuelo et al. 2008)

investigation (Fig. 4) of alfalfa plants appeared a damage in root hairs, a shortage in infective root zone, necrosis symptoms, a swelling of root tip and a reduction in rhizobial infections by 90% which led to a decline in a nodulation in the presence of As. A pot experiment was conducted by Athar and Ahmad (2002) to study the toxicity of Cd, Pb, Cu, Zn, Cr, and Ni added separately or in combination on chickpea-Rhizobium and greengram-Bradyrhizobium systems and metal uptake pattern by legume grains. They found chickpea-Rhizobium system was comparatively more sensitive to heavy metal toxicity than greengram-Bradyrhizobium system in terms of decreased yield, low nitrogen content in plant tissues, and the protein content in grains. The phytotoxicity was apparently due to the susceptibility of the symbiotic apparatus against toxic doses of heavy metals. Metal uptake by grains seemed to be directly related to the concentration of heavy metals and was greater in the case of an individual metal added separately than in combination.

From molecular biology point of view, the presence of any toxic metals/metalloids could induce changes in the expression of nodulin genes associated with processes that take place in the epidermis and the outer cortical cells, and the expression of genes associated with events that take place in the inner cortical cells is less affected (Lafuente et al. 2010).

4 Metals Phytoremediation by Leguminous Plants

In general, the reaction and sensitivity toward heavy metals differ from one set of plants to other. Plants grown in polluted area react with metals in two ways: some plants tend to accumulate heavy metals, while others could be able to exclude heavy metals (Baker 2008). Considering the sensitivity to the heavy metal, plants can be characterized as (1) metal excluders, (2) metal indicators, and (3) metal hyperaccumulators (Bleeker et al. 2003; Prasad and De Oliveira Freitas 2003; Ghosh 2005). Hyperaccumulator plants are preferable in phytoremediation strategy because it is based on phytoextraction, could able to tolerate high concentrations of specific metal, and accumulates metals in their tissues without any toxicity symptoms (Pence et al. 2000; Callahan et al. 2006; Zhang et al. 2010). The ability of hyperaccumulator plants to translocate metals to the aerial organs from roots exceeds the concentration of metals in shoots than in roots and exceeds the metal concentration in the soil. On the contrary, non-accumulator plants (excluders) are preferable for phytostabilization because it prevents the metals to be accumulated in shoots by avoiding the uptake by roots; persists the metal in root tissues, and/or does not allow the metals to translocate to the aerial organs of the plant (Pajuelo et al. 2008). In phytoremediation, the usage of metal excluders plants makes the metals not completely removed but is immobilized in soil and in turn prevents leaching, erosion, and metal transfer into the food chain which needs the polluted areas to be continuously monitored since metal (Kidd et al. 2009).

Although many legumes exert tolerance toward heavy metals, others are classified as the metal excluders and could accumulate very low amount of the metals in shoot tissues (Del Río et al. 2002; Pastor et al. 2003; Gupta et al. 2007). Most of the legumes have recorded undetectable amounts of heavy metals in harvested grains (Wani et al. 2007). Most of the legumes tend to take up and store the heavy metals in root cells and chelate it in aid of molecules such as glutathione, phytochelatins, metallothioneins, organic acids, histidine, nicotinamine, etc., therefore, only a small quantity of metal is loaded into the xylem and is translocated to the shoots (Salt et al. 1998; Mejáre and Bülow 2001; Ebbs et al. 2002). Such features, that legumes behave under stress of heavy-metal polluted sites, make the usage of it in metal legume-based phytoremediation projects does not pose any risk to the food chain (Pajuelo et al. 2008).

Plants that belong to leguminous family (Fabaceae), including species of the genera *Vicia*, *Cytisus*, *Astragalus*, *Lupinus*, etc., could be able to habitat in several metal contaminated sites and they often harbor metal-tolerant rhizobia (Prasad and De Oliveira Freitas 2003). One of the legumes that firstly colonizes in severely contaminated soils after a mine spill was some of *Medicago* species (Carrasco et al. 2005). Long-term study on polluted environments, that have a toxicity with spill of the Aznalcóllar mine (southern Spain), to survey plant species could be survived under stress of heavy metal revealed that leguminous plants are predominant up to 15 species of 99 different plant species (Del Río et al. 2002). Several advantages make legumes act as a pioneer phytoremediator of heavy metals listed by de-Bashan et al. (2012). The advantages include improving soil characteristics via contaminants immobilization, organic matter increment, additional N-compounds and reformation of rhizosphere flora. The soil improvement provides a suitable environment for the growth of other plant species possible under harsh conditions. Furthermore, legume plants stimulate the diversity of microorganisms like rhizobacteria and arbuscular mycorrhiza fungi (AMF) to immobilize metal and enhance the growth of plants.

Finally, they help in improvement and stabilization of the ecology of the polluted environment.

5 Metal Resistance and PGP Properties Pathways in Rhizobia Microsymbionts

Plant growth promoting microorganisms are vital in the remediation of heavy metal polluted sites as they have a variety of ways to endure metal toxicity. The microorganisms were exploited to sequester, precipitate, or change the oxidation state of numerous heavy metals (Gupta and Joia 2016; Kang et al. 2016). Four mechanisms were suggested for microbial bioremediation: (1) toxic metals are sequestered by cell wall components or by intracellular metal binding proteins and peptides such as metallothioneins (MT) and phytochelatins along with compounds such as bacterial siderophores which are mostly catecholates, compared to fungi that produce hydroxamate siderophores; (2) alteration of biochemical pathways to block metal uptake; (3) conversion of metals to innocuous forms by enzymes; and (4) reduction of intracellular concentration of metals using precise efflux systems (Gupta and Joia 2016; Ojuederie and Babalola 2017).

Siderophores are compounds produced by bacteria able to chelate iron enhance mobility and reduce metals bioavailability to be removed from soil. Sulfate-reducing bacteria like *Desulfovibrio desulfuricans* can convert sulfate to hydrogen sulfate which then reacts with heavy metals such as Cd and Zn to form insoluble forms of these metal sulfides (Chibuike and Obiora 2014). Biomolecules that constitute surface of microbial cell wall have negatively charged functional groups, like hydroxyl, phosphate, carbonyl groups, etc., which could readily bind to heavy metal ions (Dixit et al. 2015).

Other components present in gram-negative bacteria (rhizobia, for example) represent active sites act as ligands for binding metal ions and in turn ultimate remediation from contaminated environments (Fomina and Gadd 2014; Gupta et al. 2015). Peptidoglycan layers of gram-positive cell walls contain the amino acids alanine and glutamic acid as well as meso-di-aminopimelic acid and teichoic acid. While enzymes, glycoproteins, lipopolysaccharides, lipoproteins and phospholipids are contained in gram-negative cell walls (Ayangbenro and Babalola 2017). Microbes also have the ability to detoxify metals through enzymatic and non-enzymatic actions using heavy metals and trace elements as terminal electron acceptors obtain energy they need (Dixit et al. 2015). Bacteria produce exopolysaccharides to make biofilm production which play vital role in the biosorption and biomineralization of metal ions (Dong et al. 2013).

Rhizobia are a subset of plant growth promoting rhizobacteria (PGPR) that may have a vital role in the phytoremediation of pollutants in the environment. The performance of rhizobia with legumes could be enhanced by coinoculation with other PGP microorganisms (Soad et al. 2016) or application of organic fertilizers (Ali et al. 2018). The rhizobia have the ability to directly enhance phytoremediation through nitrogen fixation and production of the plant growth promoting factors which act to increase metal uptake and to translocate it from soil to plant due to the change in bioavailability (Hao et al. 2014). On the other hand, the redox state of metals could be immobilized/changed by action of extracellular polymeric substance (EPS) production and enzyme activities of microbial metabolism which enhance to lessen toxicity of metals in plants. Also, some rhizobia indirectly help phytostabilization of metals by their ability to adsorb and accumulate metals such as in the case of Cd with symbiosis between *Azorhizobium caulinodans* and *Sesbania rostrata* (Zhengwei et al. 2005). Work done by Pereira et al. (2006) suggested that there is a relationship between Rhizobium's tolerance, heavy metal soil contamination, and alterations in protein pool. They add, the analysis of protein alterations seems to be a good indicator to estimate the level of stress imposed on Rhizobium populations submitted to heavy-metal contamination.

6 Genetically Engineered Microorganisms for Metals Bioremediation

Processes that exploit microorganisms to remediate metals from contaminated sites not always yield acceptable results, like Hg metal cannot be cleaned up by native bacteria from its inhabitant environment. However, technology like recombinant DNA could play a vital role in bioremediation of heavy metal contamination as it enhances the remediation process (Ojuederie and Babalola 2017). The role of genetic engineering in bioremediation aims to modify plants, microorganisms, and enzymes so that they would be useful tools for degradation of harmful substances (Wołejko et al. 2016). Many efforts have been made to genetically engineer the bacteria to cleanup various heavy metals like As, Cd, Cu, Fe, Hg, and Ni (Verma and Singh 2005; Azad et al. 2014). Recombinant DNA technology aims to produce genetically engineered microorganisms (GEMs) through inserting foreign genes from other ones of the same or different species into their genome. These engineered microorganisms have been used to obtain super strains that are able to compete for bioremediation of contaminated environment by possessing enhanced ability to breakdown a variety of contaminants (Dixit et al. 2015). Genetically engineered *Escherichia coli* strain M109 and *Pseudomonas putida* containing the merA gene have been included in many studies to effectively remediate Hg-contaminated soils and sediments (Barkay et al. 2003; Deckwer et al. 2004). The merA gene and ArsM gene are widely used to remove Hg and As, respectively, from contaminated soils (Marconi et al. 1997; Liu et al. 2011).

Nowadays, there are new metabolic pathways that enable GEMs to detoxify heavy metals or make it less toxic and intern enhance their remediation such as constructing new pathways and replacement of existing gene sequences and introducing single genes operons into the microorganism (Ojuederie and Babalola 2017). Metallothionein or metallopeptides are cysteine-rich proteins synthesized under heavy metal stress in prokaryotes and eukaryotes. The expression of metallothionein or metallopeptides genes could be considered to overcome the affinity and a biosorptive ability of bacterial cells for toxic metals as a promising technology for the expansion of bacterium-based biosorbents (Silver 1996; Singh et al. 2011). The role of metallothionein has been appeared in lead-resistant bacterial isolates such as *Salmonella choleraesuis*, *Proteus penneri*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus penneri,* and *Providencia rettgeri* which were isolated from soil polluted with car battery waste (Tunali et al. 2006).

7 Improving the Legume–Rhizobium Symbiosis for Bioremediation of Heavy Metals by Genetic

Many attempts seek to enhance the growth of legumes under toxic concentrations of heavy metals using genetic engineering of their rhizobial microsymbionts by inserting new genes conferring heavy-metal resistance into the rhizobium (Fagorzi et al. 2018). A genetically modified *Medicago truncatula*, expressed a metallothionein gene from *Arabidopsis* in its roots, was inoculated with wild type of *Sinorhizobium medicae* which resulted in elevated Cu tolerance. Copper tolerance was further increased using a *S. medicae* strain expressing the *P. fluorescence* copAB Cu resistance genes, resulting in elevated Cu accumulation in the plant roots (Pérez-Palacios et al. 2017). A strain of *Rhizobium leguminosarum bv. trifolii* was genetically engineered by transferring arsenite [As(III)] S-adenosylmethionine methyltransferase gene (CrarsM) from the alga *Chlamydomonas reinhardtii* to test bioremediation under free living conditions and in symbiosis with red clover plants (Zhang et al. 2017). Engineered rhizobia expressing an algal arsM gene can methylate and volatilize As, providing a proof of concept for potential future use of legume-rhizobia symbionts for As bioremediation. An ACC deaminase that overproduces *Sinorhizobium meliloti* strain increased Cu tolerance and promoted plant growth of the host plant *Medicago lupulina* in the presence of 200 mg kg^{-1} Cu^{2+} throughout different stages of symbiosis due to reduced production of ethylene by the host plant, in turn decreasing stress perception (Kong et al. 2015). The pSinA plasmid isolated from an As-contaminated gold mine was introduced to non-symbiotic *Ensifer* sp. which resulted in the construction of As-tolerant legume symbionts for use in arsenic remediation (Drewniak et al. 2008; Romaniuk et al. 2017). Genetic and genomic studies of heavy-metal resistant rhizobia show that although relatively few genes act as the main player intolerance, a much larger set of genes may be involved in maximizing fitness in heavy metal-rich growth conditions. Some of these genes, such as the systems for Ni^{2+} efflux in *S. meliloti*, may also contribute to a linkage between metal homeostasis and nitrogen-fixation efficiency (Fagorzi et al. 2018). The genetic engineering of rhizobia has to consider many additional features such as the genotype rhizobia and host plant as well as the ecology of rhizobium including soil and root microbiome (Drewniak et al. 2008).

8 Conclusion

Heavy metals are a term usually assigned as a group name for metals and metalloids. The persistence of heavy metals in cultivated soil converts it from contamination to pollutants and causes toxicity and public health threats. Rhizoremediation is preferred in cleanup of heavy metals than physicochemical methods because it is eco-friendly, cost effective, no chemicals and the side effect is little or negligible. Rhizobia with their hosts of legume plants present a good model for rhizoremediation of heavy metals with less accumulation of metals in the plant shoots or gain which make it safer. Genetic engineering with its techniques could enhance the rhizoremediation by producing super strains of rhizobia that are capable to remediate wide range of metals by keeping their characters as plant growth promoting bacteria.

References

Ahmad JU, Goni MA (2010) Heavy metal contamination in water, soil, and vegetables of the industrial areas in Dhaka, Bangladesh. Environ Monit Assess 166:347–357. https://doi.org/10.1007/s10661-009-1006-6

Ahmed W (2015) Evaluations of some trace metal levels from the leaves of *Salix nigra* in hayatabad industrial Estate Peshawar, Khyber Pakhtunkhwa Pakistan. Am J Biomedic Life Sci 3:21–24. https://doi.org/10.11648/j.ajbls.s.2015030201.13

Al Agely A, Sylvia DM, Ma LQ (2005) Mycorrhizae increase arsenic uptake by the hyperaccumulator Chinese brake fern (*Pteris vittata* L.). J Environ Qual 34:2181–2186. https://doi.org/10.2134/jeq2004.0411

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Ali OAM, El-Tahlawy YA, Abdel-Gwad ASH (2018) Impact of compost tea types application on germination,nodulation, morphological characters and yield of two lentil cultivars. Egyptian J Agron (EJA), volume 40, The 15th International Conference on Crop Science, Ain Shams University, Egypt, pp 1–19. https://doi.org/10.21608/AGRO.2018.5678.1126

Aoun M, El Samrani AG, Lartiges BS, Kazpard V, Saad Z (2010) Releases of phosphate fertilizer industry in the surrounding environment: Investigation on heavy metals and polonium-210 in soil. J Environ Sci 22:1387–1397. https://doi.org/10.1016/S1001-0742(09)60247-3

Atafar Z, Mesdaghinia A, Nouri J, Homaee M, Yunesian M, Ahmadimoghaddam M, Mahvi AH (2010) Effect of fertilizer application on soil heavy metal concentration. Environ Monit Assess 160:83–89. https://doi.org/10.1007/s10661-008-0659-x

Athar R, Ahmad M (2002) Heavy metal toxicity in legume-microsymbiont system. J Plant Nutr 25:369–386. https://doi.org/10.1081/PLN-100108842

Ayangbenro AS, Babalola OO (2017) A new strategy for heavy metal polluted environments: a review of microbial biosorbents. Int J Environ Res Public Health 14:94–110. https://doi.org/10.3390/ijerph14010094

Azad MAK, Amin L, Sidik NM (2014) Genetically engineered organisms for bioremediation of pollutants in contaminated sites. Chin Sci Bull 59:703–714. https://doi.org/10.1007/s11434-013-0058-8

Baker AJM (2008) Accumulators and excluders -strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654. https://doi.org/10.1080/01904168109362867

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384. https://doi.org/10.1016/s0168-6445(03)00046-9

Basta NT, Ryan JA, Chaney RL (2005) Trace element chemistry in residual-treated soil: key concepts and metal bioavailability. J Environ Qual 34:49–63. https://doi.org/10.2134/jeq2005.0049dup

Bibi M, Hussain M (2005) Effect of copper and lead on photosynthesis and plant pigments in black gram [*Vigna mungo* (L.) Hepper]. Bull Environ Contam Toxicol 74:1126–1133. https://doi.org/10.1007/s00128-005-0698-8

Bielen A, Remans T, Vangronsveld J, Cuypers A (2013) The influence of metal stress on the availability and redox state of ascorbate, and possible interference with its cellular functions. Int J Mol Sci 14:6382–6413. https://doi.org/10.3390/ijms14036382

Blake RC II, Choate DM, Bardhan S, Revis N, Barton LL, Zocco TG (1993) Chemical transformation of toxic metals by a *Pseudomonas* strain from a toxic waste site. Environ Toxicol Chem 12:1365–1376. https://doi.org/10.1002/etc.5620120806

Bleeker PM, Schat H, Vooijs R, Verkleij JAC, Ernst WHO (2003) Mechanisms of arsenate tolerance in *Cytisus striatus*. New Phytol 157:33–38. https://doi.org/10.1046/j.1469-8137.2003.00542.x

Callahan DL, Baker AJM, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. JBIC J Biol Inorg Chem 11:2–12. https://doi.org/10.1007/s00775-005-0056-7

Cárdenas L, Martínez A, Sánchez F, Quinto C (2008) Fast, transient and specific intracellular ROS changes in living root hair cells responding to Nod factors (NFs). Plant J 56:802–813. https://doi.org/10.1111/j.1365-313X.2008.03644.x

Carrasco JA, Armario P, Pajuelo E, Burgos A, Caviedes MA, López R, Chamber MA, Palomares AJ (2005) Isolation and characterisation of symbiotically effective Rhizobium resistant to arsenic and heavy metals after the toxic spill at the *Aznalcóllar pyrite* mine. Soil Biol Biochem 37:1131–1140. https://doi.org/10.1016/j.soilbio.2004.11.015

Chaney RL (1983) Plant uptake of inorganic waste constituents. In: Parr JF, Marsh PB, Kla JM (eds) Land treatment of inorganic wastes. Noyes Data Corporation, Park Ridge, pp 50–76

Chapman PM, Wang F, Janssen CR, Goulet RR, Kamunde CN (2003) Conducting ecological risk assessments of inorganic metals and metalloids: current status. Hum Ecol Risk Assess 9:641–697. https://doi.org/10.1080/713610004

Chen T, Liu X, Zhu M, Zhao K, Wu J, Xu J, Huang P (2008) Identification of trace element sources and associated risk assessment in vegetable soils of the urban-rural transitional area of Hangzhou, China. Environ Pollut 151:67–78. https://doi.org/10.1016/j.envpol.2007.03.004

Chibuike GU, Obiora SC (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. Appl Environ Soil Sci 2014:1–12. https://doi.org/10.1155/2014/752708

DalCorso G (2012) Heavy metal toxicity in plants. In: Furini A (ed) Plants and heavy metals. Springer, Dordrecht, pp 1–25

de-Bashan LE, Hernandez J-P, Bashan Y (2012) The potential contribution of plant growth-promoting bacteria to reduce environmental degradation – a comprehensive evaluation. Appl Soil Ecol 61:171–189. https://doi.org/10.1016/j.apsoil.2011.09.003

Deckwer WD, Becker FU, Ledakowicz S, Wagner-Dobler I (2004) Microbial removal of ionic mercury in a three-phase fluidized bed reactor. Environ Sci Technol 38:1858–1865. https://doi.org/10.1021/es0300517

Del Rı́o M, Font R, Almela C, Vélez D, Montoro R, De Haro Bailón A (2002) Heavy metals and arsenic uptake by wild vegetation in the Guadiamar river area after the toxic spill of the *Aznalcóllar* mine. J Biotechnol 98:125–137. https://doi.org/10.1016/S0168-1656(02)00091-3

Dixit R, Wasiullah MD, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7:2189–2212. https://doi.org/10.3390/su7022189

Dong G, Wang Y, Gong L, Wang M, Wang H, He N, Zheng Y, Li Q (2013) Formation of soluble Cr (III) end-products and nanoparticles during Cr(VI) reduction by *Bacillus cereus* strain XMCr-6. Biochem Eng J 70:166–172. https://doi.org/10.1016/j.bej.2012.11.002

Drewniak L, Matlakowska R, Sklodowska A (2008) Arsenite and arsenate metabolism of *Sinorhizobium sp*. M14 living in the extreme environment of the Zloty Stok Gold mine. Geomicrobiol J 25:363–370. https://doi.org/10.1080/01490450802402836

Duffus JH (2002) "Heavy metals" a meaningless term? (IUPAC technical report). Pure Appl Chem 74:793–807. https://doi.org/10.1351/pac200274050793

Ebbs S, Lau I, Ahner B, Kochian L (2002) Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens* (J. & C. Presl). Planta 214:635–640. https://doi.org/10.1007/s004250100650

Euliss K, Ho C-h, Schwab AP, Rock S, Banks MK (2008) Greenhouse and field assessment of phytoremediation for petroleum contaminants in a riparian zone. Bioresour Technol 99:1961–1971. https://doi.org/10.1016/j.biortech.2007.03.055

Facchinelli A, Sacchi E, Mallen L (2001) Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environ Pollut 114:313–324. https://doi.org/10.1016/s0269-7491(00)00243-8

Fagorzi C, Checcucci A, DiCenzo GC, Debiec-Andrzejewska K, Dziewit L, Pini F, Mengoni A (2018) Harnessing rhizobia to improve heavy-metal phytoremediation by legumes. Gene 9:542. https://doi.org/10.3390/genes9110542

Fomina M, Gadd GM (2014) Biosorption: current perspectives on concept, definition and application. Bioresour Technol 160:3–14. https://doi.org/10.1016/j.biortech.2013.12.102

Ganesan V (2012) Rhizoremediation: a pragmatic approach for remediation of heavy metal-contaminated soil. In: Zaidi A, Wani PA, Khan MS (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Vienna, pp 147–161

Ghosh M (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3:1–18. https://doi.org/10.15666/aeer/0301_001018

Gonzaga MIS, Santos JAG, Ma LQ (2006) Arsenic phytoextraction and hyperaccumulation by fern species. Sci Agric 63:90–101. https://doi.org/10.1590/S0103-90162006000100015

Goswami NN, Rattan RK (1992) Soil health – key to sustained agricultural productivity. Fert News 37:53–60

Gupta A, Joia J (2016) Microbes as potential tool for remediation of heavy metals: a review. J Microb Biochem Technol 8:364–372. https://doi.org/10.4172/1948-5948.1000310

Gupta AK, Dwivedi S, Sinha S, Tripathi RD, Rai UN, Singh SN (2007) Metal accumulation and growth performance of *Phaseolus vulgaris* grown in fly ash amended soil. Bioresour Technol 98:3404–3407. https://doi.org/10.1016/j.biortech.2006.08.016

Gupta VK, Nayak A, Agarwal S (2015) Bioadsorbents for remediation of heavy metals: current status and their future prospects. Environ Eng Res 20:1–18. https://doi.org/10.4491/eer.2015.018

Hao X, Taghavi S, Xie P, Orbach MJ, Alwathnani HA, Rensing C, Wei G (2014) Phytoremediation of heavy and transition metals aided by legume-rhizobia symbiosis. Int J Phytoremediation 16:179–202. https://doi.org/10.1080/15226514.2013.773273

Hawkes SJ (1997) What is a "heavy metal"? J Chem Educ 74:1374–1374. https://doi.org/10.1021/ed074p1374

Hecht-Buchholz C, Brady DJ, Asher CJ, Edwards DG (1990) Effects of low activities of aluminium on soybean (*Glycine max*). In: van Beusichem ML (ed) Plant nutrition — physiology and applications: proceedings of the eleventh international plant nutrition colloquium, 30 July–4 August 1989, Wageningen, The Netherlands. Springer, Dordrecht, pp 335–343

Jayasumana C, Fonseka S, Fernando A, Jayalath K, Amarasinghe M, Siribaddana S, Gunatilake S, Paranagama P (2015) Phosphate fertilizer is a main source of arsenic in areas affected with chronic kidney disease of unknown etiology in Sri Lanka. Springerplus 4:90–98. https://doi.org/10.1186/s40064-015-0868-z

Kang C-H, Kwon Y-J, So J-S (2016) Bioremediation of heavy metals by using bacterial mixtures. Ecol Eng 89:64–69. https://doi.org/10.1016/j.ecoleng.2016.01.023

Kelepertzis E (2014) Accumulation of heavy metals in agricultural soils of Mediterranean: Insights from Argolida basin, Peloponnese, Greece. Geoderma 221-222:82–90. https://doi.org/10.1016/j.geoderma.2014.01.007

Kidd P, Barceló J, Bernal MP, Navari-Izzo F, Poschenrieder C, Shilev S, Clemente R, Monterroso C (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67:243–259. https://doi.org/10.1016/j.envexpbot.2009.06.013

Kong Z, Mohamad OA, Deng Z, Liu X, Glick BR, Wei G (2015) Rhizobial symbiosis effect on the growth, metal uptake, and antioxidant responses of *Medicago lupulina* under copper stress. Environ Sci Pollut Res 22:12479–12489. https://doi.org/10.1007/s11356-015-4530-7

Kopittke PM, Dart PJ, Menzies NW (2007) Toxic effects of low concentrations of Cu on nodulation of cowpea (*Vigna unguiculata*). Environ Pollut 145:309–315. https://doi.org/10.1016/j.envpol.2006.03.007

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant-Microbe Interact 17:6–15. https://doi.org/10.1094/MPMI.2004.17.1.6

Lafuente A, Pajuelo E, Caviedes MA, Rodríguez-Llorente ID (2010) Reduced nodulation in alfalfa induced by arsenic correlates with altered expression of early nodulins. J Plant Physiol 167:286–291. https://doi.org/10.1016/j.jplph.2009.09.014

Lambert M, Leven BA, Green RM (2000) New methods of cleaning up heavy metal in soils and water. In: Environmental science and technology briefs for citizens. Kansas State University, Manhattan

Liu S, Zhang F, Chen J, Sun G (2011) Arsenic removal from contaminated soil via biovolatilization by genetically engineered bacteria under laboratory conditions. J Environ Sci 23:1544–1550. https://doi.org/10.1016/S1001-0742(10)60570-0

Lorito M, Woo SL, Garcia I, Colucci G, Harman GE, Pintor-Toro JA, Filippone E, Muccifora S, Lawrence CB, Zoina A, Tuzun S, Scala F (1998) Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. Proc Natl Acad Sci U S A 95:7860–7865. https://doi.org/10.1073/pnas.95.14.7860

Ma J, Lv C, Xu M, Chen G, Lv C, Gao Z (2016) Photosynthesis performance, antioxidant enzymes, and ultrastructural analyses of rice seedlings under chromium stress. Environ Sci Pollut Res Int 23:1768–1778. https://doi.org/10.1007/s11356-015-5439-x

Maheswari M, Yadav SK, Shanker AK, Kumar MA, Venkateswarlu B (2012) Overview of plant stresses: mechanisms, adaptations and research pursuit. In: Venkateswarlu B, Shanker AK, Shanker C, Maheswari M (eds) Crop stress and its management: perspectives and strategies. Springer, Dordrecht, pp 1–18

Maiti S, Ghosh N, Mandal C, Das K, Dey N, Adak MK (2012) Responses of the maize plant to chromium stress with reference to antioxidation activity. Braz J Plant Physiol 24:203–212. https://doi.org/10.1590/s1677-04202012000300007

Marconi AM, Kieboom J, de Bont JAM (1997) Improving the catabolic functions in the toluene-resistant strain *Pseudomonas putida* S12. Biotechnol Lett 19:603–606. https://doi.org/10.1023/A:1018366126573

Mejáre M, Bülow L (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19:67–73. https://doi.org/10.1016/S0167-7799(00)01534-1

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. https://doi.org/10.1111/1574-6976.12028

Mico C, Recatala L, Peris M, Sanchez J (2006) Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. Chemosphere 65:863–872. https://doi.org/10.1016/j.chemosphere.2006.03.016

Mulligan CN, Yong RN, Gibbs BF (2001) An evaluation of technologies for the heavy metal remediation of dredged sediments. J Hazard Mater 85:145–163. https://doi.org/10.1016/s0304-3894(01)00226-6

Nriagu JO (1990) Global metal pollution: poisoning the biosphere? Environ Sci Policy Sustain Dev 32:7–33. https://doi.org/10.1080/00139157.1990.9929037

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Env Res Pub He 14:1504. https://doi.org/10.3390/ijerph14121504

Pajuelo E, Rodríguez-Llorente ID, Dary M, Palomares AJ (2008) Toxic effects of arsenic on Sinorhizobium–*Medicago sativa* symbiotic interaction. Environ Pollut 154:203–211. https://doi.org/10.1016/j.envpol.2007.10.015

Pastor J, Hernández AJ, Prieto N, Fernández-Pascual M (2003) Accumulating behaviour of *Lupinus albus* L. growing in a normal and a decalcified calcic luvisol polluted with Zn. J Plant Physiol 160:1457–1465. https://doi.org/10.1078/0176-1617-01007

Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Proc Natl Acad Sci 97:4956–4960. https://doi.org/10.1073/pnas.97.9.4956

Pereira SIA, Lima AIG, Figueira EMAP (2006) Heavy metal toxicity in *Rhizobium leguminosarum* biovar viciae isolated from soils subjected to different sources of heavy-metal contamination: Effects on protein expression. Appl Soil Ecol 33:286–293. https://doi.org/10.1016/j.apsoil.2005.10.002

Pérez-Palacios P, Romero-Aguilar A, Delgadillo J, Doukkali B, Caviedes MA, Rodríguez-Llorente ID, Pajuelo E (2017) Double genetically modified symbiotic system for improved Cu phytostabilization in legume roots. Environ Sci Pollut Res 24:14910–14923. https://doi.org/10.1007/s11356-017-9092-4

Prasad MNV, De Oliveira Freitas HM (2003) Metal hyperaccumulation in plants - biodiversity prospecting for phytoremediation technology. Electron J Biotechnol 6:3. https://doi.org/10.2225/vol6-issue3-fulltext-6

Praveen A, Pandey VC, Marwa N, Singh DP (2019) Chapter 15 - rhizoremediation of polluted sites: harnessing plant–microbe interactions. In: Pandey VC, Bauddh K (eds) Phytomanagement of polluted sites. Elsevier, London, pp 389–407

Rafati M, Khorasani N, Moattar F, Shirvany A, Moraghebi F, Hosseinzadeh S (2011) Phytoremediation potential of *Populus Alba* and *Morus alba* for cadmium, chromium and nickel absorption from polluted soil. Int J Environ Res 5:961–970. https://doi.org/10.22059/ijer.2011.453

Reinhold-Hurek B, Bünger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hotspots for microbial activity. Annu Rev Phytopathol 53:403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Roberts TL (2014) Cadmium and phosphorous fertilizers: the issues and the science. Proc Eng 83:52–59. https://doi.org/10.1016/j.proeng.2014.09.012

Romaniuk K, Dziewit L, Decewicz P, Mielnicki S, Radlinska M, Drewniak L (2017) Molecular characterization of the pSinB plasmid of the arsenite oxidizing, metallotolerant *Sinorhizobium sp*. M14 - insight into the heavy metal resistome of sinorhizobial extrachromosomal replicons. FEMS Microbiol Ecol 93:215. https://doi.org/10.1093/femsec/fiw215

Sabiha J, Mehmood T, Chaudhry MM, Tufail M, Irfan N (2009) Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. Microchem J 91:94–99. https://doi.org/10.1016/j.microc.2008.08.009

Saha JK, Selladurai R, Coumar MV, Dotaniya ML, Kundu S, Patra AK (2017) Agriculture, soil and environment. In: Soil pollution - an emerging threat to agriculture. Springer, Singapore, pp 1–9

Sakizadeh M, Faraji F, Pouraghniyayi MJ (2015) Quality of groundwater in an area with intensive agricultural activity. Expos Health 8:93–105. https://doi.org/10.1007/s12403-015-0185-3

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol 49:643–668. https://doi.org/10.1146/annurev.arplant.49.1.643

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130. https://doi.org/10.1016/S0098-8472(98)00058-6

