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Plant-Metal Interactions

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Preface

Metals have a widespread occurrence in the environment and are the basis of life on earth. The essential metals like zinc play several crucial roles in plants that their unavailability or deficiency leads to severe retardation of growth and development of the plant. However, at the same time, there are other metals like arsenic, cadmium, and chromium whose contamination has emerged as a problem of unprecedented scale in the last few decades. Due to the importance of metals in plants' life, the vast amount of research has been conducted on pant-metal interactions, which include morphological, anatomical, and physiological evaluation of plants' performance against deficiency or excess of a metal. The modern sophisticated techniques and instrumentation have led to rapid and thorough understating of responses of plants at gene, protein, and metabolite levels. This has resulted in enormous advancements in the field of "*plant-metal interactions*" from organ to tissue to cellular level responses with in-depth information about genes to proteins and metabolites. The present book includes a total of 13 chapters, dealing with various aspects of plant-metal interactions and present up-to-date knowledge on the subject.

The metal levels inside the plants are regulated via increased synthesis of a number of metal-complexing proteins, molecules, and peptides. In addition, the role of antioxidant enzymes is vital in the scavenging of toxic reactive oxygen species generated in response to toxic metals or in the presence of excess of essential metals. The holistic processes of metal homeostasis and detoxification are regulated through complex and intricate signaling and regulatory mechanisms. Chapter 1 of the book deals with transcriptomic, proteomic, and metabolomic approaches to delineate in-depth plant-metal interactions and gives an overview of metal-induced stress responses in plants and plants' mechanisms. Chapters 2, 3, and 4 deal with signaling-related aspects of metals in plants and with the roles of metal scavengers in plants and lucidly present the concepts and up-to-date knowledge on the topic. Furthermore, there are unique plants which are adapted to harsh conditions of metals and environment. Some plants can uptake and accumulate metals in exceptionally high amounts, while others can withstand excessive saline environments. Such hyperaccumulators and halophytic plants have been the keys to investigate tolerance mechanisms of plants by comparative evaluation with nonaccumulators and glycophytes, respectively. Chapters 5 and 6 discuss the mechanisms of metal uptake and the accumulation and tolerance in hyperaccumulator and halophytic plants.

Arsenic contamination and toxicity are of great concern due to natural reasons behind the recent surge of arsenic in soil and groundwater in Southeast Asian countries. Arsenic toxicity to humans through rice is an important issue. Chapter 7 discusses the important aspects of arsenic uptake, transport, and detoxification in plants, while Chap. 8 discusses the metabolic modulations elicited in plants in response to arsenic stress. These chapters enhance our understanding of the arsenic problem to a great detail. The interactions of plants with another widely present contaminant metal, that is, chromium, are also discussed in fine detail in Chap. 9. Achieving sustainable and high crop yields is desirable, and therefore, understanding the impact of metals on crop production is crucial. Chapter 10 deals with the sources and effects of metals in crop productivity and discusses the impacts of both essential metals like copper, zinc, and nickel, and toxic metals such as cadmium, arsenic, chromium, lead, and mercury. In addition, Chap. 11 deals with the aspects of plant-microbe interactions in the context of metal tolerance of plants, while Chaps. 12 and 13 deal with phytostabilization and nanomaterials as a sustainable and feasible approach to deal with metal contamination issues.

The book therefore comprises a unique combination of chapters on various aspects and will provide the reader a comprehensive view of plant-metal interactions. The book covers metals whose contamination is widespread and which are of global environmental and public health concern like arsenic, cadmium, and chromium. This book would act as a guiding textbook for undergraduate and postgraduate students and as a mean to understand the latest research trends for doctoral students.

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Sudhakar Srivastava is an Assistant Professor. He obtained his M.Sc. in Botany from the University of Lucknow in 2002 and his Ph.D. in Botany from the CSIR-National Botanical Research Institute (CSIR-NBRI) and University of Lucknow in 2008. After obtaining his Ph.D., he served as Scientific Officer in Bhabha Atomic Research Centre (BARC), Mumbai, from June 2009 to March 2014, and then moved to the Institute of Environment and Sustainable Development (IESD), Banaras Hindu University (BHU), in April 2014. He has more than 16 years of research experience in the field of plant-metal interactions with major interest on arsenic stress responses of plants. He has published 65 research articles and 20 review and letter articles in high-impact international and national journals. He has also published 20 book chapters in the books of international repute and few general articles in popular magazines and websites. He has also received several awards for his contributions, among which the notable ones are Young Scientist Award of the National Academy of Sciences, India (NASI), Allahabad, in 2011, Young Scientist Award from Uttar Pradesh Council of Science & Technology (UPCST) in 2013–2014, and Young Scientist Award from Science and Engineering Research Board (SERB) in 2015. He is an Associate Member of the National Academy of Agricultural Sciences (NAAS) and a Life Member of several societies as well as nongovernmental organizations. He is also an Associate Editor of reputed international journals, such as *Acta Physiologiae Plantarum* and *Frontiers in Ecology and Evolution*. He is also an Expert Reviewer of several journals and project funding agencies.

Ashish K. Srivastava is presently working as Scientific Officer in Bhabha Atomic Research Centre, Mumbai. His research is focused on developing strategies for enhancing crop resilience toward different abiotic stresses. The stimulatory potential of thiourea (a reactive oxygen species scavenger) has been demonstrated for enhancing stress tolerance and crop productivity through lab and small-scale field experiments and by conducting multilocation field trials at salt- and drought-affected fields of Rajasthan. A small-scale technology has been developed for increasing yield and reducing arsenic accumulation from rice grains. Besides, thiourea has been used as a chemical probe for delineating redox regulatory components of stress tolerance. He has proposed a bifurcative model of thiourea action wherein redox homeostasis acts as “central” point to regulate gene expression through miRNA and hormones. To summarize, he is employing the combination of physiology, molecular biology, and biotechnology to understand crops behavior under stress and develop suitable strategy to minimize stress-induced yield losses in different crop plants. He has more than 40 research and review articles and book chapters to his credit. He has been awarded Young Scientist Award of the National Academy of Sciences, India (NASI), Allahabad, in 2018, Young Scientist Medal from the Indian National Science Academy (INSA) in 2014, and Young Scientist Award from the Department of Atomic Energy (DAE) in 2014. He has also received President International Fellowship Award from the Chinese Academy of Sciences, China, in 2016 and EMBO Short-Term Fellowship in 2011.

Penna Suprasanna is a Senior Scientist and Head of Plant Stress Physiology and Biotechnology Group, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, and is Professor, Homi Bhabha National Institute, Department of Atomic Energy, Mumbai. He made significant contributions to crop biotechnology research through radiation-induced mutagenesis, plant cell and tissue culture, genomics, and abiotic stress tolerance. His research on radiation-induced mutagenesis and in vitro selection in sugarcane yielded several agronomically superior mutants for sugar yield and stress tolerance. He has made intensive efforts to apply radiation mutagenesis techniques in vegetatively propagated plants through collaborative research projects with several national and international bodies (IAEA, Vienna). He is also serving as the DBT Expert/Nominee on Biosafety committees. He is the Recipient of the “Award of Scientific and Technical Excellence” by the Department of Atomic Energy, Government of India and is the Fellow of Maharashtra Academy of Sciences, Andhra Pradesh Academy of Sciences, Telangana Academy of Sciences, and Association of Biotechnology. He has published more than 250 research papers/articles in national and international journals/books, edited two Springer books, and has guided several doctoral students. His research is focused on molecular biology of abiotic stress tolerance and salt-stress adaptive mechanisms in plants. The research group led by him has successfully identified novel microRNAs and plant bioregulators besides validating the concept of redox regulation toward abiotic stress tolerance and crop productivity.

An Integrated Transcriptomic, Proteomic, and Metabolomic Approach to Unravel the Molecular Mechanisms of Metal Stress Tolerance in Plants

Parul Parihar, Samiksha Singh, Rachana Singh, G. Rajasheker, P. Rathnagiri, Rakesh K. Srivastava, Vijay Pratap Singh, Penna Suprasanna, Sheo Mohan Prasad, and P. B. Kavi Kishor

1 Introduction

Rapid rate of industrialization, urbanization, intensive agriculture, and mining has been causing widespread contamination of our soil and aquifer resources with heavy metals. This is a problem of paramount importance since metal pollution can lead to dangerous consequences by entering into the food chain (Rascio and Navari-Izzo 2011). Some of the metals may be required for plants as micronutrients (Cu, Fe, Mg, Mn, Ni, Zn) for normal growth and development, but many (Ag, As, Cd, Co, Cr, Hg, Pb, Sb, Se) may not be essential for plant growth or may have unknown functions in the plant systems (Rascio and Navari-Izzo 2011). But, if heavy metals are present in excess amount, they may hamper the metabolic pathways and disrupt the functions of enzymes (Hossain et al. 2012a, b, Ali et al. 2013). Enhanced levels

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of heavy metals generate reactive oxygen species (ROS) or non-free radical species such as singlet oxygen and hydrogen peroxide and also cytotoxic compounds such as methylglyoxal which cause oxidative stress (Hossain et al. 2012a, b, Sytar et al. 2013). Heavy metal stress also leads to programmed cell death due to membrane and DNA damage, ion leakage, and redox imbalance (Rascio and Navari-Izzo 2011). But, over a period of time, plants have acquired diverse mechanisms to cope with adverse effects of heavy metal stress. They may avoid metal stress to preclude the onset of stress either by excluding or preventing the entry (Viehweger 2014). Dalvi and Bhalerao (2013) reported that plants prevent the entry of metals by immobilizing them with mycorrhizal association and complexing them by organic acids secreted from roots. If metals enter into the plant systems, plants use other tolerance mechanisms for getting rid of them. These strategies include metal sequestration into vacuoles (Patra et al. 2004) and metal binding to cell walls, biosynthesis of phytochelatins, and metallothioneins (John et al. 2009). Apart from metal sequestration mechanisms, plants synthesize osmolytes such as proline and activate antioxidant defense mechanisms (Manara 2012).Thus, plants adapt a wide range of strategies to overcome heavy metal toxicity. But, the intricate molecular mechanisms that are associated with such strategies are not fully known. Advances in the study of transcriptome, using next-generation sequencing methods, matrix-assisted laser desorption ionization (MALDI) time-of-flight, time-of-flight (MALDI-TOF-TOF) for the study of proteome are assisting us to dissect out different pathways and complex network of genes and proteins. This chapter describes an account of the biosynthesis of two important ligand peptides, phytochelatins, and metallothioneins that are used by plants, their functions in metal detoxification, and how transcriptomic, proteomic, and metabolomic data are helping us to unravel some of the molecular mechanisms associated with heavy metal toxicity. Some directions are also provided at the end for forming a future framework of research.

2 Heavy Metal-Induced Nutritional and Water-Deficit Stresses

2.1 Nutritional Stress in Metal-Exposed Plants

Heavy metal stress causes transient deficiencies of nutrients in plants due to spatial and temporal variations in plant nutrient demands and supplies. The movement of mineral elements into the plant system may in turn depend upon several factors like diffusion of elements, transport of bulk soil solution, etc. (Marschner 1995). While most of the nutrients (e.g., N, P, K, S, Mg) are transported through phloem, calcium and boron are usually supplied through the xylem to the growing parts (Nazar et al. 2012). Thus, both xylem and phloem should function efficiently under nutrient deficiency caused by metal stress. Heavy metals like cadmium compete with Ca, Mg, and Fe in transport process across membrane via ZIP and NRAMP family members or Ca channels and transporters (Llamas et al. 2000, Perfus-Barbeoch et al. 2002). Further, Gothberg et al. (2004) pointed out that the competition between nutrients and toxic metals for binding sites at the membrane or cell wall level influences the entry and distribution of these metals. Sun and Shen (2007) showed that the reduction decrease of Mn, Fe, Mg, S, and P levels in cabbage plants sensitive to Cd stress is the reason for decreased growth. In *Aeluropus littoralis* (Rezvani et al. 2012), heavy metals caused an increase in macronutrient and decease in micronutrient concentrations. Yoshihara et al. (2006) reported Cd-induced iron deficiency responsive genes like *HvIDS*2pro::*GUS*, *NtFRO*1, and *NtIRT*1 in tobacco roots. Work carried out by Wu et al. (2012) shows that high Fe content in shoots under Cd stress alleviates Cd toxicity in *Arabidopsis*. Thus, nutrient deficiency in plants appears to be widespread under heavy metal stress. As pointed out by Guerinot (2000), comprehensive understanding of the functions of the ZIP and other family transporters is pivotal for enhancing mineral nutrient content and developing crop plants that accumulate or exclude toxic metals.

2.2 Water Stress in Metal-Exposed Plants

Water and inorganic nutrients absorbed through the roots support the plant growth and development. Roots are directly exposed to higher heavy metal concentrations from where they are exported to shoots and leaves (Burkhead et al. 2009, Feleafel and Mirdad 2013). Due to the presence of soluble salts including heavy metal salts, water relations would be severely affected in plants. However, Vaculik et al. (2012) reported changes in root anatomy and morphology of *Salix caprea* under Zn and Cd stress. Besides this, decreased root elongation, increased root dieback, and reduced root hair surface were also noticed in other plants (Sharma 2012, Gallego et al. 2012, Anjum et al. 2013, Feleafel and Mirdad 2013). If heavy metals are present in excess amount, they influence the flow of water by lowering the transpiration rate (Barcelo and Poschenrieder 1990). Maggio and Joly (1995), and Carvajal et al. (1996) reported reduction in pressure-induced water flux from tomato and wheat, respectively, in response to Hg. While roots of *Brassica juncea* displayed an increase in the vacuole size (Han et al. 2004), in *Lutea luteus*, Pb-induced vacuolization was recorded with high values of relative water content (Rucinska-Sobkowiak et al. 2013). In roots of *Oryza sativa* and *Pisum sativum*, extensive tissue damage was noticed in the presence of Cd and Ni (Gabbrielli et al. 1999, Lux et al. 2011, Llamas et al. 2008). Stomata are closed due to the interaction of heavy metals with guard cells and reduced osmotic potentials (Rucinska-Sobkowiak 2016). They also pointed out that ABA-induced signals play a vital role in stomatal movement. It appears that metals decelerate short-distance water transfer both in symplast and apoplast. Such a situation certainly reduces the flow of water into the vascular system and impairs the supplies to the shoot and leaves. Likewise, long-distance transport of water is hindered due to decreased hydraulic conductivity in the roots, stems, and leaves. Also, such metal-induced perturbations in water relations modulate aquaporin gene expressions in plants resulting in water loss reductions (Rucinska-Sobkowiak 2016). However, further research is needed to unravel the cross talk between signaling elements and their role in the transduction of metal-induced signals from below-the-ground parts to the aboveground parts. Such studies help to evolve plants with better resilience to metal-induced water-deficit conditions.