Saravanan A, Jeevanantham S, Narayanan VA, Kumar PS, Yaashikaa PR, Muthu CMM (2019) Rhizoremediation – a promising tool for the removal of soil contaminants: A review. J Environ Chem Eng 2019:103543. https://doi.org/10.1016/j.jece.2019.103543

Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, Rehim A, Hussain S (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721. https://doi.org/10.1016/j.chemosphere.2016.12.116

Sathishkumar M, Binupriya AR, Baik S-H, Yun S-E (2008) Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. CLEAN Soil Air Water 36:92–96. https://doi.org/10.1002/clen.200700042

Schwab AP, Banks MK (1994) Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In: Bioremediation through rhizosphere technology, vol 563. American Chemical Society, Washington, DC, pp 132–141

Shaheen R, Hassan I, Hafiz IA, Jilani G, Abbasi NA (2015) Balanced zinc nutrition enhances the antioxidative activities in *Oriental lily* cut-flower leading to improved growth and vase quality. Sci Hortic 197:644–649. https://doi.org/10.1016/j.scienta.2015.10.030

Silver S (1996) Bacterial resistances to toxic metal ions - a review. Gene 179:9–19. https://doi.org/10.1016/S0378-1119(96)00323-X

Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharm 43:246–253. https://doi.org/10.4103/0253-7613.81505

Singh S, Raju NJ, Nazneen S (2015) Environmental risk of heavy metal pollution and contamination sources using multivariate analysis in the soils of Varanasi environs, India. Environ Monit Assess 187:345. https://doi.org/10.1007/s10661-015-4577-4

Soad YSS, El-Egami HM, El-Tahlawy YA (2016) symbiotic N2-fixation efficiency of lupine plant under fungal and bacterial inoculation. Egypt J Biotechnol 52:1–16. https://doi.org/10.13140/RG.2.2.18260.04483

Tunali S, Çabuk A, Akar T (2006) Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. Chem Eng J 115:203–211. https://doi.org/10.1016/j.cej.2005.09.023

Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12:1161–1208. https://doi.org/10.2174/0929867053764635

Verma S, Kuila A (2019) Bioremediation of heavy metals by microbial process. Environ Technol Innov 14:100369. https://doi.org/10.1016/j.eti.2019.100369

Verma N, Singh M (2005) Biosensors for heavy metals. Biometals 18:121–129. https://doi.org/10.1007/s10534-004-5787-3

Wang H-Q, Zhao Q, Zeng D-H, Hu Y-L, Yu Z-Y (2015) Remediation of a magnesium-contaminated soil by chemical amendments and leaching. Land Degrad Dev 26:613–619. https://doi.org/10.1002/ldr.2362

Wångstrand H, Eriksson J, Öborn I (2007) Cadmium concentration in winter wheat as affected by nitrogen fertilization. Eur J Agron 26:209–214. https://doi.org/10.1016/j.eja.2006.09.010

Wani PA, Khan MS, Zaidi A (2007) Effect of metal tolerant plant growth promoting *Bradyrhizobium sp.* (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70:36–45. https://doi.org/10.1016/j.chemosphere.2007.07.028

Wild E, Dent J, Thomas GO, Jones KC (2005) Direct observation of organic contaminant uptake, storage, and metabolism within plant roots. Environ Sci Technol 39:3695–3702. https://doi.org/10.1021/es048136a

Wołejko E, Wydro U, Łoboda T (2016) The ways to increase efficiency of soil bioremediation. Ecol Chem Eng S 23:155–174. https://doi.org/10.1515/eces-2016-0011

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:1–20. https://doi.org/10.5402/2011/402647

Xue Z, Gao H, Zhao S (2014) Effects of cadmium on the photosynthetic activity in mature and young leaves of soybean plants. Environ Sci Pollut Res 21:4656–4664. https://doi.org/10.1007/s11356-013-2433-z

Zhang S, Chen M, Li T, Xu X, Deng L (2010) A newly found cadmium accumulator—Malva sinensis Cavan. J Hazard Mater 173:705–709. https://doi.org/10.1016/j.jhazmat.2009.08.142

Zhang J, Xu Y, Cao T, Chen J, Rosen BP, Zhao FJ (2017) Arsenic methylation by a genetically engineered Rhizobium-legume symbiont. Plant Soil 416:259–269. https://doi.org/10.1007/s11104-017-3207-z

Zhengwei Z, Fang W, Lee HY, Yang Z (2005) Responses of *Azorhizobium caulinodans* to cadmium stress. FEMS Microbiol Ecol 54:455–461. https://doi.org/10.1016/j.femsec.2005.05.006

Metallothionein-and Phytochelatin-Assisted Mechanism of Heavy Metal Detoxification in Microalgae

Shweta Tripathi and Krishna Mohan Poluri

Contents

Abstract

Microalgae are aquatic photosynthetic organisms accomplished with an ability to differentiate between essential and nonessential metal ions for their growth. This selective approach makes them the most suitable candidate for heavy metal removal from contaminated wastewaters. The different detoxification mechanisms executed by microalgae involve biosorption and bioaccumulation. With particular emphasis on bioaccumulation, the present chapter highlights the

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role of metallothionein and phytochelatins in heavy metal detoxification. These peptides are synthesized to chelate heavy metal ions and assist compartmentalization of this peptide—metal complex in cell organelles of microalgae. The present chapter covers the biosynthesis and regulation of metallothionein/phytochelatins in microalgae as a response to different heavy metals. The application of an individual or integrated omics approach revealing the detoxification mechanism in microalgae is also reviewed. In a nutshell, the chapter will provide a comprehensive understanding of the adaptive mechanism of this scintillating microorganism for mitigating heavy metals in order of priority to clean the environment.

Keywords

Microalgae · Metallothioneins · Phytochelatins · Heavy metals · Detoxification

Abbreviations

APX	Ascorbate peroxidase
As	Arsenic
BSO	Buthionine sulfoximine
Cd	Cadmium
Cd-GS2	Bisglutathionato cadmium
CE	Cation exchange
Cr	Chromium
Cys	Cysteine
Cu	Copper
ESI-Q-TOF MS	Electrospray ionization quadrupole time of flight mass spectrometry
Fe	Iron
GC-MS	Gas chromatography-mass spectrometry
GS	Glutathione synthase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
Glu	Glutamate
Gly	Glycine
HMWC	High molecular weight complex
HMT1	Heavy metal transport
^{1}H NMR	Proton nuclear magnetic resonance
Hg	Mercury
ICP-MS	Inductively coupled plasma mass spectrometry
LC-ESI-MS/MS	Liquid chromatography electrospray ionization tandem mass spectrometry
LMWC	Low molecular weight complex
Mn	Manganese
MALDI-TOF	Matrix-assisted laser desorption/ionization
MATE	Multi-antimicrobial extrusion protein

MTs	Metallothioneins
Mo	Molybdenum
MTP1	Metal transport protein
Nramp	Natural resistance-associated macrophage proteins
Pb	Lead
PC	Phytochelatin
PCR	Polymerase chain reaction
PCS	Phytochelatin synthase
ROS	Reactive oxygen species
rp-LC	Reverse phase liquid chromatography
Zn	Zinc
ZIP	Zrt-Irt-like proteins

1 Introduction

Microalgae belong to an incredible and diverse group of photosynthetic microorganisms that thrive in a wide range of habitat involving fresh, marine, brackish as well as toxic wastewaters. Microalgae being the primary constituent of the aquatic system are considered to be the most sensitive organisms against metal and metalloid toxicity. Some physiologically crucial elements such as copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are few of the prominent examples of micronutrients that facilitate unhindered metabolic activities inside the cells. The first series of transition metals (group 5-12) along with molybdenum (Mo) belong to the category of essential nutrients (Da Silva and Williams 2001). This significant abundance of essential metal ions in the periodic table cannot deny the fact that approximately one-third of the total structurally characterized proteins are metalloproteins (Clemens 2006). Other toxic and nonessential heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) may displace the essential trace elements required for proper function of metalloenzymes, thereby interfering the healthy physiological growth of cells (Tripathi et al. 2019). Additionally, higher affinity of toxic heavy metal ions for the sulfhydryl group of proteins, as well as structural similarity of As, Cr, Cd, Hg, and Pb with functional groups and essential traces metals such as phosphates, sulfates, Zn, Cu, Mn, Fe, etc., adds to the severity of the problem. These replacements adversely affect the cell by disturbing the membrane permeability, cell division, reduced enzymatic activities, and generation of reactive oxygen species (ROS) perturbing the cellular redox status leading to suppressed growth of microalgae (Gómez-Jacinto et al. 2015; Strejckova et al. 2019; Gauthier et al. 2020). Although the natural abundance of these toxic heavy metals is quite low, unprecedented increase in anthropogenic activities like mining, smelting, waste incineration, disposal of synthetic agriculture fertilizers, paint, and pigments has led to their sheer augmentation into the environment (Sharma et al. 2015). The persistent and non-biodegradable nature of heavy metals necessitate their continuous removal from soil and water bodies to avoid ecological imbalance. Land and aquatic plants, along with algae, are particularly attractive candidates for bioremediation of water bodies and soil (Gaur and Rai 2001). Among which, unicellular microalgae have emerged as a potential candidate for mitigating toxic heavy metals. Different microalgae species such as *Chlorella*, *Scenedesmus*, *Chlamydomonas*, etc., are directly exposed to metal-toxicated wastewaters and flourish without inhibition, thus displaying the metal tolerance capacity (Suresh Kumar et al. 2015; Arora et al. 2018b, 2019). To utilize the selective nature of microalgae for metal remediation to its full potential, unraveling the tolerance and bioaccumulation strategy is quintessential. This innate ability of hyperaccumulators may be explained by either genetic evolution or physiological adaptation in response to the surrounding. In the course of evolution, these microorganisms develop a suite of mechanism that regulates the uptake and accumulation of metal ions inside the cells. Physiological adaptation includes the production of osmoprotectants such as proline and glycine betaine, or antioxidant enzyme such as superoxide dismutase (SOD), ascorbate peroxidase (APX), etc. (Sytar et al. 2013; Priyadarshini et al. 2019). In contrast, genetic evolution corresponds to the differential expression of gene products, such as chelating proteins (Gómez-Jacinto et al. 2015).

Moreover, tolerance toward multiple metals in the surrounding also raises the need to decipher the explicit behavior of microalgae in response to every different metal. This resistance phenomenon could be due to concurrent tolerance of single or multiple metals with altered biosynthetic adaptive mechanisms (Gaur and Rai 2001). For example, exposure of *Chlorella vulgaris* to Cu-, Cd-, and Pb-containing solution followed phytochelatin (PC)-dependent detoxification mechanism to remove Cu and Cd ions, whereas Pb was removed by biosorption onto the cell surface (Zhang et al. 2014). Further exposure to all the three metals together suggested enhanced removal of Cd and Pb due to the production of extracellular polymeric substance on the cells of Cu-spiked *C. vulgaris* (Zhang et al. 2015).

Overall the process of metal detoxification employed by microalgae can be divided into three different mechanisms. The first mechanism suggests attachment of metal ions to the extracellular polymeric substance released on the cell wall, which prevents entry inside the cell, thereby reducing metal bioavailability and toxicity (Perales-Vela et al. 2006; Monteiro et al. 2012). The second mechanism follows the intracellular uptake of metal ions with the help of cysteine-containing peptides such as metallothioneins and/or phytochelatins to facilitate vacuolar compartmentalization of the toxic-free ions, whereas the third process involves the efflux of excess metal ions back into the surrounding (Gaur and Rai 2001; Hirata et al. 2005; Perales-Vela et al. 2006; Monteiro et al. 2012). Among these phenomena, metallothionein-assisted detoxification especially catches attention as its constitutive expression evidences an indispensable role in maintaining the metal homeostasis (Perales-Vela et al. 2006; Sharma et al. 2015).

Considering the metal detoxification mechanism of microalgae and the unique physiological role of metallothionein and phytochelatins, this chapter provides a comprehensive knowledge related to metallothionein-/phytochelatin-assisted heavy metal removal by microalgae. Mechanistic aspects related to the structure, biosynthesis, and regulation of metallothionein in the presence of heavy metal are elaborated. Along with the molecular insights, the mechanism of chelation and subsequent compartmentalization of metallic ions is also discussed. Further, recent studies comprising the application of omics-based techniques are reviewed in detail to highlight their prominent findings.

2 General Characteristics of Metallothioneins/Phytochelatins and Their Classification

Metallothioneins (MTs) are cysteine-rich proteins conferring high affinity for specific metal ions like Cd. These can be designated as molecular soldiers of cells, preventing toxicity of metal ions by the formation of organometallic complexes. MTs were first identified in horse renal cortex cells in 1957 as metal-chelating proteins (Margoshes and Vallee 1957). These metal-binding polypeptides constitute a Cys-X-Cys sequence, where "X" is any amino acid other than Cys (Cobbett 2001; Cobbett and Goldsbrough 2002). Generally, MTs are low molecular weight (<10 kDa) cysteine-rich proteins involved in sequestration of heavy metal ions in different organisms ranging from animals, bacteria, plants, yeast, fungi, and microalgae. MTs are recognized by their low molecular weight, high cysteine, and metal content with conserved patterns (Cys-X-Cys) and the absence of aromatic amino acids (Gaur and Rai 2001). The strict and constitutive arrangement of cysteine residues in MTs is the basis of their classification in three different types (Kägi and Kojima 1987; Robinson 1989; Torres et al. 2008; Gutiérrez et al. 2019).

The class I MTs have cysteine arrangements in close resemblance with horse kidney MT (not found in algae), whereas class II MTs are distantly correlated with cysteine arrangements with horse kidney MTs (present in cyanobacteria, plants, and fungi; Olafson et al. 1988). Class III MTs are enzyme catalyzed thiolate peptides, widely known as phytochelatins (present in plants and several representative groups of algae such as Chlorophyta, Chrysophyta, Xanthophyta, Euglenophta, etc. (Gekeler et al. 1988; Robinson 1989; Shaw et al. 1989). In general, as per the class devoted to different MTs, they are named as MTI, MTII, and MTIII.

2.1 Role of Metallothioneins/Phytochelatins in Metal Tolerance and Homeostasis

MTs are known for their crucial role in trafficking trace metal (like Zn, Mn, etc.) through various cellular functions. In the face of heavy metal toxicity, these MTs participate in maintaining the metal homeostasis to prevent the occurrence of intracellular oxidative stress. In addition to this, the instant generation of specific antioxidant molecules (proline, betaine, tocopherol, etc.) helps to prevent any possible damage by ROS species (Sharma et al. 2015). Among all, glutathione (GSH) pool of cells plays a crucial role in scavenging oxidative stress. Other than GSH, free thiols of MTs can scavenge ROS through MT redox cycle. Oxidation of thiolate clusters bound to metal ions results in the generation of free metal ions and MT–disulfide bond (Ruttkay-Nedecky et al. 2013). Any variation in the redox ratio of GSH pool (GSH/GSSG) generates reducing environment and leads to the formation of reduced MT-thiol compounds. This event comprises the reduction and oxidation of MT owing to a decisive role in maintaining metal homeostasis in normal cells (Souza et al. 2012).

2.2 Biosynthesis and Regulation of MTIII or Phytochelatin in Microalgae

Although plants, fungi, and some algae synthesize a different class of metal-binding thiol peptides, they share a functional analogy with mammalian MTs (Gekeler et al. 1988; Robinson 1989; Gaur and Rai 2001). Structural analysis of Cd-binding proteins from Cd-exposed plants extracts showed the occurrence of poly (y-glutamylcysteinyl)-glycine (Bernhard and Kagi 1985; Grill et al. 1985). Further characterization of Cd-chelating peptides isolated from *Chlorella fusca* and *Scenedesmus acutus* also confirmed the (γ-GluCys)$_n$-Gly structure for this peptide (Gekeler et al. 1988; Hirata et al. 2005; Clemens 2006). Polypeptides having no glycine or β-alanine in place of glycine were designated as class III metallothioneins or phytochelatins (Gaur and Rai 2001; Perales-Vela et al. 2006). Phytochelatins are mainly comprised of glutamic acid, cysteine, and glycine, with the general structure of (γ-Glu-Cys)$_n$-Gly, where $n = 2$–11 having inducible nature contrary to animal MTs. Moreover, induction of (γ-GluCys)$_2$ and (γ-GluCys)$_3$ was prominent in *C. fusca* and *S. acutus* cells exposed to higher concentrations of Ag, Cu, Cd, Pb, and Zn (Grill et al. 1986, 1987).

Structural similarity between glutathione and phytochelatins represents an interconnected pathway utilizing the same enzymes for their biosynthesis. Furthermore, the unusual γ-carboxymide linkage between glutamate and cysteine residue in the chemical structure of glutathione suggests the existence of an enzyme-mediated pathway for synthesis of phytochelatins (Gaur and Rai 2001; Perales-Vela et al. 2006). Synthesis of glutathione is a two-step enzyme-mediated process. The first step is the formation of γ-glutamylcysteine in the presence of γ-glutamylcysteine synthetase, whereas the second constitutes the catalytic synthesis of glutathione in the presence of glutathione synthetase (Gaur and Rai 2001; Hirata et al. 2001, 2005). Further, the synthesis of PC is catalyzed by PC synthase by utilizing GSH. Significance of γ-glutamylcysteine in the process of phytochelatin synthesis is explained by the supplementation of buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcysteine, in heavy metal-spiked cells. These treated cells lacking phytochelatins constitute higher sensitivity against heavy metal ions (Rauser 1990, 1995). In addition to this, mutants of *S. pombe* deficient in enzymes of GSH biosynthesis failed to synthesize phytochelatins (Mutoh and Hayashi 1988). These studies highlight the importance of crosstalk between GSH and PC synthetic pathways for mitigating heavy metal stress in microalgae. The overall biosynthetic pathway of PC from GSH and chemical structure of PC has been depicted in Fig. 1.

Over the past two decades, there have been several models explaining the catalytic action of PCS for synthesizing PCs. Grill and coworkers gave the very

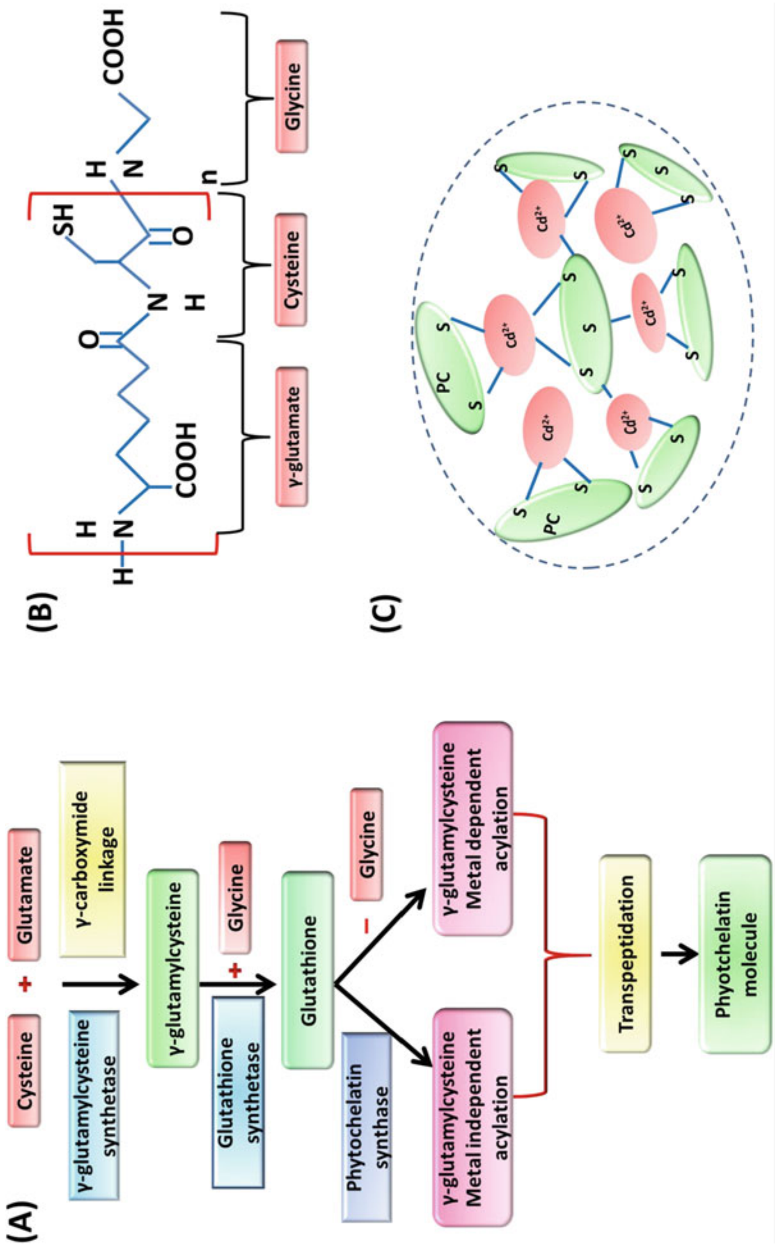


Fig. 1 (**a**) Biosynthetic pathway of PC; (**b**) chemical structure of PC, and (**c**) structure of PC–Cd complex

first model in 1989. It was suggested that PC synthase (dipeptidyl transpeptidase; EC 2.3.2.15) consists of two distinct reactions sites and requires heavy metals as its activating factor. The first step comprises the cleavage of glycine from GSH with the simultaneous generation of γ-glutamylcysteine. The next reaction supports the formation of a peptide bond between obtained γ-glutamylcysteine and other GSH or PC molecules (Gekeler et al. 1989; Hirata et al. 2005; Clemens 2006).

Later, Ha et al. in 1999 reported that N-terminal of PC synthase (PCS) is responsible for the catalytic activity of the enzyme. Mutants of the PCS gene in *Arabidopsis thaliana* named AtPCS1 lacking the C-terminal domain showed a significant synthesis of PCs solely due to N-terminal domain (Ha et al. 1999). However, C-terminal in PC synthase senses the availability of heavy metals and assists their transfer to the reactive sites in N-terminal (Ha et al. 1999; Hirata et al. 2005).

In addition to the above model, kinetic observation during the synthesis of PC by tagging AtPCS1 with FLAG unveiled the potential of Cd–GS$_2$ complex as an activator of PC synthesis. Generally, Cd–GS$_2$ complexes serve as a substrate for PC synthesis, and free Cd ions are known to activate PC synthesis. Further investigations with S-methyl GSH as substrate confirmed that substituted thiols could act as activators of PC synthesis in the absence of heavy metal ions (Vatamaniuk et al. 2000). Among all the proposed models that illustrated the mechanism and action of PCS, a recent report by Vatamaniuk et al. based on the radiolabelled substrate added unique and novel information to the existing concepts. The report suggests that PCS catalyzes acylation of γ-glutamylcysteine at two distinct sites in the enzyme. The first site is acylated in a metal-independent manner, whereas acylation at other sites requires the presence of Cd ions (Vatamaniuk et al. 2004). Especially the active sites of the catalytic domain consist of a conserved sequence of cysteine residues Cys56, Cys90, Cys109, and Cys113. Among these, Cys 56 in AtPCS1 genes holds a vital function in acylation of the first site for the formation of PC and heavy metal tolerance (Hirata et al. 2005).

3 Phytochelatin (MTIII)-Mediated Heavy Metal Detoxification in Microalgae

Presence of toxic metal ions like Cd, Pb, Hg, As, and Zn in surrounding induces the synthesis of phytochelatins in microalgae cells (Perales-Vela et al. 2006). Although microalgae employ numerous strategies to deal with heavy metal toxicity, synthesis of thiol-containing peptides is considered to be more specific and interesting. Phytochelatin or MTIII is a product of enzymatic reaction in the cytosol, but it is abundant in both intracellular and extracellular fractions (Sharma et al. 2015). The exogenous PCs inherently bind to metal ions to exudate, precipitate and stabilize them on the cell surface to prevent their cellular entry. Cytosolic PCs form complexes with metal ions to aid their compartmentalization in cell organelles, thereby reducing the toxicity (Perales-Vela et al. 2006; Priyadarshini et al. 2019). In a structural model, PCs bind to Cd ions through coordinate covalent bonds by involving the sulfur atoms of cysteine side chains. Depending upon the number of PC residues, a Cd ion can bind to 2, 3, or 4 sulfur atoms to form a stable complex (Cruz et al. 2002; Hirata et al. 2005; Clemens 2006; Fig. 1).

Another significant step in MTIII–metal complexation is the inclusion of sulfide ions (Steffens 1990; Jia et al. 2016). Sulfide ions generated during Cys synthesis provide more stability to the complex and convert it to high molecular weight complexes (Jia et al. 2016; Ahmad et al. 2019). In fact, on basis of the presence and absence of sulfide ions in MTIII–metal complex, these complexes have been divided into two categories (Hu et al. 2001; Perales-Vela et al. 2006). Low molecular weight complex (LMWC) does not carry sulfide ion and makes comparatively weaker complex with metals, whereas high molecular weight complex (HMWC) provides higher stabilization and in consequence improves detoxification (Scarano and Morelli 2002; Jia et al. 2016). Furthermore, these protein sulfide complexes are also involved in maintaining the equilibrium of these ions during heavy metal toxicity (Hirata et al. 2001, 2005). Malfunction in sulfide metabolism led to formation of insoluble metal salt precipitants rather than PC–metal complexes in marine microalgae *Tetraselmis suecica* (Perrein-Ettajani et al. 1999). This phenomenon suggests the importance of sulfide ions in detoxification mechanism. Although numerous reports are emphasizing the importance of different types of MTs to mitigate heavy metal stress in animals, plants, fungi, bacteria, and cyanobacteria (Davis and Cousins 2000; Blindauer 2011; Emamverdian et al. 2015; Hasan et al. 2017; Gutiérrez et al. 2019), but microalgae exclusively represent the type III MTs also known as phytochelatins.

Stokes et al., for the first time, discovered phytochelatin as a metal-chelating agent in *Scenedesmus acutiformis* in 1977 (Stokes et al. 1977). *Chlamydomonas reinhardtii* showed sequestration of more than 70% of cytosolic cadmium via MTIII or PC complexation (Howe and Merchant 1992). Other studies include synthesis of PCs in the presence of Pb, Hg, As, and Zn exposed microalgae cells (Hirata et al. 2001; Pawlik-Skowrońska 2001; Le Faucheur et al. 2005; Tukaj et al. 2007; Marcano et al. 2009; Kalinowska and Pawlik-Skowrońska 2010; Gómez-Jacinto et al. 2015; Strejckova et al. 2019). The production of the PC molecule in any species is specific and depends on the nature and concentration of the metal ion as well as the time of exposure (Table 1). A recent study highlighted production of PC molecules in *Chlorella vulgaris* both under individual and synergistic concentrations of Cd, Cu, and Pb (Zhang et al. 2014, 2015). Other than 50 μM Pb, all the combinations showed production of PC2 after 24 h of exposure. Surprisingly, further incubation for 1 week showed decline in content of PC2 in Cu- and Cd-spiked (200 μM and 100 μM) cells. PC3 and PC4 were explicitly observed in Cd (100 μM) and Cd Pb (100 and 50 μM) exposed culture after two weeks of incubation (Zhang et al. 2015). This study showed the time- and metal-dependent generation of PC3 and PC4. Similarly, Hg-induced phytochelatin production was observed in *Chlorella sorokiniana* with two Hg-binding phytochelatins, (des-Gly)PC2 and PC2 after 9 days of exposure to 5 mg L^{-1} $HgCl_2$ (Gómez-Jacinto et al. 2015). Another study in *Dunaliella terticola* showed strong induction of phytochelatin synthesis (PC2 to PC5) in the presence of

Table 1 List of PC synthesizing microalgae in the presence of different heavy metal ions

Heavy metal	Species name	Conc. of metal (mg L^{-1})	Exposure time	Type of PC	References
Cd (II)	*Scenedesmus quadricauda*	40 μM	3 days	PC2, PC3, and PC4	Strejckova et al. (2019)
	C. reinhardtii	70 μM	3 days	PC2	Penen et al. (2019)
	C. vulgaris	100 μM	3 days	PC2 and PC3	Zhang et al. (2014)
Cu (II)		500 μM			
Cd (II)	*Chlorella* sp.	100 μM	15 days	PC2, PC3, and PC4	Zhang et al. (2015)
Cd (II) +Pb (II)		100 μM + 50 μM			
Cu (II)		200 μM			
Cu (II) + Pb (II)		100 μM + 50 μM			
Hg (II)	*C. sorokiniana*	18 μM	9 days	PC2	Gómez-Jacinto et al. (2015)
Cu (II)	*Stichococcus minor*	5 μM	14 days	PC2	Kalinowska and Pawlik-Skowrońska (2010)
	G. terricola				
Cd (II)	*Scenedesmus rotundus*	0.04 mM	24 days	PC4	Shivaji and Dro (2019)
Zn (II)		0.2 mM			
Cd (II)	*Scenedesmus vacuolatus*	0.3–79 nM	3 days	PC2-PC6	Le Faucheur et al. (2005)
Pb (II)	*Phaeodactylum tricornutum*	10 μM	3 days	PC2 and PC4	Morelli and Scarano (2001)
Cu (II)			7 h	PC2–PC6	Morelli et al. (2005)
Cd (II)	*S. armatus*	93 μM	24 h	PC2, PC3, and PC4	Tukaj et al. (2007)
As (III, V)	*D. salina*	86 μM and 54 μM	3 days	PC2, PC3, and PC4	Wang et al. (2017)

200 μM Zn in the media in a time-dependent manner. This study also highlighted the quantification of precursors and intermediated molecules such as cysteine (Cys), y-glutamylcysteine (yEC), and GSH (Hirata et al. 2001). Similarly, Cu-induced phytochelatins were observed in *Stichococcus minor* and *Geminella terricola* isolated from Cu-polluted and nonpolluted sites, respectively. This study represented the tolerance mechanism of adapted microalgae *S. minor* in comparison to *G. terricola* (Kalinowska and Pawlik-Skowrońska 2010). Another study elucidated the arsenate-induced synthesis of PC in marine microalga *Phaeodactylum tricornutum*. This study highlighted the relationship between rate of arsenic accumulation and PC synthesis. Accumulation of metals at a higher rate in comparison to PC synthesis disturbs the detoxification process leaving excess free ions in the cells (Morelli et al. 2005). All the above studies establish that phytochelatins are the key

molecules in detoxification of excess concentration of both essential and toxic heavy metal ions.

3.1 Sequestration of the Protein–Metal Complex in Vacuole, Chloroplast, and Mitochondria

The phenomenon of compartmentalization was first discovered in yeast *Schizosaccharomyces pombe* by (Ortiz et al. 1992, 1995). PC–metal complexes finally get sequestered in the vacuoles of the cell. *Dunaliella terticola* was the first microalgae recognized with the process of PC–metal sequestration in vacuoles (Heuillet et al. 1986). Transportation of these complexes to vacuoles is facilitated by specific transporters present on wall of vacuoles. According to the literature, heavy metal transport (HMT1) protein encoded by hmt1 gene in *S. pombe* can internalize low molecular weight complexes (Ortiz et al. 1992). However, recent reports have highlighted the role of metal transport protein (MTP1) in vacuolar membrane of *Chlamydomonas*. MTP1 are constitutively expressed in vacuolar membrane for accumulation of Zn but also facilitated accumulation of divalent metals like Cd inside the vacuole (Hanikenne 2005). Reports based on electron probe microanalysis evidenced dense material inside the vacuole representing both sulfur and cadmium ions in Cd (100 mg L^{-1}) exposed *D. terticola* cells (Heuillet et al. 1986). On a similar note, *T. suecica* represented a significant accumulation of Cd ions inside the vacuole (Ballan-Dufrançais et al. 1991).

Few studies also established the accumulation of cadmium inside cells as black deposits in TEM (transmission electron microscopy) micrographs and EDX (energy dispersive X-ray) analysis in *Ankistrodesmus falcatus*, *Chlorella pyrenoidosa*, *Scenedesmus quadricauda*, and *C. reinhardtii* (Silverberg 1976; Samadani et al. 2018). Other than vacuoles, few microorganisms prefer sequestration of excess metal in chloroplast and mitochondria to prevent disturbance in cytosolic processes. For example, a different strain of *C. reinhardtii* showed preferred accumulation of MTIII-Cd ions in chloroplast rather than vacuole (Nagel et al. 1996). Few authors also reported the organelle ultrastructural variations in the presence of toxic metal ions. For example, exposure to 20 mM Cd for 72 h led to the significant increase in starch granules and vacuoles, with electron-dense black deposits in *Chlamydomonas acidophila* (Nishikawa et al. 2003). Another study showed damaged starch sheath of chloroplast due to sequestration of Cd ion in pyrenoids of *C. reinhardtii* (Penen et al. 2017, 2019). Furthermore, to maintain the regulatory networks inside the cells, these metallic complexes are eventually hydrolyzed, and excess metal ions are pumped out through active efflux transporters (ABC transporters) present in their cell walls (Lu et al. 2019). All the above studies suggested the importance of PCs as chelating agents in microalgae as well as compartmentalization of complexes into various organelles (Levy et al. 2008; Priyadarshini et al. 2019). The overall phenomenon of MTIII or PC-assisted detoxification of heavy metals has been schematically represented in Fig. 2.