3 Role of Thiol- and Non-thiol Compounds and Metal(loid) Chelation

Certain ions, though essential for plant growth and development, can cause toxicity when present in excess. However, plants have evolved multiple strategies to maintain metal homeostasis, alongside the mitigation of heavy metal stress. When plants are exposed to higher metal concentrations, either they can exclude or efflux out the metals into the xylem (xylem loading) or accumulate and sequester into vacuoles (Robinson et al. 1994; Montargès-Pelletier et al. 2008). Lasat (2002) and others (Seth et al. 2008, Cuypers et al. 2009) pointed out that exclusion of metal ligands from root symplasm into xylem vessels or sequestration into the vacuoles leads to metal homeostasis and metal-induced oxidative stress. Thiol compounds such as glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs) contain sulfhydryl (–SH) groups and bind with high affinity to many metals (Seth et al. 2012). GSH is a tripeptide (γ-Glu-Cys-Gly) and a major –SH compound and acts as a precursor for the synthesis of phytochelatins (Clemens 2006, Srivalli and Khanna-Chopra 2008). MTs are sulfur (S)-containing cysteine (Cys)-rich, gene-encoded polypeptides and bind to metals in plants (Verbruggen et al. 2009). Though PCs partake in metal detoxification, their role during normal physiology of plants in the absence of metal stress is largely unknown. In addition to thiol compounds, in a number of plants, non-thiols like organic acids (citrate, malate, oxalate, malonate, aconitate, tartarate) and amino acids such as proline, histidine, cysteine, arginine, glutamate, and nicotianamine either alone or in combination with thiol compounds, have been shown to contribute to metal chelation (Hall 2002). Thus, it appears that plants utilize a wide array of mechanisms/resources for metal sequestration/detoxification.

3.1 *Phytochelatins and Their Induction*

PCs are a family of cysteine-rich polypeptides with general structure (γ-Glu-Cys)n-X, in which X represents Gly, γ-Ala, Ser, Gln, or Glu and $n = 2–11$ depending on the organism, though the most common forms have 2–4 peptides (Gekeler et al. 1989). PCs were discovered first in *Saccharomyces pombe* after exposing them to cadmium which produced PC_2 and PC_3 (Kondo et al. 1985). They have been named as cadystin A and B, but later the name PC was adopted (Wu et al. 2014). They are mostly induced by Cd when compared to Zn (Thangavel et al. 2007) and Hg (Sobrino-Plata et al. 2009). Interestingly, more GSH has been found in Hg-exposed *Hordeum vulgare* aerial parts than in roots. However, longer-chain PCs (such as PC3, PC4, and PC5) were abundant in roots in comparison with aerial parts (Dago et al. 2014). Morelli and Scarano (2001) discovered that Pb and Cd can cause the synthesis of PC2 and PC4, respectively, in *Phaeodactylum tricornutum*. Though the –SH group of the Cys residues helps PCs to bind to the metals, the PC-metal complexes are transported into the vacuoles via ABC transporters or a group of solute transporters for detoxification (Verbruggen et al. 2009, Solanki and Dhankhar 2011).

3.2 Biosynthesis of PCs and Variations (Homo-PCs, Hydroxymethyl-PCs, and Iso-PCs)

Biosynthesis of phytochelatins can be plant or metal specific. Zenk (1996) reported that Hg, Cd, As, Ag, and Fe are strong inducers, Pb and Zn are weak inducers, and Cu and Ni are moderate inducers. It appears that Pb is a strong inducer of PCs in comparison with other metals in many plant species (Dago et al. 2014). They also reported more longer-chain PCs such as PC3, PC4, and PC5 in *Hordeum vulgare* roots than in leaves and shoots. In plants such as *Phaeodactylum tricornutum* (Morelli and Scarano 2001), exposure to Pb and Cd causes the production of PC2 and PC4, respectively. However, how Cd can stimulate PC synthesis or its activation is not currently known. Song et al. (2014) are of the opinion that essential metals like Zn, Cu, and Mn are transported into vacuoles in the form of PC2-metal complexes through ATP-binding cassette (ABC) transporters. Grill et al. (1989) pointed out that the biosynthesis of PCs is triggered by exposure of cells to metals like Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Ag^{2+}, and others. Glutamate and cysteine are the precursors for the biosynthesis of γ-glutamylcysteine, and the reaction is catalyzed by γ-glutamylcysteine (EC) synthetase (γ-ECS) in plants. Glycine molecule is then added to this dipeptide, and the enzyme associated with this biochemical conversion is ATP-dependent glutathione synthetase. γ-ECS is converted to γ-glutamylcysteine glycine (γ-Glu-Cys-Gly) which is then catalyzed by the enzyme phytochelatin synthase (PCS) to form PCs (Zenk 1996). However, variations occur in the formation of PCs. In leguminous plants (Phaseoleae tribe), phytochelatins contain β-alanine instead of glycine and are analogous to homo-GSH; therefore, they are named as homo-phytochelatins (Grill et al. 1986). If homoglutathione occurs in some plants, and in such cases, β-alanine-specific ATP-dependent enzyme homoglutathione synthase utilizes γ-Glu-Cys to produce γ-Glu-Cys-β-Ala (homo-phytochelatins as in the case of Leguminosae family). On the other hand, in members of the family Poaceae, biosynthesis of γ-Glu-Cys-Ser [(Glu-Cys)n-Ser] is common (Klapheck et al. 1992). Klapheck et al. (1992) found that hydroxymethyl-GSH(γ-Glu-Cys-Ser), which upon exposure to metals, produces homologous peptides [(Glu-Cys)n-Ser] and later named as hydroxymethyl-PCs. However, *Zea mays* is an exception to this, where γ-Glu-Cys-Glu has been noticed (Meuwly et al. 1993, 1995). Thus, C-terminal-modified PC peptides have been noticed with different residues (other than glycine) and termed as iso-phytochelatins. In addition to PCs, iso-PCs, peptides without C-terminal amino acids represented as (g-Glu-Cys)n and are called as desGly-PCs. Such desGly-PCs were noticed in *Saccharomyces pombe* (Mehra and Winge 1988) as well as in maize (Bernhard and Kagi 1987). The catalytic moiety of phytochelatin synthase (PCS) contains cysteine (Cys) residue. Wang et al. (2009) pointed out that though the C-terminal domain is not highly conserved, in all species, the termini contain multiple Cys residues that bind Cd ions with high affinity. Irrespective of their names, the major function of PCs is metal detoxification in plants. However, why such a diverse group of PCs is synthesized in varied taxa is not exactly known.

3.3 Mode of Action of PCs

It is necessary for plants to chelate metals so as to prevent the disruption of vital metabolic activities by heavy metals in the cytosol. Once metals enter the cytosol, they activate the phytochelatin synthase (PCS) enzyme, which is otherwise inactive. PCS then produce PC molecules with variable length utilizing GSH. The metal-binding peptides are then biosynthesized in the cytosol and form a metal-PC complex as detailed above. It has been pointed out later by Toppi and Gabbrielli (1999) that PCs complex with metal ions such as Cd^{2+} through the thiolic group (-SH) of cysteine and the PC-metal complexes are accumulated in the vacuole through the activity of ABC transporter family members or a group organic solute transporters (Solanki and Dhankhar 2011). DalCorso et al. (2008) found out the metal-PC complexes are effectively sequestered into vacuoles, and the heavy metals are also transported over a long distance through the xylem and phloem vessels in higher plants (Mendoza-Cozatl et al. 2008). Since both metal-tolerant and metal-sensitive plants produce PCs, it is believed widely that PCs alone are not responsible for hyperaccumulation of Zn, Ni, or Pb (Shen et al. 1997, Leopold et al. 1999). Rauser (1999) pointed out that metal ions after combining with PCs are transported to the vacuoles and form a complex with sulfides and organic acids. Formation of PC-As (III) complex in rice leaves was shown to reduce the translocation of arsenic (As) from leaves to the grains (Duan et al. 2011). Phytochelatin synthase genes have been isolated from higher plants and overexpressed in many plants. Heterologous overexpression of *Triticum aestivum TaPCS1* in rice increased the sensitivity to the metal cadmium(Cd) and accumulation in shoots but not in roots (Wang et al. 2012). However, overexpression of *Ceratophyllum demersum PCS1* (*CdPCS1*) in *Nicotiana tabacum* or *Arabidopsis thaliana* (Shukla et al. 2013) increased the synthesis of PCs and accumulation of Cd and As. Thus, PCs detoxify the heavy metals in plants, besides transporting them (Salt and Rauser 1995). However, this does not fully explain how precisely the heavy metal specificity or species specificity of hyperaccumulation takes place in certain plants as has also been pointed out by Baker et al. (2000) and Hossain et al. (2012a, b).

3.4 The Superfamily of Metallothioneins: Classification and Structure of Metallothioneins

Metallothioneins (MTs) are intracellular, gene-encoded, low molecular weight (2–7 kDa), cysteine (Cys)-rich (20–30%) polypeptides that act as metal chelators. MTs are distributed very widely in microbes (prokaryotes), fungi, and animal and plant kingdoms. Based on their sequence similarity and phylogenetic relationship, MTs have been classified into 15 families. Depending on the number of Cys regions and their distribution, they are classified again into type 1–type 4 subfamilies (Cobbett and Goldsbrough 2002, Peroza et al. 2009, Hassinen et al. 2011). MT has α and β structural domains at the C- and N-termini which are composed of Cys clusters and has the ability to bind to four metal ions very tightly with the help of mercaptide bonds (Cobbett and Goldsbrough 2002, Ruttkay-Nedecky et al. 2013), but at the same time, metal ions are easily exchanged for other proteins (Hassinen et al. 2011). MT was first reported as a cadmium-binding protein in the cortex of horse kidney by Margoshes and Valle (1957). Casterline and Barnett (1982) were the first to discover MTs in roots of soybean. However, plant MTs differ from that of animal MTs in the amino acid sequence, charge, homocysteine number, and distribution pattern (Mir et al. 2004). Type 1 MTs are characterized by the arrangement of Cys residues in the order of Cys-amino acid-Cys in N- and C-termini. On the other hand, type 2 MTs have Cys-Cys, Cys-amino acid-amino acid-Cys and Cys-amino acid-Cys in the N-terminus, and Cys-amino acid-Cys in the C-terminus. Sequence alignment of the *MT* cDNAs with that of genomic sequences showed that *MT* genes have 1–3 introns, the size ranging from 71 to 275 bp. Like other gene sequences, *MT* gene promoters also contained TATA and CAAT boxes (8–635 bp) upstream of the start codon along with other regulatory elements like MYC, ABRE-like, and MYB-binding sites (Samson and Gedamu 1997). This indicates that they are associated with abiotic stress tolerance in plants, maybe by scavenging reactive oxygen species (ROS).

3.5 Expression of MT Genes

The work of Cobbett and Goldsbrough (2002) and Hassinen et al. (2011) reveals that MT genes display a very distinct spatial and temporal expression patterns in plants. MTs are certainly constitutively expressed, but they are also stimulated by many endogenous and exogenous agents. This signifies that there is a functional divergence for MT subfamilies and is also necessary for plant survival and development. Guo et al. (2003) studied for the first time the expression of MT gene family (including senescence) in *Arabidopsis* and their responses to Cu treatment. MTs are expressed under varied abiotic stress conditions like drought, salinity, high temperature, cold, light, wounding, and senescence (reviewed in Anjum et al. 2015). Also, metals such as Cu, Cd, Pb, and Zn strongly induce plant MT gene expressions (Mehes-Smith et al. 2013). Gene expressions in plants differed depending upon metal accumulators and non-accumulators. In *Arabidopsis thaliana*, *MT1a* and *MT1b* are highly expressed in roots when exposed to Cd, Cu, and Zn (Maestri et al. 2010). But in a metal accumulator *Thlaspi caerulescens*, high levels of MT1 transcripts were noticed in leaves when compared with leaves under the influence of Cu. Likewise, type 3 MT of *Thlaspi caerulescens* showed higher Cu binding capacity compared to *Arabidopsis*. Roosens et al. (2004) on the other hand found higher expression of *MT3* genes during leaf aging and *MT4* in seeds of *A. thaliana*. Gautam et al. (2012) reported many MT genes for detoxification in rice. They noticed 11 class I MT genes in rice genome that are differentially expressed during growth. Both *MT1* and *MT2* were expressed when rice was exposed to As (V) (Nath et al. 2014). Such a tissue-specific expression of MTs reveals that they play a crucial role during plant development besides metal detoxification. In *T. caerulescens*, modifications in protein sequences of TcMT1 and TcMT3 were observed in their Cys domains. Later, Roosens et al. (2005) and Gautam et al. (2012) noted a close association between the number of Cys residues and metal tolerance. They noted that the lower the number of Cys residues, the lower the metal tolerance when *TcMT1* was overexpressed in yeast. Thus, the distribution and organization in Cys residues may have bearing on metal chelation in plants and metal homeostasis.