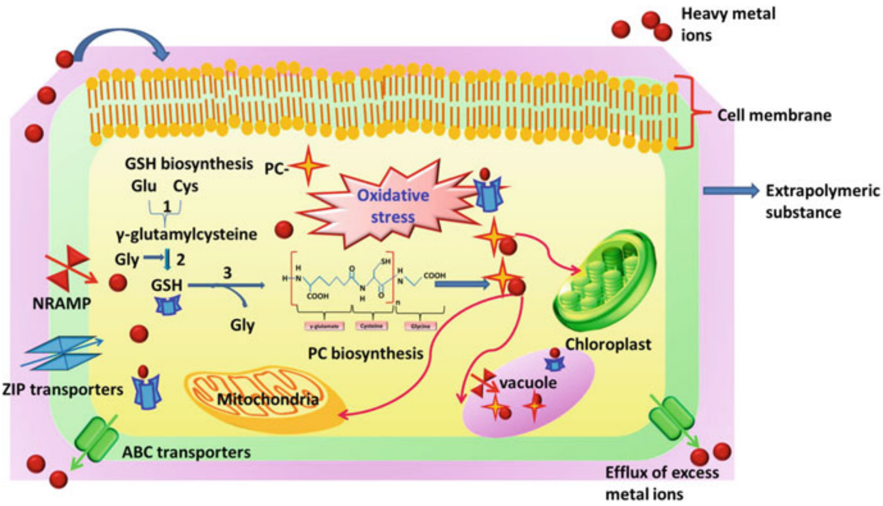
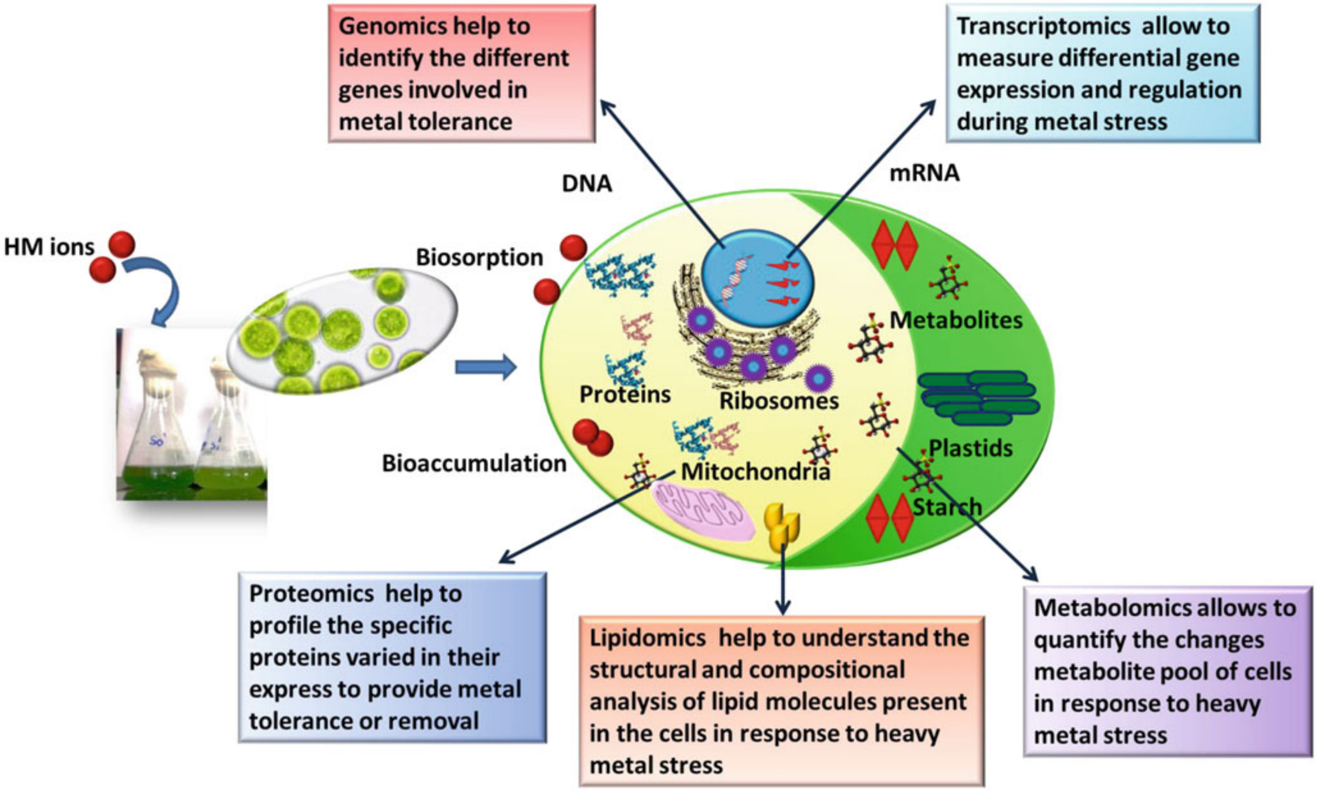


Fig. 2 Schematic showing an overall mechanism of metal detoxification and sequestration by phytochelatins in microalgae. (1) γ-glutamylcysteine synthetase; (2) glutathione synthetase; (3) phytochelatin synthase. *GSH* glutathione, *PC* phytochelatin

4 Omics-Based Techniques to Unravel the Genetic Makeup of Hyperaccumulators

The adaptive ability of microalgae to tolerate a high amount of toxic compounds like heavy metal ions requires a deep understanding of the underlying genetic makeup of the cells. Assessment of metal stress response in any organism is usually quantified with the help of few physiological parameters such as growth, toxicity profiling, cell size, phenotypic variations, etc. Some other associated biochemical changes are quantified by assays showing oxidative stress compensated by increased activities of antioxidant enzymes. However, mapping only the adverse outcomes of the toxic component in microalgae gives a faint picture of defense response mounted by the cell. To comprehend the accurate response of cells against toxicants urges for a system-based approach that allows characterizing and understanding the overall perturbation in the molecular pathways.

Recently, omics technologies have emerged as an integrated platform for providing a comprehensive description of all components in a cell in a broader picture (Arora et al. 2018c; Mishra et al. 2019; Salama et al. 2019) (Fig. 3). It generates a massive amount of data delineating the information of the genome expressed via transcribed mRNA (transcriptome) to proteins (proteome) and eventually to metabolites (metabolome), revealing the central dogma of life. Microalgae being the basis of food web and producer of oxygen are a crucial component of the ecosystem. Moreover, their tremendous application in ecotoxicological studies



Genomics help to identify the different genes involved in metal tolerance

Transcriptomics allow to measure differential gene expression and regulation during metal stress

Proteomics help to profile the specific proteins varied in their express to provide metal tolerance or removal

Lipidomics help to understand the structural and compositional analysis of lipid molecules present in the cells in response to heavy metal stress

Metabolomics allows to quantify the changes metabolite pool of cells in response to heavy metal stress

Fig. 3 Integration of omics approach and their application in heavy metal detoxification concerning toxic heavy metals has created a paradigm shift toward utilizing omics technologies (Table 2).

Genomics provides the information of nucleotide sequences representing the full genome of any organism. The sequence information enables us to understand the overall organization of the genetic arrangement covering both conserved and individual genes. Genomics also help to establish sequence similarity and evolutionary relationships among a diverse group of the organisms (Jamers et al. 2009; Mishra et al. 2019). Recently few studies have demonstrated the toxico-genomics approach underlying the molecular response of microalgae under heavy metal stress. Microarray hybridization-assisted generation of high-throughput data screening revealed differential gene expression in copper-spiked *C. reinhardtii* cells under 8–125 μM $CuSO_4$ concentration. This study identified some novel biomarkers of copper stress to elucidate the underlying molecular mechanism (Jamers et al. 2006).

Further investigation of Cd-spiked *C. reinhardtii* cells represent integrated transcriptomics and metabolomics approach to understand the molecular mechanism underlying Cd-responsive perturbations in the cells. Briefly, microarray hybridization resulted in 364 differentially expressed genes in 5 μM $CdCl_2$ suggesting glutathione S transferase, glutathione peroxidase, and thioredoxin as primary genes mounting the defense response. Furthermore, gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) based metabolomics highlighted different metabolic pool at 100 μM $CdCl_2$ concentration (Jamers et al. 2009). Similarly, comparative transcriptomics studies were performed with an extremophilic microalgae *C. acidophila* and normal *C. reinhardtii* cells to understand the mechanism of heavy metal tolerance (Puente-sánchez et al. 2018). This study presented two different mechanisms adopted by the microalgae strains. The acidophilic strains exhibited expression of certain efflux proteins responsible for adaptation to acidic environment and heavy metal detoxification, whereas increased expression of CaPCS2 (*Chlamydomonas* phytochelatin synthase) gene was the key mechanism in *C. reinhardtii* cells (Puente-sánchez et al. 2018). Another transcriptomics study in *Auxenochlorella protothecoides* UTEX234 revealed the involvement of some metal transporters involved in Cd transport inside the cells. Other than natural resistance-associated macrophage proteins (Nramp), Zrt-Irt-like proteins (ZIP) and Ca^{2+}-ATPase were also observed in facilitating entrance of Cd inside the cells. The efflux of sequestered Cd ions was assisted by multi-antimicrobial extrusion protein (MATE), cation exchange (CE), and ABC transporters in the cell membrane (Lu et al. 2019).

Proteomics study complements information obtained from genomics and transcriptomics to provide a better understanding of the whole proteome organization and protein–protein interactions inside the cell (Torres et al. 2008; Jamers et al. 2009; Salama et al. 2019). Similar to transcriptomics studies, proteomics under heavy metal stress is explored in *C. reinhardtii* cells (Gillet et al. 2006; Cid et al. 2010; Pillai et al. 2014). These studies employed 2-D electrophoresis followed by matrix-assisted laser desorption/ionization (MALDI-TOF) analysis to identify the soluble protein present in heavy metal exposed cells. For example, Cd (150 μM) spiked *C. reinhardtii* cells showed significant increase in protein related to

Table 2 Summary of omics approaches used to reveal heavy metal tolerance mechanism in different microalgae species

Metal	Species name	Omics approach	Techniques	Remarks	References
As (III and V)	*Scenedesmus* sp. *IITRIND2*	Metabolomics	^{1}H NMR	Cellular defense of cells and lipid biosynthesis	Arora et al. (2018a)
As (V)	*C. reinhardtii*	Proteomics	2-D gel electrophoresis and LC-MS/MS analysis	Arsenic-specific response in cells	Walliwalagedara et al. (2012)
Cd (II) and natural metal rich water	*Dunaliella acidophila*	Comparative transcriptomics	Illumina sequencing	Expression of oxidative stress and ROS genes	Puente-sánchez et al. (2016)
Cd (II)	*C. acidophila*	Transcriptomics	Illumina sequencing	Lipid biosynthesis	Puente-sánchez et al. (2018)
	Auxenochlorella protothecoides	Transcriptomics	Real-time qPCR	Metal transporters involved in uptake and efflux and lipid induction	Lu et al. (2019)
	C. reinhardtii	Transcriptomics and metabolomics	Microarray hybridization, GC-MS, ^{1}H NMR	Genes involved in defense mechanism Metabolites on glutathione synthesis pathway	Jamers et al. (2013)
	C. reinhardtii	Proteomics	2-D gel electrophoresis and MALDI-TOF analysis	Toxic response on whole proteome focusing redoxin targets	Gillet et al. (2006)
	Nannochloropsis oculata	Proteomics	2-D gel electrophoresis and ESI-Q-TOF MS/MS	Alteration and induction of new proteins in Cd toxicity	Kim et al. (2005)
Cr (VI)	*Pseudokirchneriella subcapitata*	Proteomics	2-D gel electrophoresis and LC-ESI-MS/MS	Cr-elicited changes in whole proteome	Vannini et al. (2009)
(Zn, Cu, Fe, Co, Ni, As, Cd, and Cr)	*Chlamydomonas* sp. *strain RT9*	Proteomics	2-D gel electrophoresis and MALDI-TOF analysis	Adaptive changes in whole proteome against metal toxicity	Cid et al. (2010)
Cu (II)	*C. reinhardtii*	Transcriptomics	Microarray hybridization	Differential expression of genes involved in glycolysis, photosynthesis, heat shock proteins, and intracellular proteolysis under copper stress	Jamers et al. (2006)

(continued)

Table 2 (continued)

Metal	Species name	Omics approach	Techniques	Remarks	References
Cd (II),Cu (II) and Pb (II) (individually)	*C. vulgaris*	Metabolomics	^{1}H NMR and LC-MS/MS quantification	Metal-responsive biomarkers such as phytochelatin and glutathione	Zhang et al. (2014)
Cd (II), Cu (II) and Pb (II) (synergy)	*Chlorella* sp.	Metabolomics and metallomics	^{1}H NMR, LC-MS/MS and ICP-MS	Metabolome profiling under multimetal toxicity	Zhang et al. (2015)
Cu (II)	*C. acidophila*	Transcriptomics	De novo transcript assembly	Genes involved in copper stress and adaptive response	Olsson et al. (2015)
Pb (II), Cd (II) and Hg (II)	*Euglena gracilis*	Proteomics	Reverse phase liquid chromatography (rp-LC) and triple-TOF spectrometer	Protein involved in tolerance and sequestration	Khatiwada et al. (2020)

glutathione synthesis, adenosine triphosphate (ATP) metabolism, chaperons whereas drastic reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase, enzymes of photosynthetic, and chlorophyll biosynthetic pathway (Gillet et al. 2006). A similar investigation was performed to elucidate the adaptive modulation in the transcriptome and proteome of silver-spiked *C. reinhardtii* cells (Pillai et al. 2014).

Metabolomics is the endpoint of the omics chain representing overall modulation in biomolecules categorized as amino acid, carbohydrates, nucleotides, organic acids, etc. (Arora et al. 2018c). Metabolic profiling is a fascinating approach to interpret the response of microalgae cells in the face of heavy metal toxicity. Techniques such as GC-MS, LC-MS (liquid chromatography-mass spectrometry), and NMR are widely employed to perform targeted or nontargeted metabolic profiling. For example, NMR-based metabolomics followed by LC-MS quantification was performed to reveal metal homeostasis in Cu-, Cd-, and Pb-spiked *C. vulgaris* cells (Zhang et al. 2014). This study highlighted the changes in metabolites in response to redox-active Cu ions and nonredox-active Cd and Pb ions. Inhibitory effect of Cu ions in synthesis of osmoprotectants such as betaine and glycerophosphocholine complemented with reduced GSH/GSSG ratio, whereas increased phytochelatin and GSH content in Cd-spiked cells were observed (Zhang et al. 2014). Recently, another study employed NMR-based metabolic profiling of arsenic-spiked cells to elucidate the differential cellular response in a novel hypertolerant and hyperaccumulator microalgae sp. named *Scenedesmus* sp. IITRIND2 (Arora et al. 2017). This species can withstand 500 mg L^{-1} of both forms of arsenic. In total, array of 45 metabolites was identified out of which 18 represented significant differences in the metabolome of As (III), As (V) spiked, and control cells to delineate the stress-responsive pathway. This study represents variation in different amino acids, citrates, sucrose content, phytochelatins, glycerol, etc. describing the adaptive and tolerance behavior of As-spiked cell (Arora et al. 2018a). In conclusion, all these studies suggest effective utilization of omics approach integrated with sophisticated analysis tools to elucidate the defense mechanism of microalgae in response to toxic insults.

5 Conclusions and Future Prospects

Elevated concentration of heavy metals in natural water bodies at an alarming rate has prompted a search for a cost-effective and ecofriendly approach. Microalgae are the primary constituent of natural water bodies with inherent ability to discriminate between essential and nonessential heavy metals. To maintain a nontoxic concentration of heavy metals inside the cell, microalgae employ MTIII- or PC-assisted complexation and store the complexes in cell organelles. Moreover, enzyme-mediated biosynthetic pathway for synthesis of PC molecules also entrenches the catalytic mechanism of PC synthase in microalgae. Although numerous studies focusing on metal detoxification are well documented in the literature, but the ultimate fate of these PC–metal complexes in cells and associated pathways with transporters still need scientific attention. Even though the applicability of omics techniques in microalgae has drastically increased in the past few years, but critical symbiotic interaction of transcriptome, proteome, and metabolome on integrated platform is still limited. The algomics techniques coupled with detailed structural characterization of MTIII and PCs in microalgae would help to elucidate the complete metabolic regulatory networks involved in metal interactions. The omics approach would also help to identify new genetic targets to engineer microalgae for its utilization in remediation at its fullest potential.

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References

Ahmad J, Ali AA, Baig MA (2019) Role of phytochelatins in cadmium stress tolerance in plants. In: Cadmium toxicity and tolerance in plants. Elsevier, London, pp 185–212

Arora N, Gulati K, Patel A (2017) A hybrid approach integrating arsenic detoxification with biodiesel production using oleaginous microalgae. Algal Res 24:29–39

Arora N, Dubey D, Sharma M (2018a) NMR-based metabolomic approach to elucidate the differential cellular NMR-based metabolomic approach to elucidate the differential cellular responses during mitigation of arsenic (III, V) in a green microalga. ACS Omega 3:11847–11856

Arora N, Gulati K, Tripathi S (2018b) Algae as a budding tool for mitigation of arsenic from aquatic systems. In: Mechanisms of arsenic toxicity and tolerance in plants. Springer, New York, pp 269–297

Arora N, Pienkos PT, Pruthi V (2018c) Leveraging algal omics to reveal potential targets for augmenting TAG accumulation. Biotechnol Adv 36:1274–1292

Arora N, Tripathi S, Pruthi V, Poluri KM (2019) An Integrated Approach of Wastewater Mitigation and Biomass Production for Biodiesel Using *Scenedesmus* sp. In: Application of microalgae in wastewater treatment. Springer, New York, pp 467–494

Ballan-Dufrançais C, Marcaillou C, Amiard-Triquet C (1991) Response of the phytoplanktonic alga *Tetraselmis suecica* to copper and silver exposure: vesicular metal bioaccumulation and lack of starch bodies. Biol Cell 72:103–112

Bernhard WR, Kagi JHR (1985) Purification and partial characterization of a typical cadmium-binding polypeptide from Zea mays. Abstr II Int Meet, Met other Low Mol Weight Met Proteins 25

Blindauer CA (2011) Bacterial metallothioneins: past, present, and questions for the future. J Biol Inorg Chem 16:1011–1024

Cid C, Garcia-Descalzo L, Casado-Lafuente V (2010) Proteomic analysis of the response of an acidophilic strain of *Chlamydomonas* sp. (Chlorophyta) to natural metal-rich water. Proteomics 10:2026–2036

Clemens S (2006) Evolution and function of phytochelatin synthases. J Plant Physiol 163:319–332

Cobbett CS (2001) Heavy metal detoxification in plants: phytochelatin biosynthesis and function. IUBMB Life 51:183–188

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cruz BH, Dıaz-Cruz JM, Šestáková I et al (2002) Differential pulse voltammetric study of the complexation of Cd (II) by the phytochelatin (γ-Glu Cys) 2Gly assisted by multivariate curve resolution. J Electroanal Chem 520:111–118

Da Silva JJRF, Williams RJP (2001) The biological chemistry of the elements: the inorganic chemistry of life. Oxford University Press, Oxford

Davis SR, Cousins RJ (2000) Metallothionein expression in animals: a physiological perspective on function. J Nutr 130:1085–1088

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015:7–9

Gaur JP, Rai LC (2001) Heavy metal tolerance in algae. In: Algal adaptation to environmental stresses. Springer, Cham, pp 363–388

Gauthier L, Tison-Rosebery J, Morin S, Mazzella N (2020) Metabolome response to anthropogenic contamination on microalgae: a review. Metabolomics 16:8

Gekeler W, Grill E, Winnacker E-L, Zenk MH (1988) Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch Microbiol 150:197–202

Gekeler W, Grill E, Winnacker EL, Zenk MH (1989) Survey of the plant kingdom for the ability to bind heavy metals through phytochelatins. Z Nat - Sect C J Biosci 44:361–369

Gillet S, Decottignies P, Chardonnet S, Le Maréchal P (2006) Cadmium response and redoxin targets in *Chlamydomonas reinhardtii*: a proteomic approach. Photosynth Res 89:201–211

Gómez-Jacinto V, García-Barrera T, Gómez-Ariza JL et al (2015) Elucidation of the defence mechanism in microalgae *Chlorella sorokiniana* under mercury exposure. Identification of Hg-phytochelatins. Chem Biol Interact 238:82–90

Grill E, Winnacker E-L, Zenk MH (1985) Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230:674–676

Grill E, Gekeler W, Winnacker E-L, Zenk HH (1986) Homo-phytochelatins are heavy metal-binding peptides of homo-glutathione containing Fabales. FEBS Lett 205:47–50

Grill E, Winnacker E-L, Zenk MH (1987) Phytochelatins, a class of heavy-metal-binding peptides from plants, are functionally analogous to metallothioneins. Proc Natl Acad Sci 84:439–443

Gutiérrez J-C, de Francisco P, Amaro F et al (2019) Structural and functional diversity of microbial metallothionein genes. Microb Divers Genomic Era 2019:387–407

Ha S-B, Smith AP, Howden R et al (1999) Phytochelatin synthase genes from *Arabidopsis* and the yeast *Schizosaccharomyces pombe*. Plant Cell 11:1153–1163

Hanikenne M (2005) A comparative inventory of metal transporters in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merolae*. Plant Physiol 137:428–446

Hasan MK, Cheng Y, Kanwar MK et al (2017) Responses of plant proteins to heavy metal stress—a review. Front Plant Sci 8:1–16

Heuillet E, Noreau A, Halpern S et al (1986) Cadmium binding to a thiol-molecule in vacuoles of *Dunaliella bioculata* contaminated with $CdCl_2$= electron probe microanalysis. Biol Cell 58:79–85

Hirata K, Tsujimoto Y, Namba T et al (2001) Strong induction of phytochelatin synthesis by zinc in marine green alga, *Dunaliella tertiolecta*. J Biosci Bioeng 92:24–29

Hirata K, Tsuji N, Miyamoto K (2005) Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. J Biosci Bioeng 100:593–599

Howe G, Merchant S (1992) Heavy metal-activated synthesis of peptides in *Chlamydomonas reinhardtii*. Plant Physiol 98:127–136

Hu S, Lau KWK, Wu M (2001) Cadmium sequestration in *Chlamydomonas reinhardtii*. Plant Sci 161:987–996

Jamers A, Van der Ven K, Moens L et al (2006) Effect of copper exposure on gene expression profiles in *Chlamydomonas reinhardtii* based on microarray analysis. Aquat Toxicol 80:249–260

Jamers A, Blust R, De Coen W (2009) Omics in algae: paving the way for a systems biological understanding of algal stress phenomena? Aquat Toxicol 92:114–121

Jamers A, Blust R, De Coen W et al (2013) An omics based assessment of cadmium toxicity in the green alga *Chlamydomonas reinhardtii*. Aquat Toxicol 126:355–364

Jia H, Wang X, Dou Y et al (2016) Hydrogen sulfide-cysteine cycle system enhances cadmium tolerance through alleviating cadmium-induced oxidative stress and ion toxicity in *Arabidopsis* roots. Sci Rep 6:1–14

Kägi JHR, Kojima Y (1987) Chemistry and biochemistry of metallothionein. In: Metallothionein II. Springer, Cham, pp 25–61

Kalinowska R, Pawlik-Skowrońska B (2010) Response of two terrestrial green microalgae (Chlorophyta, Trebouxiophyceae) isolated from Cu-rich and unpolluted soils to copper stress. Environ Pollut 158:2778–2785

Khatiwada B, Hasan MT, Sun A et al (2020) Proteomic response of *Euglena gracilis* to heavy metal exposure—Identification of key proteins involved in heavy metal tolerance and accumulation. Algal Res 45:101764

Kim YK, Yoo WI, Lee SH, Lee MY (2005) Proteomic analysis of cadmium-induced protein profile alterations from marine alga *Nannochloropsis oculata*. Ecotoxicology 14:589–596

Le Faucheur S, Behra R, Sigg L (2005) Phytochelatin induction, cadmium accumulation, and algal sensitivity to free cadmium ion in *Scenedesmus vacuolatus*. Environ Toxicol Chem Am Int J 24:1731–1737

Levy JL, Angel BM, Stauber JL et al (2008) Uptake and internalisation of copper by three marine microalgae: comparison of copper-sensitive and copper-tolerant species. Aquat Toxicol 89:82–93

Lu J, Ma Y, Xing G et al (2019) Revelation of microalgae's lipid production and resistance mechanism to ultra-high Cd stress by integrated transcriptome and physiochemical analyses. Environ Pollut 250:186–195

Marcano LBC, Carruyo IM, Montiel XM, Morales CB (2009) Effect of cadmium on cellular viability in two species of microalgae (*Scenedesmus* sp. and *Dunaliella viridis*). Springer, Cham, pp 86–93

Margoshes M, Vallee BL (1957) A cadmium protein from equine kidney cortex. J Am Chem Soc 79:4813–4814

Mishra A, Medhi K, Malaviya P, Thakur IS (2019) Omics approaches for microalgal applications: prospects and challenges. Bioresour Technol 291:121890

Monteiro CM, Castro PML, Malcata FX (2012) Metal uptake by microalgae: underlying mechanisms and practical applications. Biotechnol Prog 28:299–311

Morelli E, Scarano G (2001) Synthesis and stability of phytochelatins induced by cadmium and lead in the marine diatom *Phaeodactylum tricornutum*. Mar Environ Res 52:383–395

Morelli E, Mascherpa MC, Scarano G (2005) Biosynthesis of phytochelatins and arsenic accumulation in the marine microalga *Phaeodactylum tricornutum* in response to arsenate exposure. BioMetals 18:587–593

Mutoh N, Hayashi Y (1988) Isolation of mutants of *Schizosaccharomyces pombe* unable to synthesize cadystin, small cadmium-binding peptides. Biochem Biophys Res Commun 151:32–39

Nagel K, Adelmeier U, Voigt J (1996) Subcellular distribution of cadmium in the unicellular green alga *Chlamydomonas reinhardtii*. J Plant Physiol 149:86–90

Nishikawa K, Yamakoshi Y, Uemura I, Tominaga N (2003) Ultrastructural changes in *Chlamydomonas acidophila* (Chlorophyta) induced by heavy metals and polyphosphate metabolism. FEMS Microbiol Ecol 44:253–259

Olafson RW, Mc cubbin WD, Kay CM (1988) Primary-and secondary-structural analysis of a unique prokaryotic metallothionein from a *Synechococcus* sp. cyanobacterium. Biochem J 251:691–699

Olsson S, Puente-Sánchez F, Gómez MJ, Aguilera A (2015) Transcriptional response to copper excess and identification of genes involved in heavy metal tolerance in the extremophilic microalga *Chlamydomonas acidophila*. Extremophiles 19:657–672

Ortiz DF, Kreppel L, Speiser DM et al (1992) Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J 11:3491–3499

Ortiz DF, Ruscitti T, McCue KF, Ow DW (1995) Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J Biol Chem 270:4721–4728

Pawlik-Skowrońska B (2001) Phytochelatin production in freshwater algae *Stigeoclonium* in response to heavy metals contained in mining water; effects of some environmental factors. Aquat Toxicol 52:241–249

Penen F, Isaure MP, Dobritzsch D et al (2017) Pools of cadmium in *Chlamydomonas reinhardtii* revealed by chemical imaging and XAS spectroscopy. Metallomics 9:910–923

Penen F, Isaure MP, Dobritzsch D et al (2019) Pyrenoidal sequestration of cadmium impairs carbon dioxide fixation in a microalga. Plant Cell Environ 43(2):479–495

Perales-Vela HV, Peña-Castro JM, Cañizares-Villanueva RO (2006) Heavy metal detoxification in eukaryotic microalgae. Chemosphere 64:1–10

Perrein-Ettajani H, Amiard JC, Haure J, Renaud C (1999) Effets des métaux (Ag, Cd, Cu) sur la composition biochimique et compartimentation de ces métaux chez deux microalgues Skeletonema costatum et *Tetraselmis suecica*. Can J Fish Aquat Sci 56:1757–1765

Pillai S, Behra R, Nestler H et al (2014) Linking toxicity and adaptive responses across the transcriptome, proteome, and phenotype of *Chlamydomonas reinhardtii* exposed to silver. Proc Natl Acad Sci U S A 111:3490–3495

Priyadarshini E, Priyadarshini SS, Pradhan N (2019) Heavy metal resistance in algae and its application for metal nanoparticle synthesis. Appl Microbiol Biotechnol 103:3297–3316

Puente-sánchez F, Olsson S, Aguilera A (2016) Comparative transcriptomic analysis of the response of *Dunaliella acidophila* (Chlorophyta) to short-Term cadmium and chronic natural metal-rich water exposures. Microb Ecol 72(3):595–607

Puente-sánchez F, Díaz S, Penacho V et al (2018) Basis of genetic adaptation to heavy metal stress in the acidophilic green alga *Chlamydomonas acidophila*. Aquat Toxicol 200:62–72

Rauser WE (1990) Phytochelatins. Annu Rev Biochem 59:61–86

Rauser WE (1995) Phytochelatins and related peptides. Structure, biosynthesis, and function. Plant Physiol 109:1141–1149

Robinson NJ (1989) Algal metallothioneins: secondary metabolites and proteins. J Appl Phycol 1:5–18

Ruttkay-Nedecky B, Nejdl L, Gumulec J et al (2013) The role of metallothionein in oxidative stress. Int J Mol Sci 14:6044–6066

Salama E, Govindwar SP, Khandare RV et al (2019) Can omics approaches improve microalgal biofuels under abiotic stress? Trends Plant Sci 24:611–624

Samadani M, Perreault F, Oukarroum A, Dewez D (2018) Effect of cadmium accumulation on green algae *Chlamydomonas reinhardtii* and acid-tolerant *Chlamydomonas* CPCC 121. Chemosphere 191:174–182

Scarano G, Morelli E (2002) Characterization of cadmium- and lead-phytochelatin complexes formed in a marine microalga in response to metal exposure. BioMetals 15:145–151

Sharma R, Bhardwaj R, Handa N et al (2015) Responses of phytochelatins and metallothioneins in alleviation of heavy metal stress in plants: an overview. Plant Met Interact Emerg Remediat Tech 2015:263–283

Shaw CF, Petering DH, Weber DN, Gingrich DJ (1989) Inorganic studies of the cadmium-binding peptides from *Euglena gracilis*. Met Ion Homeost 1:313–324

Shivaji S, Dro SVL (2019) *Scenedesnus rotundus* isolated from the petroleum effluent employs alternate mechanisms of tolerance to elevated levels of cadmium and zinc. Sci Rep 9(1):8485

Silverberg BA (1976) Cadmium-induced ultrastructural changes in mitochondria of freshwater green algae. Phycologia 15:155–159

Souza PO, Ferreira LR, Pires NRX et al (2012) Algae of economic importance that accumulate cadmium and lead: a review. Braz J Pharmacogn 22:825–837

Steffens JC (1990) The heavy metal-binding peptides of plants. Annu Rev Plant Biol 41:553–575

Stokes PM, Maler T, Riordan JR (1977) A low molecular weight copper binding protein in a copper tolerant strain of *Scenedesmus acutiformis*. Trace Subst Environ Heal 11:146–155

Strejckova A, Dvorak M, Klejdus B et al (2019) The strong reaction of simple phenolic acids during oxidative stress caused by nickel, cadmium and copper in the microalga Scenedesmus quadricauda. New Biotechnol 48:66–75

Suresh Kumar K, Dahms HU, Won EJ et al (2015) Microalgae - a promising tool for heavy metal remediation. Ecotoxicol Environ Saf 113:329–352

Sytar O, Kumar A, Latowski D et al (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35:985–999

Torres MA, Barros MP, Campos SCG et al (2008) Biochemical biomarkers in algae and marine pollution: a review. Ecotoxicol Environ Saf 71:1–15

Tripathi S, Arora N, Gupta P et al (2019) Microalgae: an emerging source for mitigation of heavy metals and their potential implications for biodiesel production. In: Advanced biofuels. Elsevier, London, pp 97–128

Tukaj Z, Baścik-Remisiewicz A, Skowroński T, Tukaj C (2007) Cadmium effect on the growth, photosynthesis, ultrastructure and phytochelatin content of green microalga *Scenedesmus armatus*: a study at low and elevated CO_2 concentration. Environ Exp Bot 60:291–299

Vannini C, Marsoni M, Domingo G et al (2009) Proteomic analysis of chromate-induced modifications in *Pseudokirchneriella subcapitata*. Chemosphere 76:1372–1379

Vatamaniuk OK, Mari S, Lu YP, Rea PA (2000) Mechanism of heavy metal ion activation of phytochelatin (PC) synthase. Blocked thiols are sufficient for PC synthase-catalyzed transpeptidation of glutathione and related thiol peptides. J Biol Chem 275:31451–31459

Vatamaniuk OK, Mari S, Lang A et al (2004) Phytochelatin synthase, a dipeptidyltransferase that undergoes multisite acylation with γ-glutamylcysteine during catalysis: stoichiometric and site-directed mutagenic analysis of *Arabidopsis thaliana* PCS1-catalyzed phytochelatin synthesis. J Biol Chem 279:22449–22460

Walliwalagedara C, Keulen H, Willard B, Wei R (2012) Differential proteome analysis of *Chlamydomonas reinhardtii* response to arsenic exposure. Am J Plant Sci 3:764

Wang Y, Zhang C, Zheng Y, Ge Y (2017) Phytochelatin synthesis in *Dunaliella salina* induced by arsenite and arsenate under various phosphate regimes. Ecotoxicol Environ Saf 136:150–160

Zhang W, Tan NGJ, Li SFY (2014) NMR-based metabolomics and LC-MS/MS quantification reveal metal-specific tolerance and redox homeostasis in *Chlorella vulgaris*. Mol BioSyst 10:149–160

Zhang W, Tan NGJ, Fu B, Li SFY (2015) Metallomics and NMR-based metabolomics of *Chlorella* sp. reveal the synergistic role of copper and cadmium in multi-metal toxicity and oxidative stress. Metallomics 7:426–438

Efficacy of Duckweeds for Phytoremediation: Morpho-Physiological and Biochemical Alterations

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Abstract

Phytoremediation is considered as an advanced approach to uptake heavy metals from polluted water and to remediate the contaminated water and soil mediums using plants. Heavy metals including chromium, nickel, cadmium and lead are highly toxic metals, and their concentration is increasing via anthropogenic activities. The study exhibits the response of duckweed under metal stress on plant growth, biochemical attributes and physiology. Duckweed is one of the most suitable plant species that have local adaptation, plant root depth, detoxify heavy metals, fast growth rate and uptake of high concentrations of heavy metals. In this study, we review that heavy metal-stressed duckweed plants uptake and accumulate heavy metals due to their increased biomass. But due to the accumulation of heavy metals in plants, biochemical attributes such as chlorophyll, carotenoid, antioxidant and electrolyte leakage reduced along with the increased production of reactive oxygen species. The present study compiles the data regarding the effects of heavy metals on duckweed plants and their potential to accumulate the heavy metal in their leaves through roots. Further, it has been found that under the application of organic chelators such as citric acid, ascorbic acid and glutamic acid provides support in the development and growth of the plants parallel to increased heavy metals accumulation. Further studies at genetic and molecular level are required to understand the response of duckweed plants to heavy metals stress.