3.6 Metal(loid) Specificity and Chelation

MTs bind not only to toxic metals such as Cd but also to other transition metals that are essential for plant nutrition like Cu^+ and Zn^{2+} (Freisinger 2011). The binding of heavy metals to MTs is crucial for detoxification of heavy metals. At the same time, transport of Zn and Cu ions helps the maintenance of metal homeostasis. It appears that like in mammalian cells, plant type 1 MT protein (from *Triticum durum* and *Fucus vesiculosus*) assumes a dumbbell shape and the protein can bind very tightly to six Cd^{2+} along with four S^{2-} molecules (Zimeri et al. 2005). Besides, MT protein also binds to four Zn^{2+} ions. Jin et al. (2006) found that *MT* genes are affected by metal and oxidative stresses as well as by salt, drought, and temperature implying that they also play a role in abiotic stress. Usman et al. (2015) showed that *MT4* gene from *Hordeum vulgare* binds to zinc in the aleuronic layer of the grains and act in putative storage. However, structural information and understanding of metal-binding mechanisms in plant MTs are scanty.

3.7 Functions of MTs

In animals, MTs play a role in zinc and copper homeostasis and protection against cadmium toxicity (Vasak and Hasler 2000). In humans, while cells that contain high levels of MTs are resistant to the heavy metal cadmium, lines that synthesize low MTs are sensitive to it (Karin et al. 1983, Enger et al. 1986). Loebus et al. (2013) reported that metallothionein proteins detoxify heavy metals like cadmium and mercury in plants. Poplars were found to accumulate 800 mg of Cd and Zn per kilogram of plant weight (Laureysens et al. 2004). When willows were used in short rotation for removing metals, pollution was reduced to safer levels (Witters et al. 2009). Zhou et al. (2014) noticed homeostasis of metals such as zinc and copper under the influence of MTs. Lv et al. (2013) found out that MTs have functions like ROS scavenging (Akashi et al. 2004), and Higashimoto et al. (2009) reported protection against DNA damage in animals, plants, and microorganisms. Thus, the four different types of MTs have unique and overlapping functions in metal homeostasis, metal detoxification, and antioxidative defense. It appears that there is a functional variation for MTs, but understanding the structure-function relationship and their metal-binding attributes is highly vital for making use of MTs in genetic engineering techniques aimed at metal detoxification/environmental cleanup.

4 Heavy Metal Stress Tolerance at Molecular Level: Omics Approach

Molecular strategies are being employed to understand mechanisms of heavy metal stress tolerance using OMIC tools (Fig. 1). Plants growing in metal-contaminated sites/areas develop tolerance strategies as well as mechanisms to regulate the process of metal uptake, translocation, and accumulation. These strategies involve the role of transcriptome, proteome, and metabolome (reviewed by Singh et al. 2015). In the following section, we discussed the details how transcriptome, proteome, and metabolome regulate the process of heavy metal stress tolerance in plants.

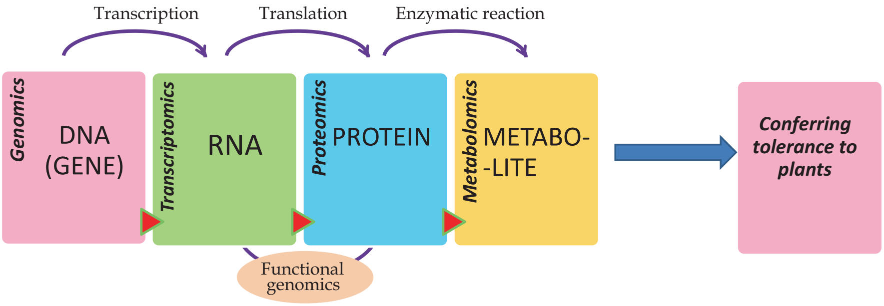

Fig. 1 An overview of omic tools involved in conferring tolerance to plants under heavy metal stress

4.1 Transcriptomics

RNA sequencing is the most important tool for developing transcriptome profile for characterizing and analyzing functionality of gene(s) (Wang et al. 2015). With the help of transcriptome analyses, the role of genes related to metal tolerance has been identified in several plant species like *Arabidopsis*, ramie, radish, and *Viola yedoensis* (Herbette et al. 2006, Xu et al. 2015, Liu et al. 2015a, Gao et al. 2015). Stressors trigger several genes and proteins to provide the plant survival mechanisms under such adverse conditions. The set of genes are either regulatory or functional, where the regulatory gene encodes transcription factor(s) while the functional gene encodes enhanced production of several metabolites like amino acids, alcohol, sugars, etc. The regulatory genes encoding transcription factors are thought to be the center of gene cluster and belong to the multigene family. It has been suggested that one transcription factor could regulate many genes, and their action involves the binding of transcription factor to the *cis*-acting elements of the target genes (Wray et al. 2003, Nakashima et al. 2009) and its further interaction with protein domain that oligomerize the transcription factors with other regulatory substances (Wray et al. 2003, Shiu et al. 2005). Several comparative transcriptome analyses have been performed with two cultivars of *Brassica rapa* under Cd stress, in order to identify the reason for heavy metal tolerance capability, and it was found that several plasma membrane-localized and tonoplast-localized transporters were overexpressed that aid the plant to survive under Cd stress (Yu et al. 2017). In another study by Liu et al. (2015a, b), regulatory role of several genes was analyzed by exploring the stress-responsive miRNA, and it was suggested that several miRNA-encoding transcription families were differentially expressed and these transcription factors were associated with the uptake and homeostasis of Cr. Transcription families like AREB/ABF, MYB, AP2/EREBP, WRKY, bHLH, bZIP, MYC, HSF, ARF, AtSR, CPP, SBP, MADS, TUB, C2C2-YABBY, C2C2-CO-like, C2C2-Gata, E2F-DP, ABI3VP1, CCAAT-HAP3, CCAAT-HAP5, C2H2, C3H, C2C2-Dof, DREB1/CBF, NAC, HB, ARID, EMF1, CCAAT-HAP2, and CCAATDR1 are known to regulate important processes under stress conditions (Shiu et al. 2005, Shameer et al. 2009). Some studies in relation with transcriptome analyses under heavy metal stress have been detailed in Table 1.

4.2 Proteomics

It basically includes the complete changes in protein profile at cellular, tissue, and organ levels. Techniques being deployed for analyzing the metal-responsive proteins involve conventional two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS). Although transcriptome analyses are helping a lot to understand the response of plants, not all the changes taking place in transcriptome are reflected in the proteome (Hossain and Komatsu 2012). For example, in a study