Keywords

Heavy metals · Duckweed · Physiology · Accumulation · Organic chelators

1 Introduction

Most techniques used to remove heavy metals from the environment are expensive, time taking and not environmentally friendly, but we require such technology that can reduce the burden of contaminants from the environment and is cost effective. For that purpose, phytoremediation is the most advanced and effective technology, which uses macrophytes to decontaminate environment (Jadia and Fulekar 2009; Farid et al. 2017a). Phytoremediation is a widely used technique to remove toxic chemicals from different growing mediums such as water, soil, physical and chemical conditions while using different plants (Wang et al. 2002; Farid et al. 2018a). There are different subgroups such as rhizofiltration, phytoextraction and phytostablization to remove or extract the heavy or toxic metals from the environmental media (Salt et al. 1995; Farid et al. 2017b). In developing countries like India and Pakistan, this technology is very suitable as it is cheap and has the potential to reap results so such plants are used that have the potential to survive and uptake metals from the contaminated medium (Farid et al. 2017c, 2019). These plants are called as hyperaccumulators (Ghosh and Singh 2005). Some heavy metals are essential for the environment, but with human activities their concentration increases and interact with plants animals and humans and disturb their normal functions causing abnormalities. Their natural path and concentration get increased (Bánfalvi 2011; Farid et al. 2017c). Most of the heavy metals released by industries into the environmental mediums cause water, air and soil pollution. Hexavalent chromium affects the plant growth, seed germination and photosynthetic process of plant and alters leaf profile (Zaheer et al. 2015). One of the most toxic elements for the plants and crops is nickel (Ni), and its concentration varies with soil solution so does its toxicity and its impacts on plant physiology (Hunter and Vergnano 1953). Phytoremediation is regarded as a new concept to remediate the environment by the use of plants as it is cheap, environment friendly and sustainable process to remove the contaminants from the environmental mediums. The most suitable plants in phytoremediation are aquatic plants as they can remove heavy metals from wastewater by up-taking them and no special environmental conditions are required (Singh et al. 2012; Sallah-Ud-Din et al. 2017). However aquatic plants have the potential or ability to accumulate heavy metals, but the level of uptake varies with each species. Various biological, physical and chemical processes take place during phytoremediation for the removal of heavy metals from water. To uptake heavy metals from the polluted water, various species of aquatic plants are introduced such as *Lemna minor* L. and microspora in the recent past. At certain level, *L. minor* has great potential to accumulate heavy metals and is easily available in brackish water (Sallah-Ud-Din et al. 2017). So due to its local availability, it is cost effective and widely used to extract heavy metals from polluted sites using phytoremediation (Hou et al. 2007).

Phytoremediation is considered as an advanced approach to remediate the contaminated water and soil mediums using plants. Hyperaccumulator plants are widely used to detoxify the polluted water and soil (Rizwan et al. 2017b). The purpose of phytoremediation is to eradicate or transport heavy metals from the water or soil to metal hyperaccumulator plants (Farid et al. 2020a). Phyto means light and the word remediation means able to cure the plants so the definition would be to cure plants by giving them light. It can also give us an idea of using plants to remediate the polluted water. Phytoremediation is a technique to use those plants that are capable enough to accumulate or uptake heavy metals making the environment less toxic (Farid et al. 2019). Generally, phytoremediation is based on phytoextraction, phytoaccumulation, phytofiltration, and phytovolatilization of heavy metals and is applied to a variety of organic and inorganic pollutants (Vamerali et al. 2010). Plants that are used in phytoremediation are used to degrade and break down the contaminant that helps the heavy metals present in wastewater to stabilize. New technology widely known as phytoremediation is used to remediate contaminated sites from organic pollutants. This technique is widely recommended for its low budget and sustainable energy benefits as it is economically feasible and more suitable for the environment. It also helped in reducing the transportation of contaminants from one media to another (Tangahu et al. 2011). Hyperaccumulator plants are naturally available in the environment and have high metal accumulation or transport capacity. These plants accumulate hundred times more than non-accumulators (Wuana and Okieimen 2011). This technique of phytoremediation is used at large scale to remediate the heavy metals from the contaminated site. Such techniques are less damaging to the environment as the process is carried out at topsoil and the remaining soil remains unaffected and is further used for agricultural purposes. Thus, phytoremediation is the best possible option as it does not damage the soil structure and even remove toxic metals, thus keeping the nutritional value of soil intact (Tangahu et al. 2011; Farid et al. 2018b).

Phytoremediation is applied to remove the toxic metals from environmental mediums like soil and water as plants accumulate or accept the heavy metals, thus remediating the environment. For this purpose, aquatic plants are used to remove the high concentration of heavy metals from the environment (Farid et al. 2015, 2019). Aquatic plants are easily preserved and low budgeted and store heavy metals in their body and still manage to survive. Duckweed is mostly present in ponds or marshy areas, and it is the free-floating plant that stays at the surface of the water. Because of its high potential to uptake or accumulate heavy metals, it has gained much importance. Almost 90% of lead can be extracted from wastewater. Duckweed increases in size if concentration of ammonia increases. It can grow at pH 6–9, and tolerance level ranges between 5 and 6 (Caicedo van der Steen et al. 2000; Singh et al. 2012).

2 Taxonomy

The present era of "resurgence of duckweed research and applications" (Lam et al. 2014; Zhao et al. 2012; Appenroth et al. 2017) is based on their practical application to remove, transfer, and uptake heavy metals. Lemnaceae comprises one of the fastest growing angiosperms (Sree et al. 2015b, 2016; Ziegler et al. 2015). In the past duckweed was used as human diet because of its high protein content in many parts of Asia (Bhanthumnavin and McGarry 1971; Cheng and Stomp 2009; Van der Spiegel et al. 2013; Appenroth et al. 1982). Due to their high protein content, they can replace soya bean products as an alternative. As exhibited by various research projects, duckweeds can be proved beneficial if given to domestic animals, e.g. sheep, cattle, rabbits, horses, waterfowls, fish and poultry (Van Dyke and Sutton 1977; Muztar et al. 1979; Landolt and Kandeler 1987; Hassan and Edwards 1992; Cheng and Stomp 2009; Anderson et al. 2011). Duckweed can also produce high starch content and can be used in biogas and biofuel plants, if suitable conditions are provided (Jain et al. 1992; Sree and Appenroth 2014; Su et al. 2014; Sree et al. 2015a; Cui and Cheng 2015). This would help in providing substitute for food crops that consume arable land and would be a cheap source for biogas plants. Duckweed plants are characterized by 37 species, and this is taken as new crop plants (Appenroth et al. 2013).

3 Heavy Metals Accumulation by *Lemna minor*

The most suitable plant species must have local adaptation, such as plant root depth, ability to toxic removal or detoxify heavy metals, fast growth rate and uptake large amounts of water for evapotranspiration (Ashraf 2010). Care should be taken in case of selection of plants because it must be considered that selected species is native to that place or not (Gordon 2003). Aquatic plant genera are known to gather metals from their surroundings, and it is well recognized that wetland species has been used for the phytoremediation of wastewater successfully. Aquatic blossoming florae can eliminate numerous metals from water including *Eichhornia crassipes* (water hyacinth), *Hydrocotyle umbellata* L. (pennywort) and *L. minor* (duckweed). The great water portion of aquatic flowers also confuses recollect of metals through burning. Many terrestrial plants roots grown hydroponically were recognized as very effective in captivating, engaged or precipitating toxic metals from contaminated wastewaters. This procedure was called rhizo-filtration. For the removal of Pb, some aquatic plant species are effectively used such as Duckweed (*L. minor*), Water hyacinth (*Eichhornia crassipes*) and Hydrilla (*Hydrilla verticillata*). Phytoextraction is effectively done if solubility and accessibility of metal in soil for root utilization are acquired. The bio-accessibility of metal mostly relies on soil properties like the ability of interchange negative ions, pipestone material, pH and soil biological stuff (Hou and Zhang 2007; Hou et al. 2007).

Extraction of metals and chemical contaminants from the soil by transferring of the contaminants into extractable parts of the plant, with the use of plants, is referred to as phyto-extraction. Preferably the hyperaccumulator plants with high mass are used in phytoremediation. These are the plants having potential to collect the huge portion of heavy metals in their above the surface parts unaccompanied by the pernicious impacts of heavy metals (Kamal et al. 2004). Involving different types of sunflower (for certain metals) and hydrangea (for aluminium [Al]), about 400 plant groups from 45 genera had been described as hyperaccumulators (Axtell et al. 2003). Aquatic macrophytes, among all the numerous plant species, gain greatest attention for phytoremediation systems. It is because of their accumulating capability of heavy metals up to 100,000 times larger than the amount of heavy metals present in wastewater. So, these types of macrophytes have been used for the treatment of heavy metals from different sources (Rai 2008).

Lemna minor consists of several small and free-floating plants on the upper surface of the water. The growth rate of *L. minor* is high and easily settled in every aquatic environment. It includes the plant family of Lemnaceae that are free-float and flowering plant. Duckmeat (*Spirodela polyrhiza*) and watermeal (*Wolffia*) are widely scattered on the ponds and used for the treatment of wastewater. These plant species are also a source of food for some animals that live in the aquatic environment (Krull 1970). *L. minor* grows very densely in nutrient-rich environment and forms a thick layer over each other. In aquatic systems, *L. minor* has been directly and indirectly affecting the properties of the wastewater. Duckweed is mostly used in phytoremediation for the phytoextraction of heavy metals from metal polluted sites.

Duckweed grows at an extensive range of temperature from 6 to 34 °C. Mostly, *L. minor* is found on municipal wastewater ponds (Sallah-ud-Din et al. 2017). *L. minor* has a great potential to accumulate heavy metals from the wastewater. It can remove about 90% of lead from the wastewater ponds. Duckweed grows under pH 6–9 and also has the capacity to tolerate a low pH level. With the increase in the ammonia concentration, the rate of growth is progressively increased (Akhtar et al. 2017). From some experimental results, it is shown that duckweeds have the potential to remove soluble Pb under various temperature ranges from 15 to 35 °C and under various pH level ranging from 5 to 8 under different Pb concentrations. It is reported that lead concentration was highest at pH 5 and maximum accumulation done less than 30 °C temperature (Gallardo et al. 1999).

4 Chemical Composition

With nutritional and environmental requirements met, duckweed plants grow very fast and can flourish for long (Cheng et al. 2002). Anyhow, growth rates of duckweed colonies will be reduced by a variety of problems: such as nutrient scarcity; toxins; higher levels of pH and temperature; overflown by overgrowth of the colony and competition from other plants for light and nutrients (Leng et al. 1995). There are many factors that affect the growth and composition of the plant. The levels of available nutrients, and species differences, can heavily influence both the quantity and quality of produced material. These differences may be interpreted in light of the existence of deficiency, optimal and toxic levels for nutrients. Little interest is shown in recent times in building an optimum nutrient range for growth of duckweed despite inconsistencies in published literature. Results from these studies showed that duckweed growth is affected by other populations primarily via nitrogen limits and increased pH. According to Leng et al. (1995) as a generalization, growth of duckweed is controlled by sunlight and temperature more than nutrient application in the water. Duckweeds can grow faster at high temperatures to trace quantity of P and N nutrients in water. Also, according to Culley et al. (1981), the reproduction and growth of duckweed are mostly influenced by the availability of macronutrients such as nitrogen, potassium and phosphorus in addition to micronutrients, light, wave action, temperature and density of plant can also tolerate pH from 3 to 10 with an optimum range of 5–7.

5 Ecological Consideration

Lemna minor has great ecological consideration due to its ability to withstand desiccation, quickly populate a new habitat and inhibit the other species competitively. In contrast to most plants, duckweeds can tolerate high concentrations of salts (up to 4000 mg/l total dissolved solids). Nutrients are equally mixed on the surface of duckweed leaf. Duckweed best grows over the pH 6.5–7.5 but can survive from pH 5 to 9 range. As a generalization, growth of duckweed is regulated by sunlight and temperature more than nutrient applications in the water. To find the nutrients like P and N, duckweeds grow faster at comparatively high temperature. Duckweed species have the ability to survive in severe conditions. Their growth rate, however, is highly dependent on the nutrient balances in the water. They can sustain and revive from nutrient loadings, higher temperatures, pH and nutrient balance. However, for duckweed to survive, four factors should be considered.

For the proper handling of crop, basic research is required that gives complete information about the life cycle of crops, when to fertilize, harvest and buffer, and which nutrients are required for the healthy growth. Strategic handling should be aimed at keeping a proper and dense cover of duckweed, a pH of 6–7 and low dissolved oxygen. In results the algal growth is being suppressed by absolute crop cover that used to minimize production of CO_2 by algal respiration and further stop its effect on pH.

An application of nutrients along with waste organic material can be given to duckweed plants. The lowest budgeted and efficient sources are wastewater pollutants from food processing plants, extensively high poultry production, cattle feedlots and homes (Ahmad et al. 2020). Solid materials like food processing wastes, manure from livestock or night soil from rural areas can be added to a pond at certain levels by mixing it with water (Jabeen et al. 2016). For initial treatment, all wastewater containing night soil or manure has to go in an anaerobic pond for a few days, before applying it to fertilize duckweed. Reduction of solids and prevention of a floating mat are required when such type of nutrients is supplied.

6 Biochemical Attributes

All plants grown under different HMs stress show reduced chlorphyll contents. With the increased level of HMs concentration, the plants' total chlorophyll content can be reduced due to the accumulation of toxic HMs. With the increased level of concentration of HMs, the total chlorophyll content of the plants decreases. The addition of citric acid along with lead concentration may promote the growth and development of plants. Citric acid provides support for the development and growth of the plants.

In all plants, the carotenoid content decreased with the addition of HMS at different concentrations. Higher the concentration of HMs, the lower will be the carotenoid content of the plants that are applied with only HMs. With the rise of HMs concentration, the plant carotenoid content can be reduced due to the accumulation of toxic HMs (Rizwan et al. 2017a). With the increasing concentration of HMs, the carotenoid content of the plants is decreased. The addition of citric acid along with HMs concentration may promote the growth and development of plants. Citric acid provides support for the development and growth of the plants (Ehsan et al. 2014; Farid et al. 2019).

In all plant samples four basic antioxidant enzymes such as catalysis (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and peroxidases (POD) were observed for the estimation of the HMs impact along with the addition of citric acid on the antioxidant potential of *L. minor*. In these plants, the concentration of

HMs increases the electrical conductivity of plants that affects their development cycle. As the HMs concentration increased, the electrical conductivity is also increased. The plant samples that are applied with greater concentration of HMs were affected more and show a higher electrical conductivity stress. The controlled treatment has low oxidative stress as compared to all other samples because control system is not applied with any metal and acid concentration. Addition of citric acid along with lead treatment reduced the electrical conductivity (Habiba et al. 2015; Farid et al. 2018a, 2019).

The malondialdehyde stress increases with the increasing concentration of HMs into the plant samples due to the toxicity of HMs. The plant samples that are applied with greater concentration of HMs were affected more and show higher malondialdehyde stress. The control treatment has low oxidative stress as a comparison to all other samples because control system is not applied with any metal and acid concentration. The plant samples that were applied with HMs concentration along with citric acid have low effect because of the exogenous effect of citric acid on malondialdehyde (Shakoor et al. 2014; Farid et al. 2020b).

7 Significance of Duckweed Plant

For hundreds of years, duckweed has been used in Asian primary production systems to produce animal feed (Leng 1999). It has around 40 species worldwide, and the major ones are of the genus *Lemna*, *Wolffia*, *Wolffiella* and *Spirodela* (Les et al. 2002). The plant is rich in both macro- and micro-elements such as calcium and chlorine and has a protein content that ranges between 6.8% and 45.0% DM (Landolt and Kandeler 1987) but has been recognized by American researchers as ideally suited for heavy metal uptake. Rates of accumulation of heavy metals differ markedly between species, as does the relationship between concentration (in water) and take-up—in some cases it is direct, while in others reciprocal (Landolt and Kandeler 1987).

Duckweed plant species are recognized all over the world because of its potential to bear heavy metals (Radić et al. 2010). Promising nominees for this method are *L. minor*, *Spirodela polyrhiza* and *Lemna gibba* (Axtell et al. 2003; Charles et al. 2006; Zhang et al. 2011). *L. minor* (duckweed) can be highly helpful in phytoremediation as it can accumulate heavy metals, fast growth, cold-bearing potential and easy to harvest (Sharma and Gaur 1995; Radić et al. 2010). Plants of duckweed that are grown in freshwater exhibit low nutritional values than in domestic wastewater. In few countries *Lemna gibba* is imported and used as food for hens as a replacement of soya and fish because it is rich in protein.

8 Relationship Between Duckweed and Heavy Metals

Lemna minor commonly named as duckweed is a high accumulator plant that has potential to accumulate and degrade higher concentration level of metals when added in the watery solution (Axtell et al. 2003). The fresh weight of plant reduces due to the heavy metal concentration and accumulation and lowers the nutrient uptake in plants under heavy metal stress (Khaliq et al. 2016). The additional citric acid expressively enhances the heavy metal accumulation by *Brassica napus* (Afshan et al. 2015). It can also enhance the cadmium accumulation by stonecrops (Huang et al. 2013). The reliable application of citric acid makes it bio-available in soil and in hydroponic media (Shakoor et al. 2014). *L. minor* is a naturally occurring free drifting plant on the marshlands and swamps, formerly studied for its ability to uptake Cr, Cu, Cd, Pb and Ni grown hydroponically (Adrees et al. 2015; Farid et al. 2017c, 2018a; Sallah-Ud-Din et al. 2017; Rizwan et al. 2017a; Ahmad et al. 2020). The concentration of HMs at different levels severely affects the growth and development of plants. The addition of citric acid increased the uptake capacity on the increasing concentration of HMs in *L. minor*.

Distribution of heavy metals in entire plant tissue is a very major characteristic, and it is very useful for the suggestion of decontamination mechanisms. The role of *L.* minor is also recently investigated to know the potential of this plant for phytoextraction (Bokhari et al. 2016). In this study, results are compiled with the recent work of *L. minor* that plays a significant part in the phytoextraction of heavy metals in aqueous media.

One of the best-known plants to accumulate heavy metals are *Lemna* plants. So, the plants have the ability to treat diverse waste systems either it is industrial leachate or muncipal wastewater. The average life of *L. minor* is almost 5–6 weeks with a production rate of 0.45 fronds each day and its mass doubled in 2–3 days (Isaksson et al. 2007). Because of its faster growth, it can be the best possible solution for phytoremediation. The solubility and movement of heavy metals must be verified to ensure their availability in the environment.

9 Different Heavy Metals Stress on Physiology and Biochemical Attribute of Duckweed

The fresh weight, dry weight, root length and leaf area were investigated and highly observed that HMs increasing concentration reduced the growth of the whole plant. *L. minor* is best known for the HMs uptake from contaminated medium. The fresh weight of *L. minor* decreased with the increasing concentration of HMs. When HMs accumulate in the parts of a plant, it reduces the fresh weight of plant by disturbing its growth and development. Afterwards, the weight of *L. minor* increases with the addition of citric acid. Citric acid increases the growth and development of plants by giving simulative support to the plants. The dry weight of all plant decreases with the increase in the HMs concentration. The dry weight of all plants that are treated with the addition of citric acid along with other HMs concentration has more weight as compared to those that are only treated with different HMs. Due to the exogenous application of citric acid, the dry weight of plant increases with citric acid and HMs (Rizwan et al. 2017b).

Some other plant gorwth and health attributes such as electrolyte leakage, chlorophyll a, b, total and carotenoids have also been observed in various studies. It has been addressed in previous studies that the heavy metal accumulation and uptake navigates the growth and biomass of the plant by ending the mineral endorsement, which disturbs the metabolic processes of the plants (Gill et al. 2015). The stress of heavy metals is the main reason for the production of reactive oxygen species, which damaged the development and affected the growth of plants (Das et al. 2014). Addition of citric acid along with HMs treatment expressively increases the fresh and dry weight of plants along with the growth of *L. minor*, which promote it as a supportive character under HMs stress. According to the recent studies, citric acid plays an important role in nutrient uptake under heavy metal accumulation (Najeeb et al. 2011).

The leaf colour of *L. minor* plants was badly affected due to lead stress in aqueous media. Due to the treatment of HMs at different concentrations, the greenish colour of all plant reduced to pale yellowish colour. Addition of citric acid along with HM treatment recovered the growth and photosynthetic pigments of plants and reduced the effect of lead on them with its exogenous applications. The leaf area of all plants is gradually reduced under heavy metal stress. When treatment of different heavy metals is applied at different concentration level, the leaf area of plants is reduced. Greater the concentration of HMs, the lower will be the leaf area. Addition of citric acid with HMs treatment supports the growth and development of plant and increases the leaf area of plants as compared to the plants that are only treated with HMs.

10 Source for Domestic Animals

Duckweed has gained a lot of attention as a food source for ruminants, fowl, fish and humans around the globe, especially in developing nations (Iqbal 1999). This has been due to its high protein content, quality of high protein, yield of protein per growing area and low fibre (Cheng and Stomp 2009). Use of duckweed in animal diets as exclusive additional feed is widely reported.) Reeve and Black (1998) carried out feeding trials on poultry and showed enhanced layer performance and quality of eggs in chickens and ducks and no harmful effects, and as a result the animals consumed the duckweed willingly and it was very helpful to their growth. Studies regarding the utilization of duckweek plant as broiler feed has also being carried out by several authors at different levels. In broiler chickens the potential nutritional value of duckweed has been known (Haustein et al. 1994).

A prime limitation to fish farming is that meals are of high biological value with high protein content that are high priced and mostly unavailable. Duckweeds that grow on water with 10–30 mg NH_3-N/L mostly have a high biological value with high protein content (Hillman and Culley 1978). Fresh duckweed is highly recommended for widespread fish farming systems that is used to remove waste by water exchange (Gaigher et al. 1984), and duckweed is efficiently converted to certain fish including carp and tilapia, i.e. the live weight (Van Dyke and Sutton 1977; Hepher and Pruginin 1979; Robinette et al. 1980; Hassan and Edwards 1992).

11 Conclusions

The present review showed that the application of citric acid along with heavy metals stress helped plants to produce more biomass and little damage to biological attributes. At heavy metal concentration without addition of chelates such as citric acid, the growth of duckweed was significantly reduced due to low nutrient availability. The biological attributes such as chlorophyll content, antioxidants and physiological characteristics were also negatively affected. However, as duckweed is locally available and a low budgeted plant it helps a lot in treating wastewater naturally.

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References

Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum MF, Irshad MK (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotoxicol Environ Saf 119:186–197

Afshan S, Ali S, Bharwana SA, Rizwan M, Farid M, Abbas F, Abbasi GH (2015) Citric acid enhances the phytoextraction of chromium, plant growth, and photosynthesis by alleviating the oxidative damages in *Brassica napus* L. Environ Sci Pollut Res 22(15):11679–11689

Ahmad R, Ali S, Rizwan M, Dawood M, Farid M, Hussain A, Wijayae L, Alyemenie MN, Ahmad P (2020) Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. Physiol Planta 168(2):289–300

Akhtar ABT, Yasar A, Ali R, Irfan R (2017) Phytoremediation using aquatic macrophytes. In: Ansari A, Gill S, Gill RR, Lanza G, Newman L (eds) Phytoremediation. Springer, Cham, pp 259–276

Anderson KE, Lowman Z, Stomp AM, Chang J (2011) Duckweed as a feed ingredient in laying hen diets and its effect on egg production and composition. Int J Poult Sci 10(1):4–7

Appenroth KJ, Augsten H, Liebermann B, Feist H (1982) Effects of light quality on amino acid composition of proteins in Wolffia arrhiza (L.) Wimm. using a specially modified Bradford method. Biochemie und Physiol der Pflanzen 177(3):251–258

Appenroth KJ, Palharini L, Ziegler P (2013) Low-molecular weight carbohydrates modulate dormancy and are required for post-germination growth in turions of Spirodela polyrhiza. Plant Biol 15(2):284–291

Appenroth KJ, Sree KS, Böhm V, Hammann S, Vetter W, Leiterer M, Jahreis G (2017) Nutritional value of duckweeds (*Lemnaceae*) as human food. Food Chem 217:266–273

Ashraf M (2010) Inducing drought tolerance in plants: recent advances. Biotechnol Adv 28 (1):169–183

Axtell NR, Sternberg SP, Claussen K (2003) Lead and nickel removal using *Microspora* and *Lemna minor*. Biores Technol 89(1):41–48

Bhanthumnavin K, Mcgarry MG (1971) Wolffia arrhiza as a possible source of inexpensive protein. Nature 232(5311):495–495

Bánfalvi G (2011) Heavy metals, trace elements and their cellular effects. In Cellular effects of heavy metals. Springer, Dordrecht, pp 3–28

Bokhari SH, Ahmad I, Mahmood-Ul-Hassan M, Mohammad A (2016) Phytoremediation potential of *Lemna minor* L. for heavy metals. Int J Phytorem 18(1):25–32

Caicedo van der Steen NP, Arce O, Gijzen HJ (2000) Effect of total ammonium nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). Wat Sci Tech 15:3829–3835

Charles AL, Markich SJ, Ralph P (2006) Toxicity of uranium and copper individually, and in combination, to a tropical freshwater macrophyte (*Lemna aequinoctialis*). Chemosphere 62 (8):1224–1233

Cheng JJ, Stomp AM (2009) Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. Clean–Soil Air Water 37(1):17–26

Cheng J, Landesman L, Bergmann BA, Classen JJ, Howard JW, Yamamoto YT (2002) Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. Trans ASAE 45(4):1003

Cui W, Cheng JJ (2015) Growing duckweed for biofuel production: a review. Plant Biol 17:16–23

Culley DD, Rejmankova E, Kvet J, Frye JB (1981) Production, Chemical quality and Use of duckweeds (*Lemnaceae*) in Aquaculture, Waste management, and animal feeds. J World Maricul Soc 12(2):27–49

Das BC, Panda A, Sahoo PK, Jena S, Padhi P (2014) Effect of chromium (VI) on wheat seedlings and the role of chelating agents. Current Sci 106(10):1387–1393

Ehsan S, Ali S, Noureen S, Mehmood K, Farid M, Ishaque W, Shakoor MB, Rizwan M (2014) Citric acid assisted phytoremediation of Cd by *Brassica napus* L. Ecotoxicol Environ Saf 106:164–172

Farid M, Ali S, Ishaque W, Shakoor MB, Niazi NK, Bibi I, Dawood M, Gill RA, Abbas F (2015) Exogenous application of EDTA enhanced phytoremediation of cadmium by Brassica napus L. Int J Environ Sci Technol 12(12):3981–3992

Farid M, Ali S, Rizwan M, Ali Q, Abbas F, Bukhari SAH, Saeed R, Wu L (2017a) Citric acid assisted phytoextraction of chromium by Sunflower; morpho-physiological and biochemical alterations in plants. Ecotoxicol Environ Saf 145:90–102

Farid M, Ali S, Rizwan M, Saeed R, Tauqeer HM, Sallah-Ud-Din R, Azam A, Raza N (2017b) Microwave irradiation and citric acid assisted seed germination and phytoextraction of Nickel (Ni) by *Brassica napus* L.; morpho-physiological and biochemical alterations under Ni stress. Environ Sci Pollut Res 24(25):2150–2164

Farid M, Ali S, Akram NA, Rizwan M, Abbas F, Bukhari SAH, Saeed R (2017c) Phytomanagement of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. Environ Sci Pollut Res 24(20):16845–16859

Farid M, Ali S, Zubair M, Saeed R, Rizwan M, Sallah-Ud-Din R, Azam A, Ashraf R, Ashraf W (2018a) Glutamic acid assisted phyto-management of silver contaminated soils through sunflower; physiological and biochemical response. Environ Sci Pollut Res 25(25):25390–25400

Farid M, Ali S, Rizwan M, Ali Q, Saeed R, Nasir T, Abbasi GH, Rehmani MIA, Ata-Ul-Karim ST, Bukhari SAH (2018b) Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. Ecotoxicol Environ Saf 151(30):255–265

Farid M, Ali S, Saeed R, Rizwan M, Ali B, Azam A, Ashraf Hussain A, Ahmad I (2019) Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. Int J Phytorem. 21(8):760–767

Farid M, Ali S, Rizwan M, Yasmeen T, Arif MS, Riaz M, Saqib M, Zia ur Rehman M, Ayub MA (2020a) Combined effects of citric acid and 5-aminolevulinic acid in mitigating chromium toxicity in sunflower (*Helianthus annuus* L.) grown in Cr spiked soil. Pak J Agri Sci 57(3):1–11

Farid M, Farid S, Zubair M, Rizwan M, Ishaq HK, Ali S, Ashraf U, Alhaithloul HAS, Gowayed S, Soliman MH (2020b) Efficacy of *Zea mays* L. for the management of marble effluent contaminated soil under citric acid amendment; morpho-physiological and biochemical response. Chemosphere 240:124930

Gaigher IG, Porath D, Granoth G (1984) Evaluation of duckweed (*Lemna gibba*) as feed for tilapia (*Oreochromis niloticus* cross *Oreochromis aureus*) in a recirculating unit. Aquaculture 41:235–244

Gallardo MT, Benson RF, Martin DF (1999) Lead accumulation by three aquatic plants. Symposia papers presented before the division of Environmental Chemistry. Am Chem Soc 39(2):46–47

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of it's by products. Asian J Energy Environ 6(4):18

Gill RA, Zang L, Ali B, Farooq MA, Cui P, Yang S, Zhou W (2015) Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. Chemosphere 120:154–164

Gordon S (2003) Alternative activation of macrophages. Nature Rev Immunol 3(1):23

Habiba U, Ali S, Farid M, Shakoor MB, Rizwan M, Ibrahim M, Abbasi GH, Hayat T, Ali B (2015) EDTA enhanced plant growth, antioxidant defense system and phytoextraction of copper by *Brassica napus* L. Environ Sci Pollut Res 22(2):1534–1544

Hassan MS, Edwards P (1992) Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrhiza*) as feed for Nile Tilapia (*Oreochromis niloticus*). Aquacult 104:315–326

Haustein AT, Gillman RH, Skillicorn PW, Hannan H, Dias F, Guevana V, Vergara V, Gastanaduy A, Gillman JB (1994) Performance of broiler chickens fed diets containing duckweed (Lemna gibba). J Agric Sci 122(2):288–289

Hepher B, Pruginin Y (1979) Guide to fish culture in Israel. 4. Fertilisation, manuring and feeding. Foreign Training Dept., Israel. 61

Hillman WS, Culley DD (1978) The uses of duckweed. Am Sci 66:442–451

Hou X, Zhang L (2007) Saliency detection: a spectral residual approach. In 2007 IEEE conference on computer vision and pattern recognition, Minneapolis, MN, USA, 17–22 June, pp 1–8

Hou W, Chen X, Song G, Wang Q, Chang CC (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*). Plant Physiol Biochem 45 (1):62–69

Huang L, Lu Y, Gao X, Du G, Ma X, Liu M, Chen Y (2013). Ammonium-induced oxidative stress on plant growth and antioxidative response of duckweed (*Lemna minor* L.). Ecol Eng 58:355–362

Hunter JG, Vergnano O (1953) Trace-element toxicities in oat plants. Annal Appl Biol 40 (4):761–777

Iqbal S (1999) Duckweed aquaculture: potentials, possibilities and limitations for combined waste water treatment and animal feed production in developing countries. SUNDEC Report No. 6/99

Isaksson R, Balogh SJ, Farris MA (2007) Accumulation of mercury by the aquatic plant *Lemna minor*. Int J Environ Stud 64(2):189–194

Jabeen N, Abbas Z, Iqbal M, Rizwan M, Jabbar A, Farid M, Ali S, Ibrahim M, Abbas F (2016) Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. Arch Agr Soil Sci 62(5):648–652

Jadia CD, Fulekar MH (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8(6):2009

Jain SK, Gujral GS, Jha NK, Vasudevan P (1992) Production of biogas from Azolla pinnata R.Br and Lemna minor L.: Effect of heavy metal contamination. Biores Tech 41(3):273–277

Kamal M, Ghaly AE, Mahmoud N, Cote R (2004) Phytoaccumulation of heavy metals by aquatic plants. Environ Int 29(8):1029–1039

Khaliq A, Ali S, Hameed A, Farooq MA, Farid M, Shakoor MB, Rizwan M (2016) Silicon alleviates nickel toxicity in cotton seedlings through enhancing growth, photosynthesis, and suppressing Ni uptake and oxidative stress. Arch Agro Soil Sci 62(5):633–647

Krull JN (1970) Aquatic plant-macroinvertebrate associations and waterflow. J Wildlife Manage 34 (4):707–718

Landolt E, Kandeler R (1987) Biosystematics investigations in the family of Duckweeds (Lamnaceae), vol 2. Veroff Geobot Institute ETH Zurich, Switzerland, pp 42–43

Lam E, Appenroth KJ, Michael T, Mori K, Fakhoorian T (2014) Duckweed in bloom: the 2nd International Conference on Duckweed Research and Applications heralds the return of a plant model for plant biology. Plant Mol Bio 84(6):737–742

Leng RA (1999) Duckweed: A tiny aquatic plant with enormous potential for agriculture and environment. Animal Production and Health Division, University of Tropical Agriculture Foundation, Phnom Penh (Combodia). FAO Rome (Italy), p 108

Leng RA, Stambolie JH, Bell R (1995) Duckweed - a potential high protein feed resource for domestic animals and fish. Livest Res Rural Develop 7(1),Corpus ID: 56119880

Les DH, Crawford DJ, Landolt E, Gabel JD, Kimball RT (2002) Phylogeny and systematics of *Lemnaceae*, the duckweed family. Syst Bot 27:221–240

Muztar AJ, Slinger SJ, Burton JH (1979) Metabolizable energy content of freshwater plants in chicken and ducks. Poult Sci 56:1893–1899

Najeeb U, Jilani G, Ali S, Sarwar M, Xu L, Zhou W (2011) Insights into cadmium induced physiological and ultra-structural disorders in *Juncus effusus* L. and its remediation through exogenous citric acid. J Hazard Mater 186(1):565–574

Radić S, Stipaničev D, Cvjetko P, Mikelić IL, Rajčić MM, Širac S, Pavlica M (2010) Ecotoxicological assessment of industrial effluent using duckweed (*Lemna minor* L.) as a test organism. Ecotoxicol 19(1):216

Rai PK (2008) Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an eco-sustainable approach. Int J Phytoremed 10(2):133–160

Reeve IJ, Black AW (1998) Improving Farmers' Management Practices: Through Learning and Group Participation: a Report for the Rural Industries Research and Development Corporation. RIRDC.

Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017a) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere 187:35–42

Rizwan M, Ali S, Qayyum MF, Ok YS, Adress M, Ibrahim M, Zia-ur-Reham M, Farid M, Abbas F (2017b) Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. J Hazard Mater 322:2–16. https://doi.org/10.1016/j.jhazmat.2016.05.061

Robinette HR, Brunson MW, Day EJ (1980) Use of duckweed in diets of channel catfish. Proceedings of the 13th annual conference SE Association. Fish Wildlife Age. pp 108–114

Sallah-Ud-Din R, Farid M, Saeed R, Ali S, Rizwan M, Tauqeer HM, Bukhari SAH (2017) Citric acid enhanced the antioxidant defense system and chromium uptake by *Lemna minor* L. grown in hydroponics under Cr stress. Environ Sci Pollut Res 24(21):17669–17678. https://doi.org/10.1007/s11356-017-9290-0

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol 109(4):1427–1433

Shakoor MB, Ali S, Hameed A, Farid M, Hussain S, Yasmeen T, Abbasi GH (2014) Citric acid improves lead (Pb) phytoextraction in *Brassica napus* L. by mitigating Pb-induced morphological and biochemical damages. Ecotoxicol Environ Saf 109:38–47

Sharma SS, Gaur JP (1995) Potential of *Lemna polyrrhiza* for removal of heavy metals. Ecologic Eng 4(1):37–43

Singh D, Tiwari A, Gupta R (2012) Phytoremediation of lead from wastewater using aquatic plants. J Agric Technol 8(1):1–11

Sree KS, Appenroth KJ (2014) Increase of starch accumulation in the duckweed *Lemna minor* under abiotic stress. Albanian J Agri Sci 11

Sree KS, Adelmann K, Garcia C, Lam E, Appenroth K-J (2015a) Natural variance in salt tolerance and induction of starch accumulation in duckweeds. Planta 241(6):1395–1404

Sree KS, Sudakaran S, Appenroth KJ (2015b) How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus Wolffia (*Lemnaceae*). Acta Physiol Planta 37(10):204

Sree KS, Bog M, Appenroth KJ (2016) Taxonomy of duckweeds (*Lemnaceae*), potential new crop plants. Emirat J Food Agric 28(5):291–302

Su H, Zhao Y, Jiang J, Lu Q, Li Q, Luo Y, Zhao H, Wang M (2014) Use of duckweed (Landoltia punctata) as a fermentation substrate for the production of higher alcohols as biofuels. Energy & Fuels 28(5):3206–3216

Tangahu BV, Abdullah S, Rozaimah S, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng 939161:1–31. https://doi.org/10.1155/2011/939161

Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Environ Chem Lett 8(1):1–17

Van der Spiegel M, Noordam MY, Van der Fels-Klerx HJ (2013) Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Comprehen Rev Food Sci Food Saf 12(6):662–678

Van Dyke JM, Sutton DL (1977) Digestion of duckweed (*Lemna spp.*) by the grass carp (*Ctenopharyngolon edella*). J Fish Biol 11:273–278

Wang Q, Cui Y, Dong Y (2002) Phytoremediation of polluted waters potentials and prospects of wetland plants. Acta Biotechnol 22(1-2):199–208

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011:402647

Zaheer IE, Ali S, Rizwan M, Farid M, Shakoor MB, Gill RA, Najeeb U, Iqbal N, Ahmad R (2015) Citric acid assisted phytoremediation of copper by Brassica napus L. Ecotox Environ Safety 120:310–317

Zhao H, Appenroth K, Landesman L, Salmeán AA, Lam E (2012) Duckweed rising at Chengdu: summary of the 1st International Conference on Duckweed Application and Research. Plant Mol Biol 78(6):627–632

Zhang X, Hu Y, Liu Y, Chen B (2011) Arsenic uptake, accumulation and phytofiltration by duckweed (Spirodela polyrhiza L.). J Environ sci 23(4):601–606

Ziegler P, Adelmann K, Zimmer S, Schmidt C, Appenroth KJ (2015) Relative in vitro growth rates of duckweeds (Lemnaceae)–the most rapidly growing higher plants. Plant Biol 17:33–41

Metals Phytoextraction by *Brassica* Species

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Contents

Abstract

The wider application of heavy metals in different industrial, agricultural, and pharmaceutical process has increased the unchecked addition of these metals in the environment. These heavy metals have become a potential hazard to environment and human health due to their mutagenic, carcinogenic, and persistent nature. Phytoextraction is an ecological approach in which plants remediate the

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metals contaminated sites by extracting pollutants and accumulating in them. *Brassica* species contain various plants which have strong potential for heavy metal removal and widely used in phytoextraction. *Brassica* species have been proved effective for remediation of even high concentration of heavy metals such as Pb, Cr, Cu, Cd, Ni, and Zn. These plants have developed effective defense mechanism composed of various types of amino acids and antioxidant enzymes activities to alleviate the metals stress without compromising on plant growth and development. The phytoextraction potential of *Brassica* species can be further enhanced by different techniques such as addition of chelating agents, organic and inorganic soil amendments, and different types of biochar.

Keywords

Brassica · Phytoextraction · Heavy metals · Antioxidants · Amino acids

Abbreviations

AlA	5-Aminolevulinic acid
APX	Ascorbate peroxidase
BR	Brassinosteroid
CA	Citric acid
CAT	Catalase
EDTA	Ethylenediaminetetraacetic acid
EL	Electrolyte leakage
GR	Glutathione reductase
GSH	Glutathione
MDA	Malondialdehyde
MTs	Metallothioneins
PCs	Phytochelatins
PGR	Plant growth regulator
POX	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase

1 Introduction

The environmental deterioration by heavy metals has become a leading health and ecological concern. The exponential use of metals in different industrial, agricultural, and domestic application has increased the human interaction with heavy metals (Mishra et al. 2019). Although heavy metals are naturally found throughout the earth crust, but anthropogenic activities have increased the addition of these heavy metals in the environment up to toxic level. The most common sources of heavy metals from human activities are industrial, agricultural, pharmaceutical, tannery, mining and smelting process. Some natural process such as volcanic eruption and weathering process also contribute to heavy metals addition in environment (Tchounwou et al. 2012). Heavy metals have higher density than water and very toxic even at low concentration. The specific characteristic of heavy metals such as solubility, accumulation, resistant to biodegradation and oxidation–reduction properties and complex formation with other elements, mutagenic and carcinogenic properties made them a severe risk to the environment, human, and other living organisms (Rai et al. 2019). The heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), and nickel (Ni) are most commonly used in different industrial process and possess serious environment issues (Mishra et al. 2019).

Cadmium (Cd) is found as natural deposits in earth in combination with other metals. Cadmium is widely used in alloy, battery, fertilizers and alloy industries. Even the low concentration of cadmium is highly toxic to ecosystem due to its ability to get concentrated in the environment. The softening of bone and fractures induced by Itai-Itai diseases is caused by accumulation of cadmium in water (Bhatnagar and Sillanpää 2009). The chronic exposure to cadmium may cause acute health problems including harm to respiratory system, lung cancer, kidney, liver, and reproductive system damage (Filipič 2012; Demim et al. 2013).

Lead (Pb) occurs naturally in trace amount in soil and water and it is constituent of earth crust. It is soft and heavy metals exist in form of sulfide, cerussite ($PbCl_2$), and galean. Wastewater discharge from industries such as electroplating industries, steel, battery manufacturing contains high amount of lead (Carolin et al. 2017). Lead tends to readily accumulate in the human body which makes it harmful. Main reported illnesses by lead are damage to kidney, nervous system, mental retardation, cancer, and abnormalities in fetus growth and development in human (Venkatesh 2004; Bellinger 2005; Riess and Halm 2007).

Chromium (Cr) is widely found in the earth and water. Chromium itself is not toxic but some of its compounds such as hexavalent forms are extremely toxic to health (Cefalu and Hu 2004). The main sources of chromium contamination are leather, tanning textile, and electroplating and glass industries. These industries waste discharge contains hexavalent (VI) and trivalent (III) form of chromium; however, as compared to Cr (III), Cr (VI) is more toxic for living organism and ecosystem. The exposure to chromium may cause damage to skin, kidney, liver, pulmonary system, and digestive system (Hu et al. 2009; Miretzky and Cirelli 2010).

Copper is essential element of human body and plays a key role in tissue, enzymes, and bone development. However at high concentration it is extremely toxic to human health. Copper is released form the mining industries, steel manufacturing, electroplating industries, paints, chemical and fertilizers industries (Awual et al. 2013; Gao et al. 2013; Mehta et al. 2015). Copper may accumulate in liver, brain, and pancreas and leads to death, other effects include headaches, kidney damage, and hair loss (Zhou et al. 2009; Tang et al. 2014).

Nickel is hard and non-biodegradable heavy metals discharged from industries such as battery manufacturing, alloy industry, printing, electroplating, and silver refining industries. The nickel badly affects the pulmonary system with symptoms of cough and shortage of breathing including skin eruption and renal edema (Mobasherpour et al. 2012; Demim et al. 2013; Malamis and Katsou 2013).

The persistence of heavy metals in the environment and associated environmental and health concerns have generated need to devise strategy for efficient remediation of these heavy metals. The phytoextraction is an innovative ecological approach to mitigate the heavy metals in which plants uptake contaminants from polluted water and/or soil by their roots (Ranieri et al. 2020; Remigio et al. 2020). These contaminants are further translocated and accumulated into aboveground biomass such as shoots. The unique ability of phytoextraction, the transfer of pollutants into shoots made this approach widely acceptable for remediation at commercial scale because harvesting of aerial part is more feasible than roots (Halim et al. 2003; Ranieri et al. 2020). Plants which are capable of accumulating high concentration of metals are called as hyperaccumulators. The hyperaccumulation ability of plants seemed due to evolutionary adaption of plants to life in unfavorable environmental conditions (Hou et al. 2017). These plants are more tolerant to heavy metals, drought, pathogens, herbivorous animals and compete with non-accumulators plants (Bhargava et al. 2012). However the detailed mechanism by hyperaccumulators to absorb and accumulate heavy metals and physiological process is not fully explored (Mahar et al. 2016a). The main characteristics of ideal hyperaccumulators plants are adaption to local environment, high capacity of metals uptake, tolerance to toxic heavy metals, well-established root system, high and rapid biomass production, adaptive to climatic conditions, resistance to pathogens and pests, easy harvesting, and less attractive for herbivores are prominent (Bhargava et al. 2012; Fan et al. 2019; Ali et al. 2013). The family *Brassicaceae* is comprised of many plants which are well known as hyperaccumulator (Mahar et al. 2016a; Li et al. 2019).

The *Brassica* (Brassicaceae) is a large group of herbaceous plants that includes world most frequently used vegetables (Verkerk et al. 2009) and it can be used for multiple purposes (Fig. 1). Among these, the most commonly used crops for human consumption are cauliflower, cabbage, broccoli, and oil seeds crops such as rapeseed and mustard (Heaney and Fenwick 1980; Verkerk et al. 2009). These vegetables and oils considered as essential sources of nutrients, elements, amino acids, and carbohydrates (Cartea et al. 2019). The *Brassica* species have been widely applied for the phytoremediation of the heavy metals contaminated soil due to its outstanding heavy metals tolerant ability, uptake and accumulation of metals, and huge above-ground biomass production (Gurajala et al. 2019; Hasanuzzaman et al. 2019; Sahay et al. 2019). The most commonly used *Brassica* species for phytoextraction are mainly *B. juncea*, *B. rapa*, *B. carinata*, *B. oleracea*, *B. napus*, and *B. nigra* (Mourato et al. 2015). *Brassica juncea*, also known as Indian mustard, is used for oil production and also has some medicinal properties. *Brassica napus* known as rapeseed is used for oil production as well as vegetable. They by-production after oil extraction is can be used as animal feed. *Brassica oleracea* composed of most common vegetables like cabbage, broccoli, and cauliflower which contain essential source of nutrients and bioactive compounds such as glucosinolates for human health. *Brassica nigra*, *B. rapa*, and *B. carinata* are mostly used for oil production (Yu 2013; Mourato et al. 2015).

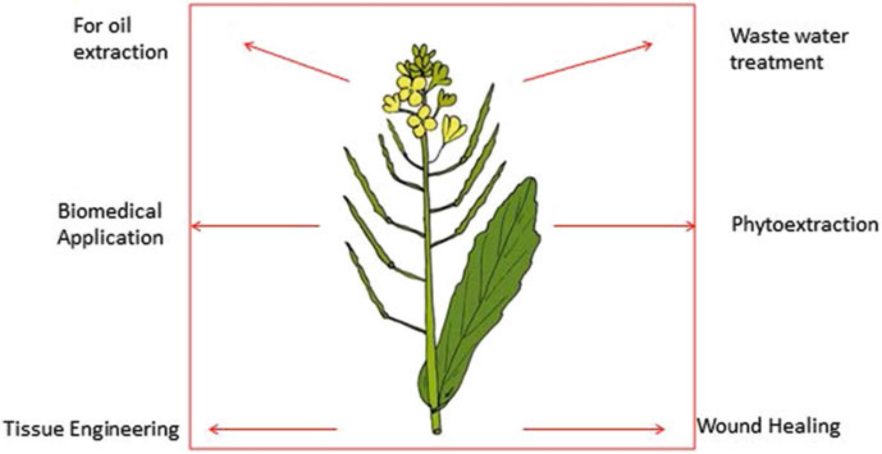

Fig. 1 Application for *Brassica* plants for different purposes

2 Heavy Metals Uptake and Phytoremediation Potential

The heavy metals accumulation in plants depends upon soil and plant characteristics. The soil properties such as pH, clay contents, cation exchange capacity, and organic matter content can influence the availability and mobility of heavy metals (Javed et al. 2019; Wang et al. 2019; Table 1). Similarly, the uptake of metals by plants may vary with plant species and genotype.

Soil condition largely influences the metals uptake and translocation in plants such as soil pH regulates the phytoextraction process in *Brassica* plants. The increasing level of pH may decrease the metals uptake of *B. juncea*. For example at pH level 5.5, the concentration of cadmium (Cd) uptake by *B. juncea* which continue to decrease with increase in pH level (Zaurov et al. 1999). The *Brassica* species also influence the soil pH level; for example, the *Brassica* species growing in rhizospheric soil reduced the pH of the soil from initial pH 5.5. In other case, contrastingly, *Brassica* species significantly upsurge the pH of acid soil, while a slight increase was observed in case of alkaline soil (Kim et al. 2010). The exact mechanism behind contrasting effect of *Brassica* on pH in different soil is not fully explored but change in pH may depend upon the type of species, soil properties, growth, and environmental conditions. The soil texture also influences the metals uptake by *Brassica* such as high metal accumulation was noticed in coarse soil as compared to fine soil. In *B. campestris*, the uptake and accumulation of Cd and other metals was influenced by concentration of metals and calcium (Ca) in the soil (Liu et al. 2007).

Many studies reported the variability in metals uptake, translocation, and accumulation by different species of *Brassica* (Guo et al. 2019; Gurajala et al. 2019). The most of the species of *Brassica* (*B. carinata, B. napus, B. oleracea, B. rapa, and*

Table 1 Application of *Brassica* species for phytoextraction of different heavy metals

Species	Heavy metal	Treatment	Duration	Experimental condition	Responses	References
Brassica juncea	Cd (0.5 mM Cd and 1.0 mM CdCl)	Citric acid	3 days	Hydroponic	Cadmium accumulation increased. CA dose improved plant growth, chlorophyll contents, and activities of antioxidant enzymes	Al Mahmud et al. (2018)
B. rapa	Cd	ATPase gene *BrHMA3*	40 days	Hydroponic and soil pot experiment	Truncated BrHMA3 plants had 2.3 and 9.3 times higher shoot Cd concentration and translocation ratio	Zhang et al. (2019a)
B. juncea	Cd (0 and 0.6 mM)	Citric acid	7 days	Seed germinator	Improved the growth of the plants, photosynthetic pigments and carotenoid contents were improved by 47.17 and 34.06%. The total phenolic and carbohydrates contents were also increased	Kaur et al. (2018)
B. juncea	Cd (0.06 mM)	Citric acid and salicylic acid (SA)	30 days	Soil	Combined dose of CA and SA reduced Cd induced damage, promoted antioxidant defense system, and resulted in membrane stabilization	Faraz et al. (2019)
B. juncea	Pb (0.25, 0.50 and 0.75 mM)	24-epibrassinolide (EBL) and SA	10 days		Combined application of EBL and SA enhances metal tolerance index by 40.07%, water content by 1.84%, and total water and lipid soluble antioxidant contents by 21.01 and 2.21%	Kohli et al. (2018)
B. species	Pb (1 mM)	Melanoidin-like products (MLP) extracted from sugarcane molasses		Hydroponic	The application of MLP detoxifies the lead ion, improved their bioavailability, and helped the plant to survive in lead solution	Hatano and Yamatsu (2018)

B. juncea	Pb	24-epibrassinolide (EBL)	Seedling and germination stage	Seed germinator	The application of EBL at concentration of 10^{-8} M was found most effective to overcome Pb stress and increased antioxidant enzymes activity and Pb uptake	Soares et al. (2020)
B. juncea	Pb (1150 mg Kg^{-1})	–	60 days after emergence	Soil	The Pb concentration in root and shoot reached up to 783 mg kg^{-1} and 94 mg kg^{-1}, respectively	Bassegio et al. (2020)
B. oleracea	Cr (200 μM)	Glycinebetaine (GB)	8 weeks	Pot experiment	Application of GB decreased oxidative stress, improved antioxidant enzyme activity, and assisted to maintain plant morphology and photosynthesis process	Ahmad et al. (2020)
B. juncea	Cr (20–80 mg kg^{-1})	Organic amendments	02 years	Soil	The organic amendments improved the yield, nutrient uptake and alleviated the Cr toxicity in plants	Kumar et al. (2020)
B. napus	Cr (VI) (2–10 mg L^{-1})	–	24 and 48 h of treatment	Hydroponic	The hairy roots alleviated the metal toxicity in the medium and fraction of Cr (VI) was intracellularly reduced to Cr (III)	Perotti et al. (2020)
B. napus	$CuCl_2$	Attapulgite clay (AC)	Short-term stress		The application of AC alleviates the physiological effects by copper stress and improved ROS contents and antioxidant enzymes activities	Zhang et al. (2019b)
B. juncea	Cu	Biochar		Green house experiment	Biochar application increased photosynthesis up to 59% and improved the water use efficiency from 52 to 182%	Gonzaga et al. (2019)

(continued)

Table 1 (continued)

Species	Heavy metal	Treatment	Duration	Experimental condition	Responses	References
B. juncea	Ni (100–800 mg L^{-1})	Ethylenediaminetetra acetic acid (EDTA) and SA	Plants were harvested at four different stages		EDTA treated plants showed 83–90% higher Ni accumulation. EDTA negatively affected all morphological parameter, whereas SA promoted plant growth but Ni accumulation was low	Kaur et al. (2019a)
B. juncea	Ni	Salicylic acid			SA application on Ni stressed plants increased antioxidant enzymes activities especially ascorbate-gluthathionate cycle and improved plant growth, photosynthesis, and mineral nutrient contents	Zaid et al. (2019)

B. campestris) showed great tolerance to most of the heavy metals with maximum tolerance to Zn as compared to Pb and Cd (Hernández-Allica et al. 2008; Gurajala et al. 2019). In another similar study, *B. napus, B. rapa*, *and B. juncea* effectively removed Zn as compared to Cu (Ebbs and Kochian 1997; Rivera-Martin et al. 2020). In a comparison of five different species of *Brassica*, namely *B. nigra, B. napus, B. carinata, B. campestris*, *and B. juncea* for phytoextraction of heavy metals (Pb, Ni, Cu, and Zn) *B. carinata* performed significantly better in removal of Zn, Pb, and Ni than other species. On the other hand, *B. juncea* showed high tolerance to metal induced toxicity for all these metals and maximum uptake of Cu (Purakayastha et al. 2008). Similarly, in a comparison of *B. juncea, B. carinata*, *and B. oleracea* to remediate heavy metals contaminated soil, *B. juncea* was found highly tolerant to metal induced toxicity (Gisbert et al. 2006). The *B. juncea* was also reported highly efficient to remove Cd as well as Cr, Cu, and Pb from highly contaminated soil (Nouairi et al. 2006; Shi et al. 2017; Gurajala et al. 2019). In remediation of Cd and Zn, the three species: *B. juncea, B. carinata*, *and B. napus* exhibited similar potential in phytoextraction of these metals (Yang et al. 2011; Benáková et al. 2017; Du et al. 2020). The accumulation pattern and potential was also reported with contrasting results by different researchers. For example, in a study, *B. napus* accumulated the maximum concentration of metals (Cd, Cu, Cr, Ni, Pb, and Zn) in the shoots instead of roots as per the distinctive behavior of an accumulator plant (Brunetti et al. 2011). The other study, reported opposite results, in which *B. juncea and B. napus* accumulated maximum concentration of Cu in roots than shoot grown in hydroponic solution (Feigl et al. 2013). However, in case of Cd, the *B. juncea* accumulated maximum concentration in shoots than roots as compared to *B. napus* from a hydroponic solution (Nouairi et al. 2006). Some species of *Brassica* are used for oil production, which is used by human and livestock animal. The uptake and accumulation of toxic metals in *Brassica* may contaminate the seed and oil obtained from these plants. However it was found that the oil extracted from the *B. napus* cultivated in highly metals contaminated soil was found very low concentration of heavy metals and safe for use (Park et al. 2012; Mourato et al. 2015). The maximum metals concentration was retained in the plants parts and residue of the oil extraction process. This highlights that *Brassica* plant can be effectively used for heavy metal remediation as well as for oil extraction (Cardone et al. 2003; Dhull et al. 2019).

The difference in phytoremediation potential and behaviors of different and same *Brassica* species is highly dependent upon experiment conditions and makes it difficult to conclude by any general assumption. The concentration of heavy metals, treatment time may influence the phytoremediation behavior of the plants. The type of experiment such as hydroponic or in soil may also alter the metals accumulation process in plants and end results. In soil the pH, organic matter contents, and cation exchange capacity may greatly impact the metal availability and mobility which is not considered in hydroponic experiments (Bian et al. 2019; Haq et al. 2020). Other factors like age of plant at contamination site, and growth period, types and range of heavy metals may also influence the phytoremediation potential of plants (Table 1).

3 Metals-Induced Toxicity

The high concentration of metals can cause metal-induced toxicity to plant resulting in direct damage to the plant and indirect damage due to oxidative stress on plants. The main damage to plants due to toxic metals is decrease in chlorophyll contents, stomatal malfunctioning, low enzymatic activity, disturbed water balance, and limited uptake of essential nutrients (Jadia and Fulekar 2009; Haq et al. 2020).

Among all other symptoms of metals-induced toxicity is responsible for stunted plant growth including deterioration of root morphology and reduced root growth (Pichhode and Asati 2020). Roots are in direct contact with toxic metals so these are the most affected part of the plants in case of metal toxicity. Many studies reported damaged and reduced roots growth in *Brassica* under high heavy metal stress (Theriappan et al. 2011; Khan et al. 2017). The high concentration of Cu may badly damage the *Brassica*'s roots structure as compared to Zn (Brunetti et al. 2011; Du et al. 2020). The Zn toxicity reduced root development in *B. oleracea* but did not affect the foliar biomass of the plant due to its maximum accumulation in roots than shoot and aerial biomass (Barrameda-Medina et al. 2014). Similarly the Zinc concentration of 500 μM badly damaged the root structure and reduced the root growth and development in *B. napus.* The highly Cd enriched growth media reduced the root and shoot growth in *B. napus* and *B. juncea*. Along it the high concentration of Cd also reduced the availability of Fe and Mn to roots and shoots resulting in stunted root and shoot growth in *Brassica* plants (Nouairi et al. 2006). The high contents of Pb (50 and 100 μM) in a hydroponic culture resulted in retarded growth of the root and shoot and reduced biomass production by *B. napus* plants.

The heavy metals stress also severely reduces the ability of plants to uptake water and essential nutrients. The reduced water intake indirectly damages the plant by limited availability of water to carry out metabolic process in plants. Similarly in *Brassica* the metal induced toxicity may badly damage the water uptake potential of plants. Under Pb toxicity the water contents of *B. Juncea* were radically decreased resulting in stunted growth of the plants (Zaier et al. 2010). The *Brassica* just like other plants also reduces the water intake under metal stress due to its self-defense mechanism. The amino acids, soluble sugars, and proline have a protective role and control the osmotic potential. Along it, the reactive oxygen species (ROS) also perform a key role in detoxification of heavy metals during uptake process (Keunen et al. 2013; Kamran et al. 2019). The *B. napus* plant under Cd stress showed increase in concentration of amino acids and proline; however, the increased level of Cd (500 μM) reduced the concentration of these protective substance which was mainly due to the damage to the plant metabolism process (Sharma and Dietz 2006).

The high extent of heavy metals is extremely toxic to photosynthesis and badly damages the photosynthetic process (Singh et al. 2016). Chlorosis and reduction in photosynthetic pigments are the most common consequence of heavy metals stress due to inadequate chlorophyll synthesis, increased destruction to chlorophyll, inhibition of essential enzymes for chlorophyll process, and disruption to Calvin cycle (Rai et al. 2016; Souri et al. 2019). The toxic metals also reduce the availability of essential nutrients and elements for photosynthesis process by their inhibition and competition effects. In *B. napus* and *B. juncea* both the Cd induced toxicity reduced the photosynthesis which was mainly due to inhibition of essential enzymes for formation of chlorophyll and disturbance in the availability of essential elements such as Fe, Mn, Mg, and Zn (Benáková et al. 2017; Chowdhary et al. 2018). In another study, the Cd toxicity in *B. napus* resulted in leaf chlorosis due to interference of Cd with cell division process and disrupted replication of chloroplast leading to reduced chloroplast per cell (Kaur et al. 2017a). Similarly, the Zn and Cd high concentration resulted in reduced chlorophyll and carotenoids contents in *B. napus* plants (Kutrowska et al. 2017). The Zn and Cd toxicity increased the ROS production and reduced the chlorophyll synthesis by inhibiting the essential enzymes mandatory for carotenoids and chlorophyll synthesis. The high concentration of Zn also interrupts the Fe metabolism and promotes the chlorosis in plants. The high concentration of Cr also reported to cause damage to photosynthesis process and net decrease in photosynthesis rate in *B. napus* plants (Perotti et al. 2020).