Table 1 Regulation at transcriptome level in heavy metal-stressed plants

Plant	Metal	Transcription factors involved	Plants response	Reference
Brassica rapa	Cd	ZIPs, P_{1B}-type ATPase, and MTPs	Enhanced expression of transcription factors associated with plasma membrane (i.e., *ZIP2*, *ZIP3*, *IRT1*, *HMA2,* and *HMA4*) and tonoplast (i.e., *CAX4*, *HMA3*, *MRP7*, *MTP3,* and *COPT5*)	Yu et al. (2017)
Phytolacca americana	Cd	ZRT/IRT, ABC transporters, expansin (EXP), nicotianamine synthase (NAS), natural resistance-associated macrophage protein (NRAMP), metal-nicotianamine transporter (YSL)	The expression of transcription factors associated with absorption, transportation, and accumulation of heavy metals	Chen et al. (2017)
Oryza sativa	Cd	GST, MT, and DREB (drought-responsive element-binding protein) 1E, DREB/C-repeat binding factor (CBF), NRAMP1 (natural resistance-associated macrophage protein), HLH DNA-binding domain containing transcription factor (Os04g0301500)	Expression of Cd-responsive metal transporter gene was enhanced that helped the seedlings to survive under Cd stress	Oono et al. (2016)
Sedum alfredii	Cd	ARF4 (auxin response factor 4) and AAP3 (amino acid permease 3)	Enhanced expression of two hub genes, ARF4 (auxin response factor 4) and AAP3 (amino acid permease 3), played significant role in regulating Cd stress	Han et al. (2016)
Raphanus sativus	Cr	SPLs, MYBs, ERFs, and bZIPs	Enhancement in transcriptional processes like SPLs, MYBs, ERFs, and bZIPs. Expression of these transcription factors aids the plant under Cr stress	Liu et al. (2015a)
Oryza sativa	Cd	DREB/CBF, bZIP, NAC, AP2/ERFs (AP37, AP59), C2H2 zinc finger (ZFP252), TIFY (TIFY11), and MYB (Myb4)	Expression of transcripts related to metal transport which was found to be linked with tolerance under Cd stress	Oono et al. (2014)

(continued)

Table 1 (continued)

Plant	Metal	Transcription factors involved	Plants response	Reference
Arabidopsis thaliana	As	WRKY6	Coordinated action of transcription factors associated with uptake and transport of As as well as expression of some factors involved in signaling under stress condition	Castrillo et al. (2013)
Oryza sativa	Al	ASR5	Expression of transcription factors associated with Al tolerance was found to be enhanced thereby conferring resistance to the plant under stress condition	Arenhart et al. (2013)
Sedum alfredii	Cd	MYB, bHLH, bZIP	Upregulation in the expression of these transcription factors helped the plants to cope up with the damage induced by Cd and also helped in hyperaccumulating the metal	Gao et al. (2013)
Oryza sativa	As	ATP-binding cassette (ABC), phosphate transporter (PT), metal transporters (MT), ABC subfamily G (ABCG), DEGs, cation exchanger (CAX), cation diffusion facilitator (CDF), zinc iron permease (ZIP), copper transporter (CTR), and natural resistance-associated macrophage protein (NRAMP)	The expression of these transcription factors suggests toward their regulatory role under As stress. Apart from this, some transcriptional regulation took place at the level of hormonal signaling and lipid metabolism, and their further regulation was carried by miRNA	Yu et al. (2012)
Arabidopsis thaliana	Cu, Cd	WRKY22, WRKY25, and WRKY29	Overexpression of these transcription factors was found to be involved in enhancing stress responses by regulating MAPK and oxylipin signaling	Opdenakker et al. (2012)
Oryza sativa	–	ZIP39	Endoplasmic reticulum (ER) stress-responsive genes were found to be overexpressed and that helped the plants in regulating stress response	Takahashi et al. (2012)
Arabidopsis thaliana	Cd	bHLH38 and bHLH39	Expression of bHLH38 and bHLH39 enhanced Cd tolerance in plants as well as improved the homeostasis of Fe	Wu et al. (2012)

on *Arabidopsis*, abundance in proteins of Zn and Mg transporters was noticed; however, at transcriptome level, the factors were not found to be abundant (Elbaz et al. 2006). These differences in transcriptome and proteome expression could be resultant of changes at posttranscriptional and translational level (DalCorso et al. 2013). Therefore, it is mandatory to analyze the changes taking place at proteome levels and identify the target proteins under heavy metal detoxification process. Proteins directly participate in the detoxification process and expression profile of several antioxidants for detoxification of ROS and molecular chaperons for re-establishing the functionality of proteins, which were found to be enhanced (Zhao et al. 2011, Sharmin et al. 2012, Wang et al. 2012). Semane et al. (2010) reported that large subunit of RuBisCO-binding protein, proteins related with oxygen evolution, PS I and II were found to enhance under heavy metal stress. In a proteome study by Kieffer et al. (2008), Cd was found to affect the expression of proteins of carbon metabolism and oxidative stress in poplar plants. Moreover, accumulation of heat shock proteins, chaperons, foldases, and pathogenesis-related (PR) proteins was differentially regulated in roots and shoots (Kieffer et al. 2009). Another proteome analysis in arsenic-stressed *Anabaena* species showed abundance in phytochelatins that conferred tolerance (Pandey et al. 2012). Similar to this, Bona et al. (2011) showed that arsenic hyperaccumulator *Pteris vittata* enhances accumulation of phytochelatins as well as glutathione. Some studies in relation with proteome analyses under heavy metal stress have been shown in Table 2.

4.3 Metabolomics

Metabolome includes the role of metabolites like ascorbate, glutathione, phenols, proline, amino acids, etc. in conferring tolerance to heavy metal-stressed plants, and identification and quantification of these metabolites are referred to as metabolomics. Metabolites are important growth-regulating component of cells that are not only involved in regulating normal growth and development process but also aid the plant in combating the stress generated due to abiotic factors, especially heavy metals. The role of several amino acids like histidine and proline has been investigated, and studies showed that these are involved in chelating metal ions. Proline is reported to accumulate under metal stress (Yusuf et al. 2012) and maintain the redox status of cell by enhancing the synthesis of glutathione (Siripornadulsil et al. 2002). Similar to proline, histidine has also been reported to play an important role under heavy metal stress (Sharma and Dietz 2009). Chia et al. (2015) showed that Cd stress enhances the proline as well as histidine accumulation in *Chlorella vulgaris*, and accumulation of these metabolites helps in chelating the metal ions. Apart from amino acids, organic acids are also involved in conferring metal tolerance in the cellular system. Among the organic acids, malate, citrate, and others have been reported to play a crucial role in chelating the metal ions (Rauser 1999). Xu et al. (2012) showed that treatment with citrate enhanced the Cd accumulation in leaves rather than roots that might be linked to its sequestrating mechanism. In a recent

Table 2 Regulation at proteome level in heavy metal-stressed plants

Plant	Metal	Method employed	Plants response	Reference
Glycine max	Cd	2-DE, nano-liquid chromatography, tandem mass spectrometry	Abundance in glutamine synthetase ascorbate peroxidase, catalase, and superoxide dismutase that aids the seedlings to survive damages under cadmium stress. Increase in the molecular chaperone expressions for refolding of proteins and for their stabilization	Hossain et al. (2012a, b)
Glycine max	Cd	IPG, 2-DE, nanoLC-MS/MS	Cd-chelating proteins and amino acids were found to enhance; moreover, proteins for biosynthesis of lignin were also upregulated	Ahsan et al. (2012)
Lycopersicon esculentum	Cd	IPG, 2-DE, MALDI-TOF-MS, LIFT TOF-TOF	Cd stress enhanced the protein amount twofold higher, and out of these polypeptides some were identified for glycolytic pathway, TCA cycle, and respirations; moreover, some corresponded to proteins involved in detoxification	Rodriguez-Celma et al. (2010)
Oryza sativa	Cd	IPG, 2-DE, MALDI-TOF MS	Differential regulation of proteins like NADH-ubiquinone oxidoreductase, putative vacuolar proton-ATPase, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, enolase, formyltetrahydrofolate synthetase, hypothetical protein OsJ_009523, glutathione reductase, alpha-1,4-glucan-protein synthase, guanine nucleotide-binding protein subunit beta-like protein, endo-1,3-beta-glucanase, L-ascorbate peroxidase 1	Lee et al. (2010)