The *Brassica* plants have great ability to tolerate metals toxicity and even under metal toxicity can selectively absorb the essential nutrients (Kutrowska et al. 2017). However, the high concentration of metal can decrease the ability of plant to uptake essential nutrients required to carry out metabolism process. In *B. napus* and *B. rapa* plants high concentration of Cu and Zn decreases the availability of Fe and Mn to the plants (Rahmani et al. 2016; Zlobin et al. 2017). Although these elements were found in good concentration in roots, but metals-induced toxicity interrupted the translocation of these essential element to above-ground parts of plants. Similarly, the Pb stress in *B. juncea* predominately reduced the concentration of essential nutrients (Gurajala et al. 2019). The Cu toxicity in *B. juncea* and *B. napus* decreased the availability of essential nutrients such as Fe, Co, Mn, and Zn (Feigl et al. 2013). The non-availability or reduced availability of these essential nutrient under metal stressed plants may negatively affect the photosynthesis process, carotenoid production and leading to appearance of chlorosis in *Brassica* plants.

4 Defense Mechanism

Plants have multiple defense systems to endure and detoxify the heavy metals toxicity (Rizwan et al. 2018). Hyperaccumulators plants have the ability to concentrate the metals in their tissue and initiate the detoxification mechanism for these metals (Reeves et al. 2018). Plants can sequester the toxic ions in the vacuole and salt glands through their non-selective salt resistance mechanisms (Shrivastava et al. 2019). Some plants also have the ability to excrete the excessive salts through their salt glands and these glands also have the ability to secrete toxic elements such as Pb, Zn, Cd on the leaf surface to detoxify these metals (Kadukova et al. 2008; Amari et al. 2017). The plants detoxify the heavy metals in above-ground parts by making heavy metals complexation with ligands and prohibiting the metals from cytoplasm and shifting these metals to inactive compartment (Rezania et al. 2016). The *Brassica* plants have the ability to concentrate the metals in the cell wall and vacuoles and prohibit its accumulation in chloroplast to protect the plants (Carrier et al. 2003; Mourato et al. 2015). In a study, *B. napus* under stress of Cd and Cu for 15 days, a small amount of Cd accumulated in cell wall, while the major amount of Cu was sequestered in vacuole and cell wall (Mwamba et al. 2016). Ligands such as organic acids, amino acids and metallothioneins (MTs) and phytochelatins (PCs) contribute to metals binding and sequestration (Rizwan et al. 2018). A universal mechanism of detoxification in plants is the binding of metals with organic acids and sequestration them into vacuole (Shrivastava et al. 2019). It is widely reported that under the stress of the metals (Cd, Ni, and Pb) the concentration of different organic acids was significantly amended in roots and shoots of the *B. juncea* (Amari et al. 2016; Shi et al. 2017) and contributed to translocation and chelation of these metal complexes (Mnasri et al. 2015). The metal stress induced by Cd in *Brassica* plants can regulate the production of PCs, which can chelate with Cd ions and sequester these metal complexes to the vacuole (Jinadasa et al. 2016). In Cd tolerant cultivar *B. oleracea* and *B. napus* the production of PCs and glutathione (GSH) was increased in response to Cd stress (Bernard et al. 2016; Jinadasa et al. 2016). The similar phenomenon was observed in *B. juncea* in which PCs and GSH contents were boosted in root and shoots in response to Cd stress (Kaur et al. 2019b). The production of PCs also varies at species as well as at plants parts level in *Brassica*. For example, in *B. juncea*, PCs produces almost four times in higher amount in roots than shoot (Ehsan et al. 2014). On the other hand, *B. napus* almost produced double amount of PCs in shoots than *B. juncea*. In *B. juncea* under Cd stress, the overall production of PCs was high as compared to *B. napus* (Nouairi et al. 2009).

Amino acids such as proline and histidine performed a key role to shield the plants from metal stress and toxicity (Szabados and Savoure 2010; Sytar et al. 2019). Proline has ability to protect the essential proteins and cell membrane from damage by heavy metals. It is well reported that plants increase the production of proline under stress of Pb, Cd, Co, and Ag (Rastgoo and Alemzadeh 2011). In *B. juncea* and *B. pekinensis* the proline contents were increased under Cd stress and proline which suggests that proline have key role in the metals tolerance of *Brassica* plants (Sharmila et al. 2017). Besides proline, some other amino acids such as glutamine, alanine, arginine, and osmoprotectant also found to perform a key role in the plant tolerance against metal stress (Kaur et al. 2017b).

Plants have a common well-developed antioxidants mechanism composed of superoxide dismutase (SOD), peroxidase (POX) to respond ROS-induced oxidative stress (Seneviratne et al. 2019). In this process SOD, peroxidase, and catalase (CAT) detoxify the H_2O_2 to H_2O and prevent the formation of $OH^{\bullet}$ radicals (Ghori et al. 2019). Among the many others, glutathione (GSH) is one of the most important intracellular antioxidant molecules commonly noticed in all cell compartments (chloroplast, cytosol, endoplasmic reticulum, vacuole, and mitochondria) responsible for removal of H_2O_2 in different cell compartments (Ghori et al. 2019; Seneviratne et al. 2019). Similarly, *Brassica* plants respond to metal stress by their well-developed antioxidants mechanisms (Chowdhary et al. 2018; Rizwan et al. 2018). The metals stress in *Brassica* species regulates the intensity of antioxidants enzymes depending upon the plant species and ROS type (Ahmad et al. 2020). The important components responsible for induced metal tolerance in *Brassica* are glutathione reductase (GR) and ascorbate peroxidase (APX) and glutathione (GSH). In *B. juncea* production of SOD, glutathione reductase (GR), and ascorbate peroxidase (APX) was boost under high concentration of Cd (Ehsan et al. 2014). Gill et al. (2011) described that an increase in enzymatic activity of CAT, APX, and GR and lower activity of SOD was noticed in tolerant cultivars of *B. juncea* in contrast to other cultivars (Gill et al. 2011). In a study, *B. juncea* under Cu stress, APX and GSH contents were increased while CAT contents were decreased (Chigbo et al. 2013). Similarly, *B. juncea* under Pb and Cd stress also showed higher content of APX, SOD and lower content of CAT (Cuypers et al. 2010).

5 Techniques to Enhance Phytoremediation Potential

Phytoremediation potential of *Brassica* plants can be enhanced by improving the availability of metals, alleviating the metal toxicity in the plants, enhanced accumulation and translocation in plants without compromising on plants growth and development (Table 1).

5.1 Addition of Chelating Agents

The phytoremediation efficiency of the plants can be enhanced by using organic and synthetic chelating agents. A widespread phenolic compound and plant growth regulator (PGR) salicylic acid (SA) has been proved effective to alleviate stress induced by metal (Gill et al. 2015; Sarwar et al. 2017; Sharma et al. 2020). The application of SA in maize significantly reduced the negative effect of Cd on chlorophyll contents, enhanced proline level, and improved the water balance in the leaves (Singh et al. 2019). The foliar application of SA on *Brassica* plants decreased the Cd induced toxicity by regulating the key metabolism process and antioxidants production (Faraz et al. 2019). This foliar spray of SA on *B. napus* plants affected by Cd enhanced the proline and micronutrient contents and reduced the H_2O_2, MDA, and electrolyte leakage (EL) contents, and minimized the damage to chloroplast. The exogenous application of 5-aminolevulinic acid (ALA) enhanced the metal tolerance of *B. napus* under Cd stress by improving the activities of antioxidants enzymes and reducing the oxidative stress (Ahmad et al. 2017; Gill et al. 2015). In the same way, application of brassinosteroid (BR) and methyl jasmonate on *B. napus* under metals stress assisted in alleviating the metal stress by promoting photosynthesis process, proline contents, and antioxidants enzymes activities (Hayat et al. 2007; Ali et al. 2014). Additionally, metal chelators boost the potential of the plants to uptake heavy metals in huge concentration by increasing the metals availability in the soil (Sarwar et al. 2017).

The utilization of organic acid such as citric acid and malic acid improved the metals availability in the soil for the plant (Table 1). However in case of *Brassica* it was observed that organic acid application in soil may reduce the plant growth and yield due to increased availability and accumulation of metals in plants (Kaur et al.

2018). The application of citric acid (CA) on *B. napus* prompted the plant biomass, antioxidants enzymes activities and reduced the oxidative stress. This foliar application of CA enhanced the metals uptake and accumulation in roots, shoots, and leaves of the plants (Ehsan et al. 2014). The exogenous application of ethylenediaminetetraacetic acid (EDTA) is widely studied due to its ability to form complex with heavy metals and enhance the metals phytoextraction (Guo et al. 2019; Mahmud et al. 2019). The application of EDTA in Cd enriched soil enhanced the Cd availability and also increased the translocation of EDTA from roots to shoots in *B. juncea* (Guo et al. 2019). In *B. rapa*, EDTA is found very effective to increase the bioavailability of Pb and Cd and accumulation in foliar plants parts (Rizwan et al. 2018).

5.2 Organic and Inorganic Amendments

There are many approaches to enhance phytoextraction of *Brassica* species as shown in (Fig. 2). Application of organic/inorganic amendments is an effective strategy to boost the phytoextraction potential of *Brassica* plants and to improve the growth and development of plant by alleviating the metals stress (Pérez-Esteban et al. 2014; Tang et al. 2015). At highly contaminated site enriched with toxic amount of Zn, Cu, and Pb, the application of cow manure and compost increased the phytoremediation potential of *B. juncea* by regulating the pH of the soil (Clemente et al. 2005). Similarly, application of compost derived from municipal solid waste upgraded the phytoremediation potential of *B. napus* to extract the heavy metals from the

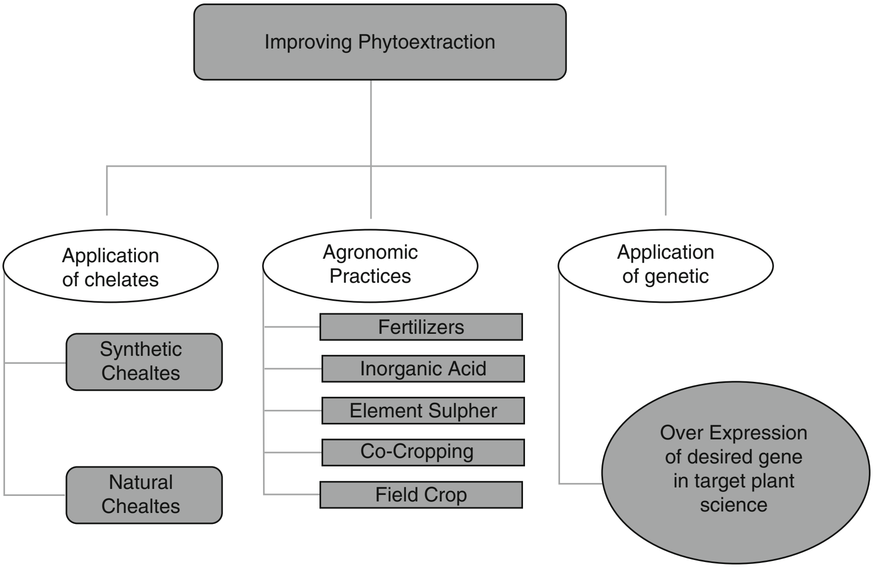

Fig. 2 Strategies to improve phytoextraction potential of *Brassica* species

treatment site (Brunetti et al. 2011). The application of biosolids in a soil contaminated with Cd and Pb increased the phytoremediation potential of *B. rapa* and increased the fresh and dry biomass, and overall accumulation in the roots, shoots, and leaves (Chen et al. 2010). Contrastingly, application of poultry manure decreases the Cd uptake and accumulation in the *B. chinensis* by limiting the availability Cd and transforming it to organic bound forms (Han-Song et al. 2010). The poultry manure application increased the pH of the soil, increased chlorophyll and carotenoids, and boosted the activity of antioxidant enzymes resulted in higher biomass production (Han-Song et al. 2010). The *B. juncea* plants under the Cr stress (20–80 mg kg^{-1} soil) were applied with three different types of organic amendments (vermicompost, farm yard manure, and sewage sludge). The application of these organic amendments resulted in an increase in plant growth, high yield per plant, dry mass accumulation, high harvest index, and significant nutrients uptake (Kumar et al. 2020).

The exogenous spray of decreased the Cd accumulation in the shoots and improved the chlorophyll contents, enzymatic activity, and high biomass production (Zong et al. 2017b). In the same way, chitooligosaccharide reduced the Cd uptake by the plants and limited it accumulation in the cell compartments and regulated enzymatic activities to detoxify the Cd induced toxicity (Zong et al. 2017a).

The addition of inorganic amendments could also minimize the metals uptake in *Brassica* plants by transforming the soil metals speciation (Paul and Chaney 2017; Yao et al. 2017). In *B. campestris*, addition of red mud in Cd enriched soil minimizes the uptake of Cd by plant as a consequence of Cd sorption (Li et al. 2016). Similarly, addition of mineral in the soil also influenced the metal phytoextraction in *Brassica* species. The exogenous application of Ca on *B. juncea* increased the plant growth and biomass production, photosynthesis, mineral contents and reduced the oxidative stress and uptake of Cd (Ahmad et al. 2015). Similarly, application of magnesium (Mg) in hydroponic solution presented the same results for *B. rapa* (Blasco et al. 2015). The addition of chloride to highly metals contaminated soil increased the metals accumulation in the shoots of *B. juncea* by formation of metal-chloride complex (Lopez-Chuken et al. 2010). In case of *B. rapa*, the addition of sulfur in metals contaminated soil decreased the pH of the soil and reduced the availability of metals by immobilizing them (Mahar et al. 2016b). Selenium decreases the metals uptake and accumulation in the plant parts, improved the plant growth and antioxidants enzymes activities, and mineral nutrients in *B. rapa*, *B. napus*, *B. campestris*, *B. oleracea*, and *B. juncea* (Thiruvengadam and Chung 2015; Ahmad et al. 2016; Ding et al. 2017).

5.3 Biochar

The application of biochar is another efficient strategy to alleviate the metals toxicity and phytoremediation potential of *Brassica* plants (Houben et al. 2013; Zheng et al. 2019). The mixing of biochar in the soil improves the soil quality, restricts the availability of the metals, and improves the plant growth. The adding of biochar

(5 and 10%) in metals contaminated soil with Zn, Cd, and Pb decreased the availability of these metals to *B. napus* and increased the plant biomass (Houben et al. 2013). The application of biochar in combination with *Brassica* is more useful when the focus is on phytostabilization of metals than phytoremediation (Mourato et al. 2015). The application of poultry derived biochar in multi contaminated soil with Cd, Cu, and Zn although did not increase the metals uptake in *B. napus* but increased the solubility of these metals in the soil (Marchand et al. 2016). Similarly, the addition of biochar in soil contaminated with Cu, Ni, Pb, and Zn assisted the *B. juncea* to grow at this highly contaminated soil and minimize uptake in plants by reducing the bioavailability of these metals in the soil (Rodríguez-Vila et al. 2016). The addition of biochar composed of two types of crop residues (coconut husk and orange shell) in Cu contaminated soil, enhanced the plants biomass by 170% in *B. juncea*. It also decreased the concentration of Cu by 51% in the soil and enhanced the water use efficiency of the plants by 52–182% (Gonzaga et al. 2019). In the same way, the addition of derived biochar derived from rice straw in metals polluted soil increased the plant growth and decreased the availability and uptake of these metals in different *Brassica* species (Niu et al. 2015).

6 Conclusion

The *Brassica* species have great potential to ameliorate the multi metals contaminated soil. The plants have strong defense system to alleviate the biotic and abiotic stress induced by metal toxicity. The antioxidant enzymes and different amino acids are main component of defense system to protect the plants from harmful effect of heavy metals. The performance of *Brassica* species under metal stress varies with species, cultivar, soil characteristics, and exposure duration. Different chelating agents, exogenous application of different organic and inorganic soil amendments, and biochar can be applied to boost the phytoextraction potential of the plants. Further field research should be conducted to explore the potential of soil amendments in combination with *Brassica* species to increase the bioavailability and removal of heavy metals. The in-depth study of *Brassica* plants in relation to heavy metals at physiological, metabolic, and genetic level will give better explanation of the heavy metal removal and metals toxicity alleviation process. Overall, the hyperaccumulation ability, high biomass production, and tolerance to heavy metal toxicity made the *Brassica* species a perfect choice for phytoextraction.

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References

Ahmad P, Sarwat M, Bhat NA, Wani MR, Kazi AG, Tran L-SP (2015) Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. PLoS One 10:e0114571

Ahmad P, Allah EA, Hashem A, Sarwat M, Gucel S (2016) Exogenous application of selenium mitigates cadmium toxicity in *Brassica juncea* L. (Czern & Cross) by up-regulating antioxidative system and secondary metabolites. J Plant Growth Regul 35:936–950

Ahmad R, Ali S, Hannan F, Rizwan M, Iqbal M, Hassan Z, Akram NA, Maqbool S, Abbas F (2017) Promotive role of 5-aminolevulinic acid on chromium-induced morphological, photosynthetic, and oxidative changes in cauliflower (*Brassica oleracea botrytis* L.). Environ Sci Pollut Res 24:8814–8824

Ahmad R, Ali S, Abid M, Rizwan M, Ali B, Tanveer A, Ahmad I, Azam M, Ghani MA (2020) Glycinebetaine alleviates the chromium toxicity in *Brassica oleracea* L. by suppressing oxidative stress and modulating the plant morphology and photosynthetic attributes. Environ Sci Pollut Res 27:1101–1111

Al Mahmud J, Hasanuzzaman M, Nahar K, Bhuyan MB, Fujita M (2018) Insights into citric acid-induced cadmium tolerance and phytoremediation in *Brassica juncea* L.: coordinated functions of metal chelation, antioxidant defense and glyoxalase systems. Ecotoxicol Environ Saf 147:990–1001

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881

Ali B, Gill RA, Yang S, Gill MB, Ali S, Rafiq MT, Zhou W (2014) Hydrogen sulfide alleviates cadmium-induced morpho-physiological and ultrastructural changes in *Brassica napus*. Ecotoxicol Environ Saf 110:197–207

Amari T, Ghnaya T, Sghaier S, Porrini M, Lucchini G, Attilio Sacchi G, Abdelly C (2016) Evaluation of the Ni 2+ phytoextraction potential in *Mesembryanthemum crystallinum* (halophyte) and Brassica juncea. J Bioremed Biodegr 7:1–7

Amari T, Ghnaya T, Abdelly C (2017) Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. S Afr J Bot 111:99–110

Awual MR, Yaita T, El-Safty SA, Shiwaku H, Suzuki S, Okamoto Y (2013) Copper (II) ions capturing from water using ligand modified a new type mesoporous adsorbent. Chem Eng J 221:322–330

Barrameda-Medina Y, Montesinos-Pereira D, Romero L, Ruiz JM, Blasco B (2014) Comparative study of the toxic effect of Zn in *Lactuca sativa* and *Brassica oleracea* plants: I. Growth, distribution, and accumulation of Zn, and metabolism of carboxylates. Environ Exp Bot 107:98–104

Bassegio C, Campagnolo MA, Schwantes D, Gonçalves Junior AC, Manfrin J, Schiller AP, Bassegio D (2020) Growth and accumulation of Pb by roots and shoots of *Brassica juncea* L. Int J Phytoremediation 22:134–139

Bellinger DC (2005) Teratogen update: lead and pregnancy. Birth Defects Res A Clin Mol Teratol 73:409–420

Benáková M, Ahmadi H, Dučaiová Z, Tylová E, Clemens S, Tůma J (2017) Effects of Cd and Zn on physiological and anatomical properties of hydroponically grown *Brassica napus* plants. Environ Sci Pollut Res 24:20705–20,716

Bernard F, Dumez S, Brulle F, Lemière S, Platel A, Nesslany F, Cuny D, Deram A, Vandenbulcke F (2016) Antioxidant defense gene analysis in *Brassica oleracea* and Trifolium repens exposed to Cd and/or Pb. Environ Sci Pollut Res 23:3136–3151

Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manag 105:103–120

Bhatnagar A, Sillanpää M (2009) Applications of chitin-and chitosan-derivatives for the detoxification of water and wastewater—a short review. Adv Colloid Interf Sci 152:26–38

Bian F, Zhong Z, Zhang X, Yang C, Gai X (2019) Bamboo–an untapped plant resource for the phytoremediation of heavy metal contaminated soils. Chemosphere 246:125750

Blasco B, Graham NS, Broadley MR (2015) Antioxidant response and carboxylate metabolism in *Brassica rapa* exposed to different external Zn, Ca, and Mg supply. J Plant Physiol 176:16–24

Brunetti G, Farrag K, Rovira PS, Nigro F, Senesi N (2011) Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. Geoderma 160:517–523

Cardone M, Mazzoncini M, Menini S, Rocco V, Senatore A, Seggiani M, Vitolo S (2003) *Brassica carinata* as an alternative oil crop for the production of biodiesel in Italy: agronomic evaluation, fuel production by transesterification and characterization. Biomass Bioenergy 25:623–636

Carolin CF, Kumar PS, Saravanan A, Joshiba GJ, Naushad M (2017) Efficient techniques for the removal of toxic heavy metals from aquatic environment: a review. J Environ Chem Eng 5:2782–2799

Carrier P, Baryla A, Havaux M (2003) Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil. Planta 216:939–950

Cartea E, Haro-Bailón D, Padilla G, Obregón-Cano S, del Rio-Celestino M, Ordás A (2019) Seed oil quality of *Brassica napus* and *Brassica rapa* germplasm from Northwestern Spain. Foods 8:292

Cefalu WT, Hu FB (2004) Role of chromium in human health and in diabetes. Diabetes Care 27:2741–2751

Chen H-L, Lai H-Y, Wang S-M, Kuo Y-C, Lu C-J (2010) Effect of biosolids and Cd/Pb interaction on the growth and Cd accumulation of *Brassica rapa* grown in Cd-contaminated soils. Water Air Soil Pollut 206:385–394

Chigbo C, Batty L, Bartlett R (2013) Interactions of copper and pyrene on phytoremediation potential of *Brassica juncea* in copper–pyrene co-contaminated soil. Chemosphere 90:2542–2548

Chowdhary P, Yadav A, Singh R, Chandra R, Singh D, Raj A, Bharagava RN (2018) Stress response of *Triticum aestivum* L. and *Brassica juncea* L. against heavy metals growing at distillery and tannery wastewater contaminated site. Chemosphere 206:122–131

Clemente R, Walker DJ, Bernal MP (2005) Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): the effect of soil amendments. Environ Pollut 138:46–58

Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ (2010) Cadmium stress: an oxidative challenge. BioMetals 23:927–940

Demim S, Drouiche N, Aouabed A, Benayad T, Dendene-Badache O, Semsari S (2013) Cadmium and nickel: assessment of the physiological effects and heavy metal removal using a response surface approach by *L. gibba*. Ecol Eng 61:426–435

Dhull S, Ishu DM, Kadian M, Yadav M (2019) Methods for the authentication and characterization of canola (*Brassica napus*; *Brassicaceae*), corn (*Zea mays*; *Poaceae*) and cotton Seed (*Gossypium hirsutum*; *Malvaceace*) oils. Clin J Pharmacol Pharmacother 1:1008

Ding Y, Wang Y, Zheng X, Cheng W, Shi R, Feng R (2017) Effects of foliar dressing of selenite and silicate alone or combined with different soil ameliorants on the accumulation of As and Cd and antioxidant system in *Brassica campestris*. Ecotoxicol Environ Saf 142:207–215

Du J, Guo Z, Li R, Guo D, Lahori AH, Wang P, Liu X, Wang X, Ali A, Zhang Z (2020) Screening of Chinese mustard (*Brassica juncea* L.) cultivars for Cd/Zn phytoremediation and research on physiological mechanisms. Environ Pollut 261:114213

Ebbs SD, Kochian LV (1997) Toxicity of zinc and copper to Brassica species: implications for phytoremediation. J Environ Qual 26:776–781

Ehsan S, Ali S, Noureen S, Mahmood K, Farid M, Ishaque W, Shakoor MB, Rizwan M (2014) Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. Ecotoxicol Environ Saf 106:164–172

Fan Y, Li Z, Zhou T, Zhou S, Wu L, Luo Y, Christie P (2019) Phytoextraction potential of soils highly polluted with cadmium using the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. Int J Phytoremediation 21:733–741

Faraz A, Faizan M, Sami F, Siddiqui H, Hayat S (2019) Supplementation of salicylic acid and citric acid for alleviation of cadmium toxicity to *Brassica juncea*. J Plant Growth Regul 39:641–655

Feigl G, Kumar D, Lehotai N, Tugyi N, Molnár Á, Ördög A, Szepesi Á, Gémes K, Laskay G, Erdei L (2013) Physiological and morphological responses of the root system of Indian mustard (*Brassica juncea* L. Czern.) and rapeseed (*Brassica napus* L.) to copper stress. Ecotoxicol Environ Saf 94:179–189

Filipič M (2012) Mechanisms of cadmium induced genomic instability. Mutat Res Fundam Mol Mech Mutagen 733:69–77

Gao J, Liu F, Ling P, Lei J, Li L, Li C, Li A (2013) High efficient removal of Cu (II) by a chelating resin from strong acidic solutions: complex formation and DFT certification. Chem Eng J 222:240–247

Ghori N-H, Ghori T, Hayat M, Imadi S, Gul A, Altay V, Ozturk M (2019) Heavy metal stress and responses in plants. Int J Environ Sci Technol 16:1807–1828

Gill SS, Khan NA, Tuteja N (2011) Differential cadmium stress tolerance in five Indian mustard (*Brassica juncea* L.) cultivars: an evaluation of the role of antioxidant machinery. Plant Signal Behav 6:293–300

Gill RA, Ali B, Islam F, Farooq MA, Gill MB, Mwamba TM, Zhou W (2015) Physiological and molecular analyses of black and yellow seeded *Brassica napus* regulated by 5-aminolivulinic acid under chromium stress. Plant Physiol Biochem 94:130–143

Gisbert C, Clemente R, Navarro-Avinó J, Baixauli C, Ginér A, Serrano R, Walker DJ, Bernal MP (2006) Tolerance and accumulation of heavy metals by *Brassicaceae* species grown in contaminated soils from Mediterranean regions of Spain. Environ Exp Bot 56:19–27

Gonzaga MIS, da Silva PSO, de Jesus Santos JC, de Oliveira Junior LFG (2019) Biochar increases plant water use efficiency and biomass production while reducing Cu concentration in Brassica juncea L. in a Cu-contaminated soil. Ecotoxicol Environ Saf 183:109557

Guo D, Ali A, Ren C, Du J, Li R, Lahori AH, Xiao R, Zhang Z, Zhang Z (2019) EDTA and organic acids assisted phytoextraction of Cd and Zn from a smelter contaminated soil by potherb mustard (*Brassica juncea*, Coss) and evaluation of its bioindicators. Ecotoxicol Environ Saf 167:396–403

Gurajala HK, Cao X, Tang L, Ramesh TM, Lu M, Yang X (2019) Comparative assessment of Indian mustard (*Brassica juncea* L.) genotypes for phytoremediation of Cd and Pb contaminated soils. Environ Pollut 254:113085

Halim M, Conte P, Piccolo A (2003) Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere 52:265–275

Han-Song C, Huang Q-Y, Li-Na L, Peng C, Liang W, Ming L (2010) Poultry manure compost alleviates the phytotoxicity of soil cadmium: influence on growth of pakchoi (*Brassica chinensis* L.). Pedosphere 20:63–70

Haq S, Bhatti AA, Dar ZA, Bhat SA (2020) Phytoremediation of heavy metals: an eco-friendly and sustainable approach. Cham. Springer, pp. 215–231.