(continued)

Table 2 (continued)

Plant	Metal	Method employed	Plants response	Reference
Hordeum vulgare	Cd	iTRAQ labeling, MALDI-TOF/TOF MS	Enhancement in inorganic pyrophosphatase and a gamma-tonoplast intrinsic protein (gamma-TIP) ratio of a CAX1a and natural resistance-associated macrophage protein (NRAMP), an ABC transporter homolog to AtMRP3	Schneider et al. (2009)
Ectocarpus siliculosus	Cu	IPG, 2-DE, MALDI-TOFMS	Proteins of PS II (proteins basically involved in OEC stabilization, glycolysis, and pentose phosphate), HSP70, and vBPO for protein stabilization and detoxification of ROS were enhanced	Ritter et al. (2010)
Oryza sativa	Cu	IPG, 2-DE, MALDI-TOF MS	Putative cytochrome and metallothionein proteins were upregulated	Zhang et al. (2009)
Lupinus albus	B	IPG, 2-DE, LC-MS/MS	Important proteins involved in glycolysis, TCA cycle and oxidation-reduction, and cell division were found to decline under deficiency of B	Alves et al. (2011)
Chlamydomonas reinhardtii	As	2DE, MALDI-TOF-MS, LC-MS/MS	Proteins were differentially modulated under As stress	Walliwalagedara et al. (2012)
Anabaena sp.	As	IPG, 2-DE, MALDI-TOF, and LC-MS	Proteins involved in maintaining glycolysis (FBA II, PGK, TK, FBPase, ATP synthase, Prx, Trx, oxidoreductase), pentose phosphate pathway, and calvin cycle were upregulated to overcome the As stress	Pandey et al. (2012)
Oryza sativa	As	IPG, 2-DE, MALDI-TOF MS, ESI-MS/MS	Proteins involved in metabolism and energy-generating processes were found to enhance under As stress, and some important proteins like that are involved in synthesis of RuBisCO, ribonucleoproteins were found to decline	Ahsan et al. (2010)

(continued)

Table 2 (continued)

Plant	Metal	Method employed	Plants response	Reference
Miscanthus sinensis	Cr	2DE, MALDI-TOF, MALDI-TOF-TOF	Proteins like that of nitrate reductase, adenine phosphoribosyltransferase, formate, and IMPase were found to accumulate, as these are considered as Cr-responsive proteins	Sharmin et al. (2012)
Zea mays	Cr	2DE, MALDI-TOF-MS-MS	Differential expression in proteins leads to Cr toxicity	Wang et al. (2013)
Pseudokirchneriella subcapitata	Cr	IPG, 2-DE, LC-ESI-MS/MS	Target proteins like RuBisCO activase, RuBisCO, and light-harvesting protein complex that are involved in photosynthetic processes and amino acid metabolism were affected by Cr stress	Vannini et al. (2009)
Suaeda salsa	Hg	2-DE, MALDI-TOF, TOF-MS	Proteins associated with photosynthesis, stress responses, energy metabolism, signaling, etc. were altered	Liu et al. (2013)
Oryza sativa	Hg	2DE, ESI-MS/MS	Differential expression of proteins involved in homeostasis of redox status and hormones, molecular chaperon activity, transcriptional processes and regulation	Chen et al. (2012)
Arabidopsis thaliana	Zn	iTRAQ	Proteins related with FRO2, a ferric-chelate reductase, IRT1, an iron and zinc transporter, and V-ATPase were enhanced to aid the plant under Zn stress.	Fukao et al. (2011)
Populus sp.	Zn	2DE-MS	Proteins associated with photosynthesis, S assimilation, metabolism of sugar, chaperons and antioxidants proteins like APX, DHAR, GST were differentially regulated	Lingua et al. (2012)

(continued)

Table 2 (continued)

Plant	Metal	Method employed	Plants response	Reference
Brassica juncea	Ni	2DE, MALDI-TOF-MS	Proteins related with S metabolism, photosynthesis and ATP generation, antioxidants were enhanced to aid the plant under Zn stress	Wang et al. (2012)
Hordeum vulgare	Mn	2DIEF/SDS-PAGE, 2D blue native BN/SDS-PAGE	Proteins related to universal stress protein (Usp) family, i.e., inorganic pyrophosphatase, a probenazole-inducible protein (PBZ1), a chloroplast translational elongation factor (Tu), and ribosomal protein were differentially regulated	Führs et al. (2010)

study, the role of malic acid (a dicarboxylic acid) was investigated under Cr stress and found that this organic acid upregulates the activity of antioxidants, viz., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione peroxidase (GPX), and the enzymes of the glyoxalase system as well as accumulation of metabolites, viz., ascorbate and glutathione, with a reduction in the accumulation of ROS (Mahmud et al. 2017). Plants deploy the synthesis of low molecular weight chelators, an important strategy for metal detoxification in cells, as these chelators scavenge heavy metal and dump them into the vacuole thereby reducing these metals from cytosol. Among the important chelators, two important heavy metal-binding proteins have been recognized, viz., PCs and MTs as mentioned above (Cobbett and Goldsbrough 2002). Biosynthesis and the functions of these two proteins are discussed in detail in the above sections. With the help of proteomic as well as metabolomics study, it has been suggested that phytochelatins are involved in detoxification of metal ions. Some studies in relation with metabolome analysis under heavy metal stress have been given in Table 3.

5 Conclusions and the Way Forward

Heavy metals accumulate in soils because of intense human activities. The basic aim of studying the heavy metal pollution/contamination and hyperaccumulator species is to unravel the physiological and molecular mechanisms underlying metal stress tolerance. Thus, one can explore the possibility of using such plants ultimately to sequester/remove metals from the sites of contamination/metal-rich areas.