Hasanuzzaman M, Matin MA, Fardus J, Hasanuzzaman M, Hossain MS, Parvin K (2019) Foliar application of salicylic acid improves growth and yield attributes by upregulating the antioxidant defense system in Brassica campestris plants grown in lead-amended soils. Acta Agrobot 72:1765

Hatano K-i, Yamatsu T (2018) Molasses melanoidin-like products enhance phytoextraction of lead through three Brassica species. Int J Phytoremediation 20:552–559

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot 60:33–41

Heaney RK, Fenwick GR (1980) Glucosinolates in *Brassica* vegetables. Analysis of 22 varieties of Brussels sprout (*Brassica oleracea* var. gemmifera). J Sci Food Agric 31:785–793

Hernández-Allica J, Becerril JM, Garbisu C (2008) Assessment of the phytoextraction potential of high biomass crop plants. Environ Pollut 152:32–40

Hou D, Wang K, Liu T, Wang H, Lin Z, Qian J, Lu L, Tian S (2017) Unique rhizosphere micro-characteristics facilitate phytoextraction of multiple metals in soil by the hyperaccumulating plant *Sedum alfredii*. Environ Sci Technol 51:5675–5684

Houben D, Evrard L, Sonnet P (2013) Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (*Brassica napus* L.). Biomass Bioenergy 57:196–204

Hu J, Chen C, Zhu X, Wang X (2009) Removal of chromium from aqueous solution by using oxidized multiwalled carbon nanotubes. J Hazard Mater 162:1542–1550

Jadia CD, Fulekar M (2009) Phytoremediation of heavy metals: recent techniques. Afr J Biotechnol 8:921

Javed MT, Tanwir K, Akram MS, Shahid M, Niazi NK, Lindberg S (2019) Phytoremediation of cadmium-polluted water/sediment by aquatic macrophytes: role of plant-induced pH changes. In: Cadmium toxicity and tolerance in plants. Elsevier, Amsterdam, pp 495–529

Jinadasa N, Collins D, Holford P, Milham PJ, Conroy JP (2016) Reactions to cadmium stress in a cadmium-tolerant variety of cabbage (*Brassica oleracea* L.): is cadmium tolerance necessarily desirable in food crops? Environ Sci Pollut Res 23:5296–5306

Kadukova J, Manousaki E, Kalogerakis N (2008) Pb and Cd accumulation and phyto-excretion by salt cedar (*Tamarix smyrnensis* Bunge). Int J Phytoremediation 10:31–46

Kamran M, Malik Z, Parveen A, Huang L, Riaz M, Bashir S, Mustafa A, Abbasi GH, Xue B, Ali U (2019) Ameliorative effects of biochar on rapeseed (*Brassica napus* L.) growth and heavy metal immobilization in soil irrigated with untreated wastewater. J Plant Growth Regul 39:266–281

Kaur R, Yadav P, Sharma A, Thukral AK, Kumar V, Kohli SK, Bhardwaj R (2017a) Castasterone and citric acid treatment restores photosynthetic attributes in *Brassica juncea* L. under Cd (II) toxicity. Ecotoxicol Environ Saf 145:466–475

Kaur R, Yadav P, Thukral AK, Walia A, Bhardwaj R (2017b) Co-application of 6-ketone type brassinosteroid and metal chelator alleviates cadmium toxicity in *B. juncea* L. Environ Sci Pollut Res 24:685–700

Kaur R, Yadav P, Thukral AK, Sharma A, Bhardwaj R, Alyemeni MN, Wijaya L, Ahmad P (2018) Castasterone and citric acid supplementation alleviates cadmium toxicity by modifying antioxidants and organic acids in *Brassica juncea*. J Plant Growth Regul 37:286–299

Kaur L, Sharma S, Gadgil K (2019a) Response of Indian mustard (*Brassica juncea* arawali) plants under nickel stress with special reference to nickel phytoextraction potential. EQA-Int J Environ Qual 34:17–33

Kaur P, Bali S, Sharma A, Kohli SK, Vig AP, Bhardwaj R, Thukral AK, Abd_Allah EF, Wijaya L, Alyemeni MN (2019b) Cd induced generation of free radical species in *Brassica juncea* is regulated by supplementation of earthworms in the drilosphere. Sci Total Environ 655:663–675

Keunen E, Peshev D, Vangronsveld J, Van Den Ende W, Cuypers A (2013) Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. Plant Cell Environ 36:1242–1255

Khan S, Khan N, Bano B (2017) In-sights into the effect of heavy metal stress on the endogenous mustard cystatin. Int J Biol Macromol 105:1138

Kim K-R, Owens G, Kwon S (2010) Influence of Indian mustard (*Brassica juncea*) on rhizosphere soil solution chemistry in long-term contaminated soils: a rhizobox study. J Environ Sci 22:98–105

Kohli SK, Handa N, Sharma A, Gautam V, Arora S, Bhardwaj R, Alyemeni MN, Wijaya L, Ahmad P (2018) Combined effect of 24-epibrassinolide and salicylic acid mitigates lead (Pb) toxicity by modulating various metabolites in *Brassica juncea* L. seedlings. Protoplasma 255:11–24

Kumar V, Sharma PK, Jatav HS, Singh SK, Rai A, Kant S, Kumar A (2020) Organic amendments application increases yield and nutrient uptake of mustard (*Brassica juncea*) grown in chromium-contaminated soils. Commun Soil Sci Plant Anal 51:149–159

Kutrowska A, Małecka A, Piechalak A, Masiakowski W, Hanć A, Barałkiewicz D, Andrzejewska B, Zbierska J, Tomaszewska B (2017) Effects of binary metal combinations on zinc, copper, cadmium and lead uptake and distribution in *Brassica juncea*. J Trace Elem Med Biol 44:32–39

Li P, Peng X, Luan Z, Zhao T, Zhang C, Liu B (2016) Effects of red mud addition on cadmium accumulation in cole (*Brassica campestris* L.) under high fertilization conditions. J Soils Sediments 16:2097–2104

Li L, Long M, Islam F, Farooq MA, Wang J, Mwamba TM, Shou J, Zhou W (2019) Synergistic effects of chromium and copper on photosynthetic inhibition, subcellular distribution, and related gene expression in *Brassica napus* cultivars. Environ Sci Pollut Res 26:11827–11,845

Liu Y, Kong GT, Jia QY, Wang F, Xu RS, Li FB, Wang Y, Zhou HR (2007) Effects of soil properties on heavy metal accumulation in flowering Chinese cabbage (*Brassica campestris* L. ssp. chinensis var. utilis Tsen et Lee) in Pearl River Delta, China. J Environ Sci Health B 42:219–227

Lopez-Chuken UJ, Young SD, Sanchez-Gonzalez MN (2010) The use of chloro-complexation to enhance cadmium uptake by *Zea mays* and *Brassica juncea*: testing a “free ion activity model” and implications for phytoremediation. Int J Phytoremediation 12:680–696

Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li R, Zhang Z (2016a) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Saf 126:111–121

Mahar A, Wang P, Ali A, Guo Z, Awasthi MK, Lahori AH, Wang Q, Shen F, Li R, Zhang Z (2016b) Impact of CaO, fly ash, sulfur and Na2S on the (im) mobilization and phytoavailability of Cd, Cu and Pb in contaminated soil. Ecotoxicol Environ Saf 134:116–123

Mahmud J, Hasanuzzaman M, Nahar K, Rahman A, Fujita M (2019) EDTA reduces cadmium toxicity in mustard (*Brassica juncea* L.) by enhancing metal chelation, antioxidant defense and glyoxalase systems. Acta Agrobot 72:1722

Malamis S, Katsou E (2013) A review on zinc and nickel adsorption on natural and modified zeolite, bentonite and vermiculite: examination of process parameters, kinetics and isotherms. J Hazard Mater 252:428–461

Marchand L, Pelosi C, González-Centeno MR, Maillard A, Ourry A, Galland W, Teissedre P-L, Bessoule J-J, Mongrand S, Morvan-Bertrand A (2016) Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol. Chemosphere 156:150–162

Mehta D, Mazumdar S, Singh S (2015) Magnetic adsorbents for the treatment of water/wastewater—a review. J Water Process Eng 7:244–265

Miretzky P, Cirelli AF (2010) Cr (VI) and Cr (III) removal from aqueous solution by raw and modified lignocellulosic materials: a review. J Hazard Mater 180:1–19

Mishra S, Bharagava RN, More N, Yadav A, Zainith S, Mani S, Chowdhary P (2019) Heavy metal contamination: an alarming threat to environment and human health. In: Environmental biotechnology: for sustainable future. Springer, Singapore, pp 103–125

Mnasri M, Ghabriche R, Fourati E, Zaier H, Sabally K, Barrington S, Lutts S, Abdelly C, Ghnaya T (2015) Cd and Ni transport and accumulation in the halophyte *Sesuvium portulacastrum*: implication of organic acids in these processes. Front Plant Sci 6:156

Mobasherpour I, Salahi E, Ebrahimi M (2012) Removal of divalent nickel cations from aqueous solution by multi-walled carbon nano tubes: equilibrium and kinetic processes. Res Chem Intermed 38:2205–2222

Mourato MP, Moreira IN, Leitão I, Pinto FR, Sales JR, Martins LL (2015) Effect of heavy metals in plants of the genus *Brassica*. Int J Mol Sci 16:17975–17,998

Mwamba T, Ali S, Ali B, Lwalaba J, Liu H, Farooq M, Shou J, Zhou W (2016) Interactive effects of cadmium and copper on metal accumulation, oxidative stress, and mineral composition in *Brassica napus*. Int J Environ Sci Technol 13:2163–2174

Niu L-q, Jia P, Li S-p, Kuang J-l, He X-x, Zhou W-h, Liao B, Shu W-s, Li J-t (2015) Slash-and-char: an ancient agricultural technique holds new promise for management of soils contaminated by Cd, Pb and Zn. Environ Pollut 205:333–339

Nouairi I, Ammar WB, Youssef NB, Daoud DBM, Ghorbal MH, Zarrouk M (2006) Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. Plant Sci 170:511–519

Nouairi I, Ammar WB, Youssef NB, Miled DDB, Ghorbal MH, Zarrouk M (2009) Antioxidant defense system in leaves of Indian mustard (*Brassica juncea*) and rape (*Brassica napus*) under cadmium stress. Acta Physiol Plant 31:237–247

Park J, Kim J-Y, Kim K-W (2012) Phytoremediation of soil contaminated with heavy metals using *Brassica napus*. Geosystem Eng 15:10–18

Paul AL, Chaney RL (2017) Effect of soil amendments on Cd accumulation by spinach from a Cd-mineralized soil. J Environ Qual 46:707–713

Pérez-Esteban J, Escolástico C, Moliner A, Masaguer A, Ruiz-Fernández J (2014) Phytostabilization of metals in mine soils using *Brassica juncea* in combination with organic amendments. Plant Soil 377:97–109

Perotti R, Paisio CE, Agostini E, Fernandez MI, González PS (2020) CR(VI) phytoremediation by hairy roots of *Brassica napus*: assessing efficiency, mechanisms involved, and post-removal toxicity. Environ Sci Pollut Res 27:9465–9474

Pichhode M, Asati A (2020) Effect of heavy metals on plants: an overview. Int J Appl Innov Eng Manage 5:2319

Purakayastha T, Viswanath T, Bhadraray S, Chhonkar P, Adhikari P, Suribabu K (2008) Phytoextraction of zinc, copper, nickel and lead from a contaminated soil by different species of *Brassica*. Int J Phytoremediation 10:61–72

Rahmani F, Peymani A, Daneshvand E, Biparva P (2016) Impact of zinc oxide and copper oxide nano-particles on physiological and molecular processes in *Brassica napus* L. Indian J Plant Physiol 21:122–128

Rai R, Agrawal M, Agrawal S (2016) Impact of heavy metals on physiological processes of plants: with special reference to photosynthetic system. In: Plant responses to xenobiotics. Springer, Singapore, pp 127–140

Rai PK, Lee SS, Zhang M, Tsang YF, Kim K-H (2019) Heavy metals in food crops: health risks, fate, mechanisms, and management. Environ Int 125:365–385

Ranieri E, Moustakas K, Barbafieri M, Ranieri AC, Herrera-Melián JA, Petrella A, Tommasi F (2020) Phytoextraction technologies for mercury-and chromium-contaminated soil: a review. J Chem Technol Biotechnol 95:317–327

Rastgoo L, Alemzadeh A (2011) Biochemical responses of Gouan (‘*Aeluropus littoralis*’) to heavy metals stress. Aust J Crop Sci 5:375

Reeves RD, van der Ent A, Baker AJ (2018) Global distribution and ecology of hyperaccumulator plants. In: Agromining: farming for metals. Springer, Cham, pp 75–92

Remigio AC, Chaney RL, Baker AJ, Edraki M, Erskine PD, Echevarria G, van der Ent A (2020) Phytoextraction of high value elements and contaminants from mining and mineral wastes: opportunities and limitations. Plant Soil 449(1-2):11

Rezania S, Taib SM, Din MFM, Dahalan FA, Kamyab H (2016) Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. J Hazard Mater 318:587–599

Riess ML, Halm JK (2007) Lead poisoning in an adult: lead mobilization by pregnancy? J Gen Intern Med 22:1212–1215

Rivera-Martin A, Broadley MR, Poblaciones MJ (2020) Soil and foliar zinc application to biofortify broccoli (*Brassica oleracea* var. italica L.): effects on the zinc concentration and bioavailability. Plant Soil Environ 66:113–118

Rizwan M, Ali S, ur Rehman MZ, Rinklebe J, Tsang DC, Bashir A, Maqbool A, Tack F, Ok YS (2018) Cadmium phytoremediation potential of *Brassica* crop species: a review. Sci Total Environ 631-632:1175–1191

Rodríguez-Vila A, Asensio V, Forján R, Covelo EF (2016) Assessing the influence of technosol and biochar amendments combined with *Brassica juncea* L. on the fractionation of Cu, Ni, Pb and Zn in a polluted mine soil. J Soils Sediments 16:339–348

Sahay S, Inam A, Iqbal S (2019) Risk analysis by bioaccumulation of Cr, Cu, Ni, Pb and Cd from wastewater-irrigated soil to Brassica species. Int J Environ Sci Technol 17:2889

Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, Rehim A, Hussain S (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721

Seneviratne M, Rajakaruna N, Rizwan M, Madawala H, Ok YS, Vithanage M (2019) Heavy metal-induced oxidative stress on seed germination and seedling development: a critical review. Environ Geochem Health 41:1813–1831

Sharma SS, Dietz K-J (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726

Sharma A, Sidhu GPS, Araniti F, Bali AS, Shahzad B, Tripathi DK, Brestic M, Skalicky M, Landi M (2020) The role of salicylic acid in plants exposed to heavy metals. Molecules 25:540

Sharmila P, Kumari PK, Singh K, Prasad N, Pardha-Saradhi P (2017) Cadmium toxicity-induced proline accumulation is coupled to iron depletion. Protoplasma 254:763–770

Shi Y, Xie H, Cao L, Zhang R, Xu Z, Wang Z, Deng Z (2017) Effects of Cd-and Pb-resistant endophytic fungi on growth and phytoextraction of *Brassica napus* in metal-contaminated soils. Environ Sci Pollut Res 24:417–426

Shrivastava M, Khandelwal A, Srivastava S (2019) Heavy metal hyperaccumulator plants: the resource to understand the extreme adaptations of plants towards heavy metals. In: Plant-metal interactions. Springer, Cham, pp 79–97

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6:1143

Singh S, Singh VP, Prasad SM, Sharma S, Ramawat N, Dubey NK, Tripathi DK, Chauhan DK (2019) Interactive effect of silicon (si) and salicylic acid (sa) in maize seedlings and their mechanisms of cadmium (cd) toxicity alleviation. J Plant Growth Regul 38:1587–1597

Soares TFSN, dos Santos Dias DCF, Oliveira AMS, Ribeiro DM, dos Santos Dias LA (2020) Exogenous brassinosteroids increase lead stress tolerance in seed germination and seedling growth of *Brassica juncea* L. Ecotoxicol Environ Saf 193:110296

Souri Z, Cardoso AA, da Silva CJ, de Oliveira LM, Dari B, Sihi D, Karimi N (2019) Heavy metals and photosynthesis: recent developments. In: Photosynthesis, productivity and environmental stress. Wiley, Hoboken, pp 107–134

Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2019) Phytohormone priming: regulator for heavy metal stress in plants. J Plant Growth Regul 38:739–752

Szabados L, Savoure A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97

Tang W-W, Zeng G-M, Gong J-L, Liang J, Xu P, Zhang C, Huang B-B (2014) Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: a review. Sci Total Environ 468:1014–1027

Tang X, Li X, Liu X, Hashmi MZ, Xu J, Brookes PC (2015) Effects of inorganic and organic amendments on the uptake of lead and trace elements by *Brassica chinensis* grown in an acidic red soil. Chemosphere 119:177–183

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. Exp Suppl 101:133–164

Theriappan P, Gupta A, Dhasarathan P (2011) Accumulation of proline under salinity and heavy metal stress in cauliflower seedlings. J Appl Sci Environ Manag 15:251–255

Thiruvengadam M, Chung I-M (2015) Selenium, putrescine, and cadmium influence health-promoting phytochemicals and molecular-level effects on turnip (*Brassica rapa* ssp. rapa). Food Chem 173:185–193

Venkatesh T (2004) The effects of environmental lead on human health-a Challenging Scenario. Health Focus 2:8–16

Verkerk R, Schreiner M, Krumbein A, Ciska E, Holst B, Rowland I, De Schrijver R, Hansen M, Gerhäuser C, Mithen R (2009) Glucosinolates in *Brassica* vegetables: the influence of the food supply chain on intake, bioavailability and human health. Mol Nutr Food Res 53:S219–S219

Wang L, Hou D, Shen Z, Zhu J, Jia X, Ok YS, Tack FM, Rinklebe J (2019) Field trials of phytomining and phytoremediation: a critical review of influencing factors and effects of additives. Crit Rev Environ Sci Technol:1–51. https://doi.org/10.1080/10643389.2019.1705724

Yang Y, Nan Z, Zhao Z, Wang S, Wang Z, Wang X (2011) Chemical fractionations and bioavailability of cadmium and zinc to cole (*Brassica campestris* L.) grown in the multi-metals contaminated oasis soil, northwest of China. J Environ Sci 23:275–281

Yao A, Wang Y, Ling X, Chen Z, Tang Y, Qiu H, Ying R, Qiu R (2017) Effects of an iron-silicon material, a synthetic zeolite and an alkaline clay on vegetable uptake of As and Cd from a polluted agricultural soil and proposed remediation mechanisms. Environ Geochem Health 39:353–367

Yu C-Y (2013) Molecular mechanism of manipulating seed coat coloration in oilseed *Brassica* species. J Appl Genet 54:135–145

Zaid A, Mohammad F, Wani SH, Siddique KM (2019) Salicylic acid enhances nickel stress tolerance by up-regulating antioxidant defense and glyoxalase systems in mustard plants. Ecotoxicol Environ Saf 180:575–587

Zaier H, Mudarra A, Kutscher D, De La Campa MF, Abdelly C, Sanz-Medel A (2010) Induced lead binding phytochelatins in *Brassica juncea* and *Sesuvium portulacastrum* investigated by orthogonal chromatography inductively coupled plasma-mass spectrometry and matrix assisted laser desorption ionization-time of flight-mass spectrometry. Anal Chim Acta 671:48–54

Zaurov DE, Perdomo P, Raskin I (1999) Optimizing soil fertility and ph to maximize cadmium removed by Indian mustard from contaminated soils. J Plant Nutr 22:977–986

Zhang L, Wu J, Tang Z, Huang X-Y, Wang X, Salt DE, Zhao F-J (2019a) Variation in the BrHMA3 coding region controls natural variation in cadmium accumulation in *Brassica rapa* vegetables. J Exp Bot 70:5865–5878

Zhang M, Ran R, Nao WS, Feng Y, Jia L, Sun K, Wang R, Feng H (2019b) Physiological effects of short-term copper stress on rape (*Brassica napus* L.) seedlings and the alleviation of copper stress by attapulgite clay in growth medium. Ecotoxicol Environ Saf 171:878–886

Zheng B-X, Ding K, Yang X-R, Wadaan MA, Hozzein WN, Peñuelas J, Zhu Y-G (2019) Straw biochar increases the abundance of inorganic phosphate solubilizing bacterial community for better rape (*Brassica napus*) growth and phosphate uptake. Sci Total Environ 647:1113–1120

Zhou Y-T, Nie H-L, Branford-White C, He Z-Y, Zhu L-M (2009) Removal of Cu^{2+} from aqueous solution by chitosan-coated magnetic nanoparticles modified with α-ketoglutaric acid. J Colloid Interface Sci 330:29–37

Zlobin IE, Kartashov AV, Shpakovski GV (2017) Different roles of glutathione in copper and zinc chelation in *Brassica napus* roots. Plant Physiol Biochem 118:333–341

Zong H, Li K, Liu S, Song L, Xing R, Chen X, Li P (2017a) Improvement in cadmium tolerance of edible rape (*Brassica rapa* L.) with exogenous application of chitooligosaccharide. Chemosphere 181:92–100

Zong H, Liu S, Xing R, Chen X, Li P (2017b) Protective effect of chitosan on photosynthesis and antioxidative defense system in edible rape (*Brassica rapa* L.) in the presence of cadmium. Ecotoxicol Environ Saf 138:271–278

Molecular Basis of Plant–Microbes Interaction in Remediating Metals and Inorganic Pollutants

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Abstract

In order to find an economical and environmentally sustainable solution, the synergistic (plant–microbe) interaction plays an important role for management of heavy metal impacted soils and inorganic pollutants. Bacterial assisted phytoremediation has a great potential for the restoration of metal contaminated environments. Resistance to multiple toxic heavy metals as well as production of plant growth promoting substances such as siderophores, ACC deaminase, and indole acetic acid are important attributes of microbes. These mechanisms have the potential for enhancing plant growth in metal stressed environments with important implications for phytoremediation. In this chapter, we have focused on molecular mechanism in phytoremediation, microbial interactions with metals and inorganic contaminants, plant–microbe interactions in phytoremediation, and symbiosis for phytoremediation of heavy metals by summarizing current knowledge of the field, covering all aspects of plant–microbes interaction in remediating metals and inorganic pollutant.

Keywords

Plant–microbes interaction · Inorganic pollutants · Molecular mechanism · PGPR characteristics · Phytoremediation

Abbreviation

ACC	1-Amino Cyclopropane-1-Carboxylate.
AMF	Arbuscular Mycorrhiza Fungi.
DTA	Diethylene Triamine Pentaacetic Acid.
EDDS	Ethylene Diamine Di Succinate.
EDTA	Ethylene Diamine Tetraacetic Acid.
HEDTA	N-Hydroxy Ethylene Diamine Triacetic Acid.
LMWC	Low Molecular Weight Compounds.
MTs	Metallothioneins.
NTA	Nitrilo Tri Acetate.
PCs	Phytochelatins.
PGPB	Plant Growth Promoting Bacteria.

1 Introduction

Rapid increase in industrialization and urbanization led to the release of contaminants both organic and inorganic in environment (WHO 2017). Among all pollutants, inorganic contaminates such as heavy metals are difficult to handle due to their non-biodegradability and persistent nature. These inorganic contaminates are naturally present in trace amounts but the anthropogenic activities are the main sources of high release of these contaminates in environment (Khan et al. 2017). Burning of fossil fuels, excessive use of fertilizers and pesticides, paints, vehicular emissions, smelting of ores are some sources of inorganic contamination (Zhang et al. 2018). These unwanted substances in the soil, water, and air affect the living organisms such as microorganisms, plants, animals, and human through food chain. The removal of these contaminates is very important for healthy environment. In this regard, phytoremediation is widely used for the removal of inorganic contaminates from different medium either through stabilization or extraction using hyperaccumulator plants (Chhajro et al. 2016).

For the efficient removal of contaminates, synthetic and natural chelating agents have been used to increase the removal ratio. However, the toxic effects of synthetic chelating agents have been observed, therefore microorganisms have been used to increase the solubility/phytoextraction of heavy metals. The fate of heavy metals in soils is directly and/or indirectly governed by soil microorganisms (bacteria and fungi). These microorganisms and their extracellular enzymes are responsible for dissolution, precipitation, complex formation, and stabilization of heavy metals in soil. Different studies have explored the functional ability of these bacteria and fungi for improved phytoextraction of heavy metals. Many soil bacteria like *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ralstonia metallidurans*, *Bacillus edaphicus*, *Bradyrhizobium sp.*, *Ochrobactrum cytisi*, *Azotobacter chroococcum*, *Bacillus megaterium*, and *Bacillus mucillaginosus* have been found efficient in increasing metal uptake and plant growth in different studies (Wu et al. 2003; Sheng et al. 2008; Braud et al. 2009; Dary et al. 2010). In phytoremediation, the intrinsic ability of plant is responsible for rhizosphere modification and uptake of metals. Plants can modify the rhizosphere through excretion of root exudates. The application of metal resistant, solubilizing, and plant growth promoting microorganisms (fungi and bacteria) can alleviate the toxicity of metals by promoting plant growth under elevated soil metals concentration and increase the phytoavailability and subsequent uptake by plant.

2 Phytoremediation

Phytoremediation (“Phyto–plant; remedium–restoring balance”) is the ecofriendly technology in which green plants are used for the removal of contaminants from the soil (Arshad et al. 2016; Dubchak and Bondar 2019). Nowadays, phytoremediation is widely used as an alternative method to chemical-based, energy intensive, conventional instrument, and expensive technologies for the restoration of contaminated soils. It is low cost, environment friendly, nondestructive, and natural technique to remove different pollutants from soil (Rehman et al. 2017; Manzoor et al. 2018) as compared to other physical and chemical approaches. Phytoremediation is used for the removal of both organic and inorganic contaminants by different mechanisms (Fig. 1) such as phytoextraction (uptake and accumulation of inorganic contaminants), phytovolatilization (conversion of volatile contaminants into less toxic form), and phytodegradation (degradation of organic contaminants).

For the removal of inorganic contaminants, phytoextraction has been widely used. Phytoextraction is “green technology that removes pollutant from

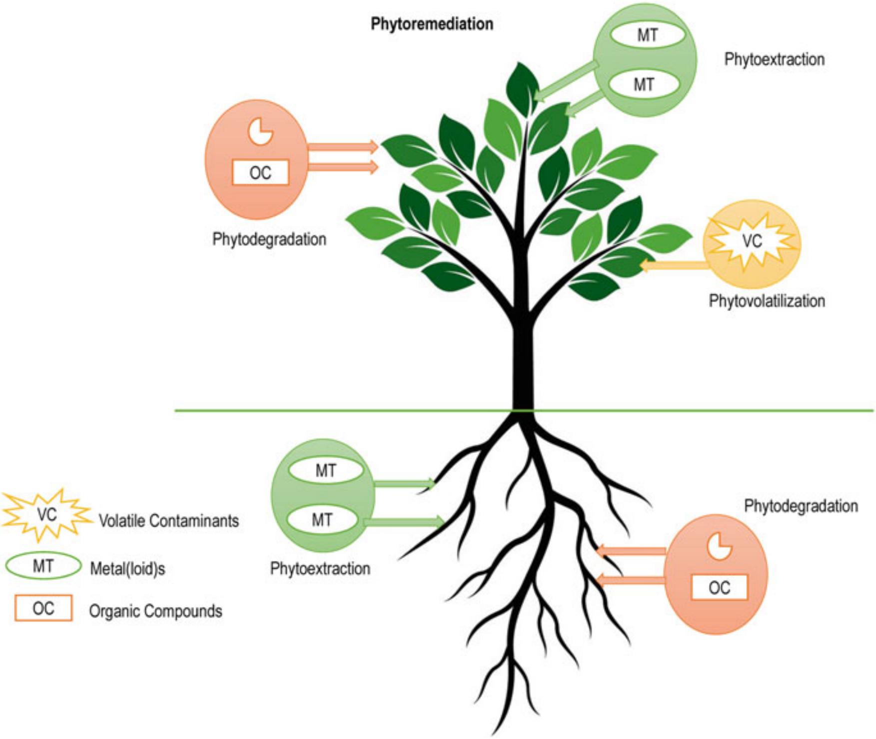

Fig. 1 Phytoremediation methods for the removal of organic and inorganic contaminants

contaminated soil by plant, assimilate and translocate to harvestable plant parts" (Chhajro et al. 2016). Due to the non-biodegradability of heavy metals, it is necessary to completely remove toxic heavy metals from the soil. For this purpose, phytoextraction is considered as a best approach, as in this technique, heavy metals are absorbed by the plant roots, transfer and accumulated in the shoots part, which is easy to handle (Bhargava et al. 2012; Sheoran et al. 2016). Metal tolerant plants also known as metallophytes can be classified into three major categories (Baker and Brooks 1989): (a) excluders, in which metal concentrations in the shoot are kept lower than metal concentration in soil; (b) accumulators, in this strategy, plants concentrate metals in above-ground plant parts; and (c) indicators, in which plants maintain a linear relationship between plant and soil metal concentrations.

Phytoextraction is the inherent ability of plants to sequester high amounts of toxic heavy metal though roots, transport and accumulate in above-ground tissues. There is a certain group of plants that can accumulate high metal concentration in aerial parts of plants without compromising biomass loss. These plants are termed as hyperaccumulators. The threshold metal concentration in plants for different metals in hyperaccumulator plant species are given in Table 1.

Table 1 Threshold metal concentrations in plants for different metals in hyperaccumulator plant species

Heavy metals	Threshold levels (mg kg^{-1}) Baker and Brooks (1989)	Revised levels (mg kg^{-1}) Van der Ent et al. (2013)
Arsenic (As)	1000	1000
Cadmium (Cd)	100	100
Cobalt (Co)	1000	300
Copper (Cu)	1000	300
Chromium (Cr)	1000	300
Lead (Pb)	1000	1000
Manganese (Mn)	10,000	10,000
Nickel (Ni)	1000	1000
Zinc (Zn)	10,000	3000

Several plant species including edible and ornamental plants have ability to uptake and accumulate high amount of heavy metals and inorganic contaminants in shoots. Some of the heavy metals hyperaccumulators are listed in Table 2.

The efficiency of phytoremediation depends on various soil and plant factors such as physicochemical characteristics of soil (pH, organic matter, dissolve organic carbon, texture), availability of heavy metals for plants uptake, capability of plant to uptake and accumulate heavy metals in shoots without toxic effects and the soil microorganisms (Shahid et al. 2014; Zhang et al. 2018; Gul et al. 2019).

3 Molecular Mechanism in Phytoremediation

Once plants have taken up the heavy metals through roots, they must have ability to transfer contaminants to shoots and stored in the vacuole. This process mainly comprises three steps such as (1) uptake of available metals from soil through roots; (2) transfer of metals through various transporter to the aerial parts; (3) finally, the transfer metals are accumulated in the vacuole (Mahmood 2010).

First step of the process is the uptake of heavy metals through roots. In this process only the available portion of metal is taken up by roots in solution. The uptake of metals depends on many factors such as water content, soil pH, organic substance and the most important is the available amount of metals. The metals are present in various forms including free ions, complexes, bonded with inorganic and organic compounds, or as a compounds like hydroxide, oxides, or silicates (Salt et al. 1995; Manzoor et al. 2019). Among all these form of metals, only soluble form is available for the plants uptake and the remaining bounded forms are taken up by the application of soil amendments (Gul et al. 2019). The available metals enter into the roots either through apoplastic or symplastic pathways. The soluble metals pass through intracellular spaces without entering into cell during the apoplastic pathway;

Table 2 Some hyperaccumulators of heavy metals and inorganic contaminants

Contaminants	Plant species	Concentration in shoots mg kg^{-1}	References
As	*Pteris vittata*	4106	Wan et al. (2016)
	Pteris vittata	6017	Han et al. (2016)
	Eleocharis acicularis	1470	Sakakibara et al. (2011)
Cd	*Pelargonium hortorum* *Pelargonium zonale*	262 186	Gul et al. (2019)
	Pelargonium roseum	1957	Mahdieh et al. (2013)
	Arabidopsis halleri	5722	Kupper et al. (2000)
	Viola principis	1201	Wan et al. (2016)
	Thlaspi caerulescens	263	Lombi et al. (2002)
	Solanum photeinocarpum	158	Zhang et al. (2011)
	Solanum nigrum	387	Sun et al. (2008)
Pb	*Pelargonium hortorum* *Mesembryanthemum criniflorum*	1028 1269	Manzoor et al. (2018)
	Viola principis	2350	Wan et al. (2016)
	Brassica juncea	10,300	Koptsik (2014)
	Pelargonium (*Atomic*)	7000	Arshad et al. (2008)
	Pelargonium hortorum *Pelargonium zonale*	1611 1545	Gul et al. (2019)
Ni	*Alyssum corsicum*	18,100	Li et al. (2003)
	Salvinia minima	16,600	Fuentes et al. (2014)
	Phyllanthus serpentinus	38,100	Chaney et al. (2010)

however, in symplastic pathway metals cross the cytoplasm (Lu et al. 2009). The absorption of heavy metal through roots is regulated by Zn and iron-regulated transporter proteins (ZIP).

The second step is the translocation of metals to the shoots. Generally, non-hyperaccumulator plants do not have capability to transfer metals to the shoots, therefore, metals are accumulated in roots. However, in hyperaccumulator plants, metals enter into xylem channel and along with water they are transferred to the shoots. It is an energy consuming process and the metals which enter through symplast pathway can only be loaded in xylem channels (Mahmood 2010).

The last step is the accumulation of metal into cell organelle. The metals are transferred to shoots and reach the leaves; before storage they are detoxified by ligands such as phytochelatins (PCs) and metallothioneins (MTs). Different metal accumulated in different parts of cell such as some metals accumulated in cell wall and some in vacuole. The prominent part for the metal accumulation is vacuole (Ghori et al. 2016).

4 Microbial Interactions with Metals and Inorganic Contaminants

Metals can be introduced into the environment naturally through erosion of rocks and volcanic eruption. Though, anthropogenic activities are mainly responsible for metal contamination, the main sources for the distribution of these metals into soil, water, and air include transportation, industrial effluents, mining, fossil fuel combustion, smelting and refining of metallic minerals, and so on. In addition, agrochemicals such as phosphate fertilizers and pesticides contained trace amount of different heavy metals are considered as an important source of agricultural soil's contamination (Agarwal 2009). Microbes can absorb heavy metals at the cell binding sites. The extracellular polymers of these microbes can form heavy metals complex by several ways. These specialized microorganisms can mineralize organic pollutants in metabolic intermediates, which are used as primary substrates for cell growth. Microbes present in soils contaminated with high amount of metals can modify the oxidation state of heavy metals by immobilizing them (Dixit et al. 2015), which makes them easy to remove. Some of the sources of metals and inorganic contaminants are shown in Fig. 2.

Some metals used as micronutrients, such as cobalt (Co), copper (Cu), and nickel (Ni), participate in numerous metabolic processes including osmosis regulation in the cell, act as a constituent of metalloenzymes, while others are not essential as they do not play any role in the growth and development, and are considered as very toxic substances (Bruins et al. 2000). The essential metals/metalloids above the required concentrations or non-essential metals at low concentrations have harmful effects (Nies 1999). These trace metals might affect the microbial cell by injuring the cell membrane, damaging the DNA' structure, disturbing cell functions, which affects enzymatic activity like dehydrogenase activity, microbial biomass, and basal respiration. But, some microorganisms have ability to develop a number of resistance mechanisms for dealing with stresses (metal). The metal resistance genes could be located on transposons, plasmids, or chromosomes (Bruins et al. 2000). Detoxification systems for the metal immobilization/mobilization or transformation have been developed as the resistance (Rajkumar et al. 2010).

5 Plant–Microbe Interactions in Phytoremediation

Plants and microorganisms cohabit or strive for existence; their correlated relations play a vital role in acclimatizing to metallic environs. Therefore, they can be explored for improving the microbial phytoremediation. The plant root exudates are beneficial sources of nutrients for microorganisms, with which they create complex communication systems. Some beneficial microorganism including fungi

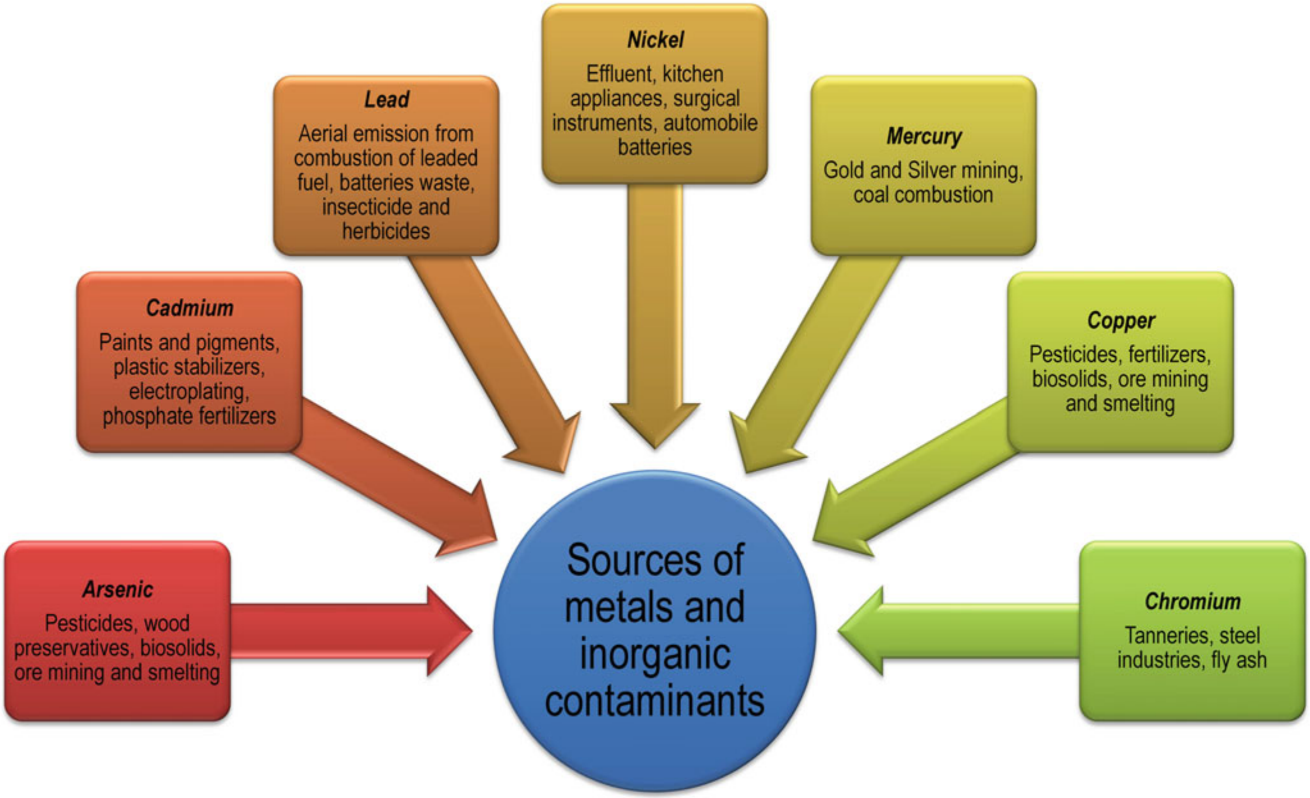

Fig. 2 Sources of metals and inorganic contaminants

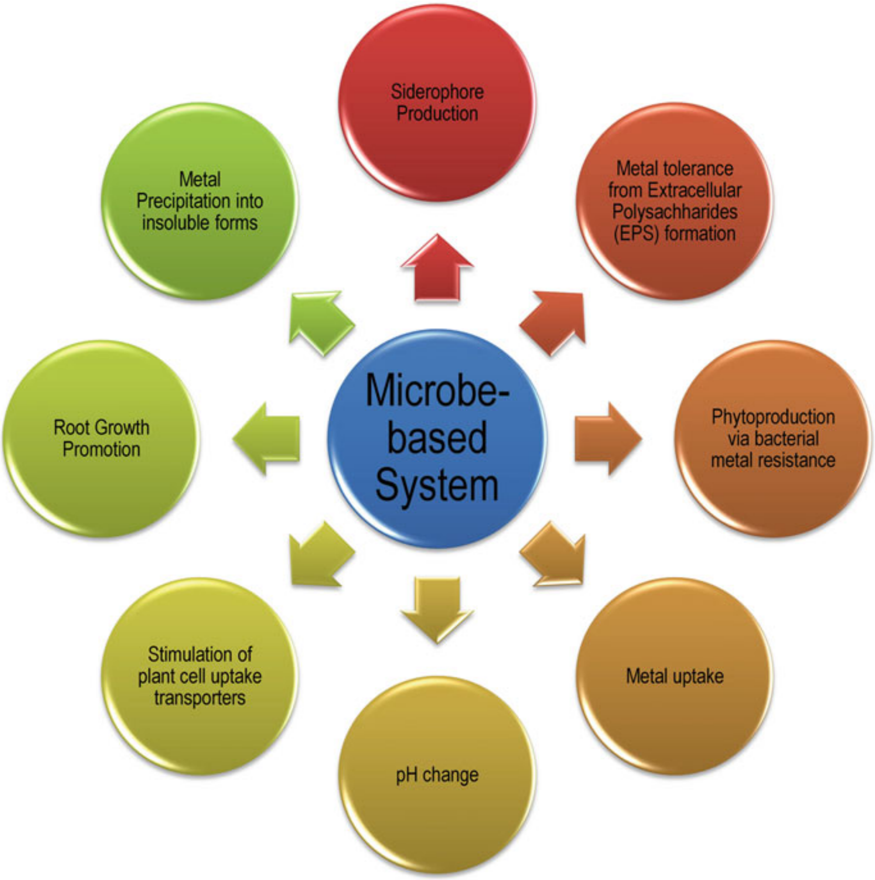

Fig. 3 Mechanisms of plant–microbe interactions that support metal phytoremediation (Stout and Nusslein 2010)

and bacteria, that promote plant growth, can attenuate the phytotoxicity of metals and indirectly stimulate plant growth by inducing defense mechanisms against plant pathogens and/or directly from plant growth through solubilization of mineral nutrients including iron (Fe), nitrogen (N), potassium (K), and phosphate (P), etc., the production of substances that promote plant growth (e.g., phytohormones) and the secretion of specific enzymes such as 1-aminocyclopropane-1-carboxylate deaminase (Ma et al. 2016). PGPM can also alter the bioavailability of the metal in soil through a variety of mechanisms, for instance, acidification, precipitation, redox reaction, chelation, and complexation. Some of the mechanisms of plant–microbe interactions that support metal phytoremediation are shown in Fig. 3.

Bioremediation with microbes or plants are economical and substitute method for the removal of heavy metals from the contaminated sites as compared to costly conventional techniques including both physical and chemical approaches (Mistry et al. 2010). The use of either plant or microbes for decontamination of metals contaminated sites has certain limitations in terms of metals extraction ratio and time. However, combined interaction between the plants and microbes, particularly bacteria that promote plant growth and root growth, can have a positive impact on promoting plant growth and eliminating pollutants from the contaminated sites (De-Bashan et al. 2012). One approach is to select natural plants that tolerate pollutants or to improve them by genetic engineering for greater tolerance to the toxic pollutants and high biomass of roots and shoots. Additional approaches might include the selection or modification of microorganism for this purpose, which can detoxify contaminants, have a favorable impact on the plant growth, or may also affect the bioavailability of contaminants (Wenzel 2009).

5.1 Plant Growth Enhancement

The groups of microorganism that inhabit plant roots are capable for the utilization of available nutrients as well as provide all necessary substances that are required for enhancing the plant growth. From the literature it has been observed that plants in combination with microbes have positive impacts on the removal of metals from the environment (Bothe 2011). In general, the plant growth promoting bacteria, participate in the phytoremediation process in two ways: (1) directly facilitate the absorption of the metal in the plant (phytoextraction) or reduce the phytoavailability of the metal contaminant for the plant (phytostabilization), and (2) indirectly, improve the biomass of the plant by conferring tolerance to the stress of the plant metal to the plant and its preferment of growth (Shahid et al. 2014).

From the above-discussed processes for the bacteria-assisted heavy metal phytoremediation, phytostabilization and phytoextraction appeared to be more effective processes for the complete removal of heavy metals from the environment. In "Phytoextraction" plants able to accumulate high amount of metals and translocate to the aerial parts are used. The above-ground part of plant in which metals are accumulated can be easily collected, handled, and disposed of either by incineration or other appropriate approaches. Although, in "phytostabilization" the metals are immobilized by the beneficial plants and microorganisms resistant to metals. The bioavailability of metals is decreased by stabilizing the metals through modification in soil; the phytoavailability of metals decreased at alkaline pH (Rajkumar et al. 2012). Furthermore, these resistant microbes might be used as inoculants for significant crops to decrease the toxic impacts of metals on agricultural land, and on crops and, at the same time also increase the crop yield and health.

Microorganisms associated with plants that accumulate metal help the plant to deal with contaminants by promoting plant growth through phosphate solubilization, N_2 fixation, siderophores production, organic acid, phytohormones. Plant growth promoting bacteria (PGPB) such as those microbes present in the rhizosphere, that are directly associated with the plants, could be classified into two different groups. Group one includes those PGPB that have direct impacts on the improvement of plant growth and development by increasing the phytoavailability of nutrients, further increase the production of different plant hormones like auxins (indole acetic acid is an auxin for various microbes) and siderophores for the phytoavailability of iron. Other group of PGPB includes those bacteria that contribute indirectly to plant condition by providing resistance to diseases against phytopathogens, known as "Biocontrol PGPB" (Hayat et al. 2010).

5.2 Metal Bioavailability

The removal of heavy metals from soil is mainly depends on the available amount of heavy metals to the plants. Plant could only uptake the bioavailable amount of inorganic pollutants and heavy metals through roots and transfer to the shoots (Rizwan et al. 2016). Various factors are involved in increasing the bioavailability of heavy metals such as soil pH, organic matter, soil texture, and dissolved organic carbon.

5.2.1 pH Modification

Soil pH plays an imperative role in increasing or decreasing the bioavailability of heavy metals. Generally, heavy metals are bounded to the soil particles and other compounds at alkaline pH and they become detached at lower or acidic pH. Therefore, soil pH plays crucial role in the mobility or stability of heavy metals (Arshad et al. 2016). Soil pH is affected by the activities of both microorganism and plants. The excretion of hydrogen ion from plant roots detached the metals from soil, acidify the rhizospheric soil pH (Kim et al. 2010), ultimately enhancing the mobility of heavy metals. *Pelargonium hortorum* lowers the soil pH by releasing hydrogen ions (Arshad et al. 2016). A recent study showed that inoculation of endophytic bacterium *Sphingomonas SaMR12* synchronized root exudates from *S. alfredii*, hence improving the Cd phytoavailability and uptake (Chen et al. 2014). In another study it has been reported that inoculation of Microbacterium sp. and Klebsiella sp. increased the Pb uptake by *Pelargonium hortorum* (Manzoor et al. 2019). This increase in Pb uptake was attributed by lowering soil pH and increasing the Pb phytoavailability.

5.2.2 Chelation

Chelating agents have been extensively studied for enhancing the metals phytoavailability and subsequent uptake by plants. The most important chelates that successfully improved the phytoextraction characteristics of treated plants include diethylenetriaminepentaacetic acid (DTPA), ethylenediaminedisuccinate (EDDS), ethylene diamine tetraacetic acid (EDTA), N-hydroxy ethylenediaminetriacetic acid (HEDTA), nitrilotriacetate (NTA), and citric acid. Application of EDTA (3.148 mmol kg^{-1}) increased shoot Pb concentration from 680.56 to 1905.57 mg kg^{-1} in *Bidens maximowicziana* (Wang et al. 2007). In another study, EDTA at 5 mmol kg^{-1} increased Pb uptake by 8.9–fold and 13.3–fold in two cultivars of *Ricinus communis* L. (Zibo-3 and Zibo-9) compared to control plants (Zhang et al. 2016).

From the literature, it is evident that EDTA is efficient in enhancing the metals mobilization and uptake. This increase in metal mobilization upon chelates application may be explained by two mechanisms: (1) adsorption of metals on free EDTA; (2) detachment of metals ions from minerals, increasing the mobilization by partially weakening the soil structure (Shahid et al. 2014).

Other chelating agents have been also used for enhancing the metals uptake by plants. Han et al. (2016) reported that application of 2 mmol kg^{-1} citric acid, significantly increased shoot, root biomass, chlorophyll a, and tolerance index by 42.8%, 51.6%, 5.1%, 11%, respectively. Recently, a study has been conducted to compare citric acid, EDDS, and EDTA in increasing the accumulation of Pb and Cd in castor bean. The application of EDDS increased plant biomass of Zibo-9 and Zibo-3 by 57.6% and 28.9%, respectively. However, biomass of Zibo-9 and Zibo-3 was decreased by 33%, 20.6% and 19.8%, 9.4%, upon EDTA and citric acid applications, respectively (Zhang et al. 2016). The inorganic fertilizers such as ammonium nitrate or urea are commonly used to increase the plant growth and yield by providing essential nutrient. Further the N-containing fertilizers lower the soil pH, ultimately increase the phytoavailability of heavy metals. In a study, it has been reported that Pb and Cd accumulation and biomass of plant were increased upon application of ammonium nitrate (Ortiz et al. 2006).

5.3 Metal Stabilization

Some microorganisms have the ability to decrease the uptake of metals by plants and translocate to the shoots by reducing their bioavailability by increasing soil pH, formation of complex compounds, or precipitation.

5.3.1 Complexation

The EPS excreted by plant related microbes are of unique importance for the formation of defensive barrier against damaging effects of metals through metal biosorption (Hou et al. 2013). The mechanisms involved in metal biosorption include adsorption, metal ion exchange, precipitation, and complexation with negatively charged functional groups (Zhang et al. 2006). EPS extracted from bacterial cultures were less efficient in metals complexation than those from sludge (Guibaud et al. 2005). However, the bacterial EPS showed high capability for metal binding and protect bacteria from metal stress. Furthermore, arbuscular mycorrhiza fungi (AMF) could produce an insoluble metal-sorbing glycol protein (glomalin) that are helpful in reducing the metal mobility and ultimately stabilized the metals in soil and therefore, considered as biostabilizer (Vodnik et al. 2008).

5.3.2 pH Modification

As discussed previously, that soil pH plays a very important role in the mobility or stability of metals. At alkaline pH the metals are bound to organic content and are not available for the plant uptake. Plants and microorganisms play key role in modification of soil pH. Some Arbuscular mycorrhizal fungi and bacteria have ability to reduce the metal availability by alkalization. The alkalinizing effect induced by Arbuscular mycorrhizal fungi through the release of OH^- can result in an active nitrate uptake by microbes and a reduction in metal phytoavailability in the rhizosphere by secreting glomalin (Giasson et al. 2008). These microorganism act as a metal sinks and create a suitable environment for the plants by reducing availability of metals.

6 Metal Uptake and Detoxification

A few microorganisms have adjusted themselves to the environment containing high amount of metals, like water, soil, or sediments having ample amount of natural metals or anthropogenic sources, like industrial effluents (Peijnenburg and Vijver 2007). Natural existence of different heavy metals has allowed microbes to adjust themselves to the different concentrations of heavy metals. They have established operative means for the regulation of essential elements (metals) within the cells. These homeostatic essential metal regulation mechanisms might play an important role in the detoxification of certain non-essential metals such as cadmium (Cd), mercury (Hg), and lead (Pb). Stress proteins can be prompted in the presence of toxic concentrations of metals (Bruins et al. 2000). Likewise, plants have also the ability to adapt to high levels of metals. Plants need numerous macronutrients such as nitrogen (N), phosphorous (P), potassium (K) and micronutrients including copper (Cu), iron (Fe), nickel (Ni), manganese (Mn), molybdenum (Mo), and zinc (Zn) for proper growth and development. They have certain nutrient retention mechanisms, like membrane transport proteins that perceive particular ions and facilitate their transport through the plant's cell membrane. Nonetheless, in some circumstances with high metal concentration in the surrounding, toxic or non-essential metals can also be absorbed (Kamaludeen and Ramasamy 2008).

The microorganisms that live near the root zone of the plant have a much greater capacity to detoxify the xenobiotics than those present in the loose soil. As the vegetable exudates act as nutritional substrates for them and, therefore, they can carry out degradation or detoxification by co-metabolism (Lasat 1999). In another scenario, plant-derived exudates assist to stimulate the existence of the associated bacteria, this further increase the efficiency of detoxification of contaminants i.e. immobilization/mobilization of non-essential metals that the plant can absorb (Kuiper et al. 2004) and accumulated in the aerial parts that can then be collected and subjected to an additional treatment for the safe discarding of the metals or the contaminant becomes less mobile and, therefore, stabilizes in the environment (Rajkumar et al. 2012).

6.1 Stress Tolerance in Plants

Plant growth promoting bacteria (PGPB) might support plants to stand against different biotic and abiotic stress including pathogens, drought, pollutants, and salinity and toxicity of pollutants. It is necessary to recognize the PGPB that can offer several assistances to its host plant by providing resistance to several pressures at the same time and ability to promote growth, and is useful for the sustainable and environment friendly agricultural activities (Dimkpa et al. 2008). A new point of view to bioremediation is the development of such crop accumulators that may be useful for the recovery of agricultural land. As a huge area of agricultural land is highly contaminated with different pollutants particularly with heavy metals, this can be of great importance compared to non-agricultural hyperaccumulators that not only require extensive times, but also do not provide productivity improvements (Wu et al. 2010).

The microbiology of the rhizosphere appears to greatly affect the resistance of the plant to different kinds of stress. Nevertheless, the significant role of nitrogen-fixing microorganisms that have been perceived for a considerable length of time, the impact of different organisms, for example, rhizobacteria and endophytes on plant development is broadly investigated today (Morrissey et al. 2004). Plants experience many environmental stresses that include both abiotic and biotic stress (pathogens, drought, salinity, floods, heavy metals). In stress, plants produce significant levels of ethylene, which makes their growth to be repressed (Glick et al. 2007a) because they cannot produce high biomass, specifically the roots. In soils polluted with metals, this is a significant deterrent to the accomplishment of bioremediation. In the event that this development restraint is dealt with, it can make it conceivable to build up a viable relationship among plants and microbes to control contamination (Arshad et al. 2007).

Plant growth promoting bacteria produces the ACC (1-aminocyclopropane-1-carboxylate) deaminase (an enzyme) that can retain ethylene levels under control and ensure that this level does not reach the point where it can affect root growth and compromise it. Therefore, the bacteria that produce ACC deaminase act as a well for ACC (which is a predecessor to ethylene) and defend the plant from high concentrations of ethylene (Glick 2005). This ACC can be emanated by plant roots and consumed by microorganisms (bacteria) that, by benefit of the enzyme ACC deaminase, divide this molecule into α-ketobutyrate and ammonia. Then, the microorganism like bacteria causes the plant to produce large amount of ACC and the plants must exude it to sustain a stability among internal and external levels of ACC. In this way, the amount of ACC is reduced within the plant assisting plant growth (Glick et al. 2007b). Honma and Shimomura were the first scientists who discovered the ACC deaminase (1978). Different research showed that ACC deaminase does not play an explicable part in the absorption of metals from the plant. However, it relieves metal-induced stress in plant and indirectly helps in the phytoremediation process (Gerhardt et al. 2006).

6.2 Sequestration and Uptake of Contaminants

The PGPRs were involved in the nutritional cycle and in the phytostimulation, which includes the fixation of atmospheric nitrogen and its supply to plants, synthesizing the siderophores that can sequester the iron (Fe) from the soil and supply it to plant cells, exhaustion of the rhizosphere Fe, induced systemic resistance, production of fungal cell wall enzymes, and competition for the root binding sites (Glick et al. 2007a). It has also been reported that siderophores help in the metal mobilization as well as promote the plant growth either directly or indirectly. The low molecular weight compounds (LMWC), specifically, ligands are responsible for binding to iron (Fe). A large diversity of fungi and bacteria secrete the anterior side for iron chelation from their surroundings under iron-deficient situations and make them available to the microbe (Gadd 2010). It has also been reported that siderophores might be involved in the absorption of non-iron metals in few bacteria. This may be important to confer the ability of metals to tolerate the bacterial cell and its sequestration inside, which provides a way to remove metals from the environment. In another case, the bacteria that produce siderophores can provide a pathway for the availability of metal in the plant by solubilizing the metal and increasing its solubility/mobility (Nair et al. 2007; Schalk et al. 2011). It is said that fluorescent pseudomonas are the rhizobacteria that produces the most studied siderophores (Alemu 2013).

7 Conclusion

Inorganic contaminants particularly heavy metals are difficult to handle or remove due to their high persistent and non-biodegradable nature. Hyperaccumulator plants are used for the removal of contaminants but the limited bioavailability of heavy metals is the main hurdle in the effective phytoremediation. For the efficient removal of contaminates, synthetic and natural chelating agents have been used to increase the removal ratio. However, the toxic effects of synthetic chelating agents have been observed. Therefore, microbial-assisted phytoextraction is preferred for the removal of heavy metals because they are environment friendly, economically feasible, without use of chemical, and have negligible negative impacts. Soil microorganisms including bacteria and fungi alter the soil pH by secreting different enzymes and increased the bioavailability of heavy metals. Among numerous techniques are used for the removal of contaminant, use of microorganisms along with hyperaccumulator plant is the efficient way for the soil cleanup.

References

Agarwal SK (2009) Heavy metal pollution. APH Publishing Corporation, New Delhi, pp 7–17

Alemu F (2013) Isolation of Pseudomonas fluorescens species from rhizospheric soil of faba bean and assessment of their siderophores production. Int J Adv Res 1(8):203–210

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25(8):356–362

Arshad M, Silvestre J, Pinelli E, Kallerhoff J, Kaemmerer M, Tarigo A, Shahid M, Guiresse M, Pradère P, Dumat C (2008) A field study of lead phytoextraction by various scented *Pelargonium* cultivars. Chemosphere 71(11):2187–2192

Arshad M, Merlina G, Uzu G, Sobanska S, Sarret G, Dumat C, Silvestre J, Pinelli E, Kallerhoff J (2016) Phytoavailability of lead altered by two *Pelargonium cultivars* grown on contrasting lead-spiked soils. Soil Sediment Contam 16(2):581–591

Baker AJM, Brooks R (1989) Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1(2):81–126

Bhargava A, Carmona FF, Bhargava M, Srivastava S (2012) Approaches for enhanced phytoextraction of heavy metals. J Environ Manage 105:103–120

Bothe H (2011) Plants in heavy metal soils. In: Detoxification of heavy metals. Springer, Berlin, pp 35–57

Braud A, Jezequel K, Bazot S, Lebeau T (2009) Enhanced phytoextraction of an agricultural Cr-and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere 74(2):280–286

Bruins MR, Kapil S, Oehme FW (2000) Microbial resistance to metals in the environment. Ecotoxicol Environ Saf 45(3):198–207

Chaney RL, Broadhurst CL, Centofanti T (2010) Phytoremediation of soil trace elements. In: Trace elements in soils, pp 311–352

Chen J, He F, Zhang X, Sun X, Zheng J, Zheng J (2014) Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil. FEMS Microbiol Ecol 87(1):164–181

Chhajro MA, Rizwan MS, Guoyong H, Kubra KA, Honging H (2016) Enhanced accumulation of Cd in castor (Ricinus communis L.) by soil applied chelators. Int J Phytorem 18:664–670

Dary M, Chamber-Perez MA, Palomares AJ, Pajuelo E (2010) "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177(1-3):323–330

De-Bashan LE, Hernandez JP, Bashan Y (2012) The potential contribution of plant growth-promoting bacteria to reduce environmental degradation–A comprehensive evaluation. Appl Soil Ecol 61:171–189

Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in Streptomyces spp. Chemosphere 74:19–25

Dixit R, Malaviya D, Pandiyan K, Singh U, Sahu A, Shukla R, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2):2189–2212

Dubchak S, Bondar O (2019) Bioremediation and phytoremediation: best approach for rehabilitation of soils for future use. In: Remediation measures for radioactively contaminated areas. Springer, Cham, pp 201–221

Fuentes II, Espadas-Gil F, Talavera-May C, Fuentes G, Santamaría JM (2014) Capacity of the aquatic fern (Salvinia minima Baker) to accumulate high concentrations of nickel in its tissues, and its effect on plant physiological processes. Aquat Toxicol 155:142–150

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156(3):609–643

Gerhardt KE, Greenberg BM, Glick BR (2006) The role of ACC deaminase in facilitating the phytoremediation of organics, metals and salt. Curr Trends Microbiol 2:61–72

Ghori Z, Iftikhar H, Bhatti MF, Minullah NM, Sharma I, Kazi AG, Ahmad P (2016) Phytoextraction: the use of plants to remove heavy metals from soil. In: Plant metal interaction. Springer, Switzerland, pp 385–409

Giasson P, Karam A, Jaouich A (2008) Arbuscular mycorrhizae and alleviation of soil stresses on plant growth. In: Siddiqui ZA, Akhtar MS, Futai K (eds) Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht, pp 99–134

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251(1):1–7

Glick BR, Cheng Z, Czarny J, Duan J (2007a) Promotion of plant growth by ACC deaminase-producing soil bacteria. In: New perspectives and approaches in plant growth-promoting Rhizobacteria research. Springer, Dordrecht, pp 329–339

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007b) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26(5-6):227–242

Guibaud G, Comte S, Bordas F, Dupuy S, Baudu M (2005) Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. Chemosphere 59:629–638

Gul I, Manzoor M, Silvestre J, Rizwan M, Hina K, Kallerhoff J, Arshad M (2019) EDTA−assisted phytoextraction of lead and cadmium by *Pelargonium* cultivars grown on spiked soil. Int J Phytorem 21(2):101–110

Han Y, Zhang L, Gu J, Zhao J, Fu J (2016) Citric acid and EDTA on the growth, photosynthetic properties and heavy metal accumulation of Iris halophila pall. Cultivated in Pb mine tailings. Int Biodeter Biodegr 128:15–21. https://doi.org/10.1016/j.ibiod.2016.05.011

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60(4):579–598

Honma M, Shimomura T (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. Agric Biol Chem 42(10):1825–1831

Hou W, Ma Z, Sun L, Han M, Lu J, Li Z et al (2013) Extracellular polymeric substances from copper-tolerance Sinorhizobium meliloti immobilize Cu2+. J Hazard Mater 261:614–620

Kamaludeen SPB, Ramasamy K (2008) Rhizoremediation of metals: harnessing microbial communities. Indian J Microbiol 48(1):80–88

Khan MA, Khan S, Khan A, Alam M (2017) Soil contamination with cadmium, consequences and remediation using organic amendments. Sci Total Environ 601–602:1591–1605

Kim S, Lim H, Lee I (2010) Enhanced heavy metal phytoextraction by Echinochloa crus-galli using rootexudates. J Biosci Bioeng 109(1):47–50

Koptsik GN (2014) Problems and prospects concerning the phytoremediation of heavy metal polluted soils: a review. Eurasian Soil Sci 47(9):923–939

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact 17(1):6–15

Kupper H, Lombi E, Zhao F, Steve PM (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75–84

Lasat MM (1999) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J Hazard Subst Res 2(1):5

Li Y-M, Chaney R, Brewer E, Roseberg R, Angle JS, Baker A, Reeves R, Nelkin J (2003) Plant Soil 249(1):107–115

Lombi E, Tearall KL, Howarth JR, Zhao FJ, Hawkesford MJ, McGrath SP (2002) Influence of iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator Thlaspi caerulescens. Plant Physiol 128(4):1359–1367

Lu L, Tian SK, Yang XE, Li TQ, He ZI (2009) Cadmium uptake and xylem loading are active processes in the hyperaccumulator *Sedum alfredii*. J Plant Physiol 166(6):579–587

Ma Y, Oliveira RS, Freitas H, Zhang C (2016) Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. Front Plant Sci 7:918

Mahdieh M, Yazdani M, Mahdieh S (2013) The potential of *Pelargonium roseum* plant for phytoremediation of heavy metals. Environ Monit Assess 185(9):7877–7881

Mahmood T (2010) Phytoextraction of heavy metals-the process and scope for remediation of contaminated soils. Soil Environ 29(2):91–109

Manzoor M, Gul I, Silvestre J, Kallerhoff J, Arshad M (2018) Screening of indigenous ornamental species from different plant families for Pb accumulation potential exposed to metal gradient in spiked soils. Soil Sediment Contam 27(5):439–453

Manzoor M, Abid R, Rathinasabapathi B, De Oliveira LM, da Silva E, Deng F, Rensing C, Arshad M, Gul I, Xiang P, Ma LQ (2019) Metal tolerance of arsenic-resistant bacteria and their ability to promote plant growth of Pteris vittata in Pb-contaminated soil. Sci Total Environ 660:18–24

Mistry K, Desai C, Patel K (2010) Chromate reduction by Vagococcus sp. isolated from Cr (VI) contaminated industrial effluent. Electron J Biol 6(1):6–12

Morrissey JP, Dow JM, Mark GL, O'Gara F (2004) Are microbes at the root of a solution to world food production. EMBO Rep 5(10):922–926

Nair A, Juwarkar AA, Singh SK (2007) Production and characterization of siderophores and its application in arsenic removal from contaminated soil. Water Air Soil Pollut 180(1-4):199–212

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51(6):730–750

Ortiz JCR, Cepeda RDV, Mireles JLR, Quintanar RM, hernandez JLG (2006) Soil nitrogen fertilization effects on phytoextraction of cadmium and lead by tobacco (Nicotiana tabacum L.). J Biorem 10(3):1–10

Peijnenburg WJ, Vijver MG (2007) Metal-specific interactions at the interface of chemistry and biology. Pure Appl Chem 79(12):2351–2366

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30(6):1562–1574

Rehman Z, Khan S, Brusseau ML, Shah MT (2017) Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. Chemosphere 168:1589–1596

Rizwan MS, Guoyong H, Kubra KA, Honging H (2016) Enhanced accumulation of Cd in castor (*Ricinus communis* L.) by soil applied chelators. Int J Phytorem 18:664–670

Sakakibara M, Ohmori Y, Ha NTH, Sano S, Sera K (2011) Phytoremediation of heavy metal-contaminated water and sediment by *Eleocharis acicularis*. Clean Soil Air Water 39:735–741

Salt DE, Smith RD, Raskin I (1995) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13(11):2844–2854

Shahid M, Pinelli E, Pourrut B, Dumat C (2014) Effect of organic ligands on lead-induced oxidative damage and enhanced antioxidant defense in the leaves of *Vicia faba* plants. J Geochem Explor 144:282–289

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156(3):1164–1170

Sheoran V, Sheoran AS, Poonia P (2016) Factors affecting phytoextraction: A review. Pedosphere 26(2):148–166

Stout L, Nusslein K (2010) Biotechnological potential of aquatic plant–microbe interactions. Curr Opin Biotechnol 21(3):339–345

Sun Y, Zhou Q, Diao C (2008) Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator Solanum nigrum L. Bioresour Technol 99:1103–1110

Van der Ent A, Baker AJ, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362(1-2):319–334

Vodnik D, Grcman H, Macek I, Van Elteren JT, Kovačevic M (2008) The contribution of glomalin-related soil protein to Pb and Zn sequestration in polluted soil. Sci Total Environ 392:130–136

Wan X, Lei M, Yang J (2016) Two potential multi-metal hyperaccumulators found in four mining sites in Hunan Province, China. CATENA 148:67–73. https://doi.org/10.1016/j.catena.2016.02.005

Wang HQ, Lu SJ, Yao ZH (2007) EDTA-enhanced phytoremediation of lead contaminated soil by Bidens maximowicziana. J Environ Sci 19(12):1496–1499

Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321(1-2):385–408

WHO (2017) Lead poisoning and health. https://www.who.int/en/news-room/fact-sheets/detail/lead-poisoning-and-health

Wu LH, Luo YM, Christie P, Wong MH (2003) Effects of EDTA and low molecular weight organic acids on soil solution properties of a heavy metal polluted soil. Chemosphere 50:819–822

Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174(1–3):1–8

Zhang DY, Wang JL, Pan XL (2006) Cadmium sorption by EPSs produced by anaerobic sludge under sulfate-reducing conditions. J Hazard Mater 138:589–593

Zhang F, Zhang H, Xia Y, Wang G, Xu L, Shen Z (2011) Exogenous application of salicylic acid alleviates cadmium toxicity and reduces hydrogen peroxide accumulation in root apoplast of *Phaseolus aureus* and *Vicia sativa*. Plant Cell Rep 30:1475–1483

Zhang H, Guo Q, Yang J, Ma J, Chen G, Chen T, Zhu G, Wang J, Zhang G, Wang X, Shao C (2016) Comparison of chelates for enhancing Ricinus communis L. phytoremediation of Cd and Pb contaminated soil. Ecotoxicol Environ Saf 133:57–62

Zhang X, Zhong B, Shafi M, Guo J, Liu C, Guo H, Peng D, Wang Y, Liu D (2018) Effect of EDTA and citric acid on absorption of heavy metals and growth of moso bamboo. Environ Sci Pollut Res 25:18846–18852