Table 3 Regulation at metabolome level in heavy metal-stressed plants

Metabolite/antioxidants	Plant	Metal	Method employed	Plants response	Reference
Sugars, amino acids, organic acids, inorganic acids	*Raphanus sativus*	Cd, Pb	GC-MS	Sugar, amino acids, and organic acids were altered under Pb or Cd stress	Wang et al. (2015)
Glutathione (GSH)	*Arabidopsis thaliana*	Cd and Hg	Biochemical estimation	GSH was found to enhance metal tolerance by maintaining the redox status of the cell for proper functioning	Sobrino-Plata et al. (2014)
Antioxidative enzymes	*Eichhorniacrassipes*	Pb	Biochemical estimation	The activity of stress-related antioxidants like SOD, CAT, and APX. was found to enhance under Pb stress	Malar et al. (2014)
Ascorbate peroxidase, glutathione reductase and glutathione,	*Brassica juncea*	Ni and Zn	Biochemical estimation	Reduction in oxidative stress was noticed due to increased activity of the APX, GR, and GSH content	Khan and Khan (2014)
ascorbate, glutathione, polyamines	*Vigna radiata*	Cd	Biochemical estimation	Enhanced activity of antioxidants, i.e., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), and glutathione peroxidase (GPX), and content of ascorbate, glutathione, phytochelatins and polyamines were noticed, and this increase helped the plant to survive under Cd stress	Nahar et al. (2016)
Proline and catalase	*Chlorella*	Pb and Cd	Biochemical estimation	Both the metabolites were found to enhance under the Pb and Cd stress	Subashchandrabose et al. (2017)
Proline	*Lactuca sativa*	Al	Biochemical estimation	Proline content was found to enhance under Al stress	Silva and Matos (2016)
SOD, POD, CAT, and APX	*Eichhorniacrassipes*	Pb	Biochemical estimation	APX and POD activity were found to enhance under the stress condition, while SOD and CAT activity were differentially regulated	Malar et al. (2014)

Metabolite/antioxidants	Plant	Metal	Method employed	Plants response	Reference
Proline, CAT, GSH, and POD	*Vicia faba*	Cd, Cu, Ni, Pb and Zn	Biochemical estimation	Enhancement in GPX activity and proline content was noticed	Nadgórska-Socha et al. (2013)
Vitamin E (α-tocopherol), ascorbate, and glutathione	*Arabidopsis thaliana*	Cd and Cu	Biochemical estimation	Differential expression of metabolites was noticed as vitamin E was found to increase, while ascorbate and glutathione content declined	Collin et al. (2008)
Amino acids (proline, histidine, and glutamine)	*Chlorella vulgaris*	Cd	Biochemical estimation	Enhanced accumulation of amino acids was noticed that helped in chelating metal ions within the cells	Chia et al. (2015)
Amino acid (proline and histidine)	*Solanum nigrum* and *Solanum torvum*	Cd	LC-MS/MS Q-TRAP	Enhanced biosynthesis as well as accumulation of amino acids, viz., proline and histidine, which suggests toward their involvement in improving Cd tolerance of the test seedlings	Xu et al. (2012)
Organic acid (citric acid)	*Solanum nigrum* and *Solanum torvum*	Cd	LC-MS/MS Q-TRAP	Biosynthesis of organic acid, i.e., citric acid, was enhanced in the seedlings treated with Cd that helped the plant to combat with the stress	Xu et al. (2012)
Organic acid (maleic acid)	*Brassica juncea*	Cr	Biochemical estimation	Exogenous supplementation improved the antioxidant as well as metabolite status within the cell thereby conferring tolerance to the cell	Mahmud et al. (2017)

In spite of an avalanche of information that has accumulated over the years with regard to heavy metal tolerance in plants, many aspects of this area still await explanation. Till date, we could not generate a plant that can withstand multiple heavy metals and useful for phytoremediation/phytomining strategies. It appears that the heavy metal-accumulating species get protection from the herbivores and other pathogens, but this needs to be further validated fully. Therefore, there is a need to generate plants either by breeding or using genetic engineering/genome editing tools immediately. Extensive research is needed under field conditions which can increase our understanding of heavy metal tolerance in plants/hyperaccumulators under various sites of metal contamination. Such an understanding would provide clues for removing the metals effectively from the contaminated sites, preventing their entry into food chain, and saving human and animal lives. The fact that metal accumulators may also serve as ideal genetic resources to develop plants with increased essential metal accumulation like Fe, Zn, and selenium and food crop biofortification for improving the health of the general public is a silver lining in this area of research.

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Conflict of Interest Authors declare that they do not have any conflict of interests regarding the publication of this paper.

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Molecular Mechanism and Signaling Response of Heavy Metal Stress Tolerance in Plants

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1 Introduction

Heavy metals are naturally occurring inorganic chemical elements exhibiting specific gravity more than five, along with metallic properties (Joshi et al. 2015; Mustafa and Komatsu 2016). Being an inorganic compound, they are essentially nonbiodegradable and are largely bound to the soil matrix and therefore remain stable in the environment (Huang et al. 2018a). Heavy metals are absorbed by plants from the environment through the root system using both passive and active transport processes and are then translocated to the aerial parts via the xylem, which in turn affects plant's metabolism and growth (Kushwaha et al. 2015). According to their function in plants, they are categorized into essential and nonessential heavy metals. Essential heavy metals such as, Mo, Mn, Fe, Co, Vn, St, Zn, Cu, and Ni are required by plants in trace amounts for plethora of biochemical and physiological processes (Barbosa et al. 2015). Nonessential heavy metals, i.e., Cr, Hg, Sb, As, Pb, Cd, Ag, and Se, do not affect any biochemical and physiological mechanism in plants (Emamverdian et al. 2015; Joshi et al. 2015).

During the last century, anthropogenic perturbations of biosphere such as agrochemicals, landfill leachates, mine tailings, industrial waste, disposal of sewage sludge and war chemicals, nuclear wastes, dust from smelters, combustion of fossil fuel, poor irrigation practices in agriculture, and rapid urbanization wreaked havoc for the accumulation and contamination of toxic metals in the food chain affecting natural biogeochemical cycles and thus becoming a serious abiotic stress factor

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globally (Kısa et al. 2016a; Seneviratne et al. 2017; Huang et al. 2018a). Heavy metals become toxic by replacing essential metals in pigments or enzymes, thereby causing irreversible structural changes as well as physiological disorders, namely, reduced photosynthesis, generation of reactive oxygen species (ROS), and cytotoxic compounds like methylglyoxal (MG), distorting the electron transport chain, lipid peroxidation, and cellular homeostasis leading to programmed cell death (PCD) (Kushwaha et al. 2015; Kisa et al. 2016b).

Tolerance against heavy metals in plants is mediated by various molecular and biochemical mechanisms that aid in the sequestration, exclusion, detoxification, and attenuation of intracellular metal ions besides chelating extracellular compounds such as glutathione (GSH), cysteine, phytochelatins (PCs), and metallothioneins (MTs) to compartmentalize them in vacuoles (Kisa et al. 2016a; Mustafa and Komatsu 2016). Plants are an essential component of food chain; thus it is requisite to understand the strategies that plants utilize to attenuate the inimical effects of heavy metals. Various "omics" approaches, which include transcriptomics, proteomics, and metabolomics, provide promising strategies to understand various signaling pathways and molecular mechanisms responsible for plant's heavy metal stress tolerance. Our chapter will provide an overview about the molecular mechanisms of heavy metal stress tolerance with a focus on their uptake, oxidation stress mitigation, signal transduction, functional genomics, and miRNA-based regulation.

2 Heavy Metal Ion Uptake and Translocation

Soil is considered as sink for heavy metals, and therefore they get accumulated at higher concentrations in this medium. However, metals show differential affinities toward different elements in soil, thus affecting bonding with other macromolecules and complex formation. In addition, different metals have different mobilities in soil. For example, Cd and Zn are known to become mobile, whereas Cu and Pb remain immobile and readily make complexes. Plants absorb micronutrients from their surrounding aquatic and terrestrial environment during the growth period. This uptake is achieved through a nonmetabolic, passive process regulated by diffusion and mass flow (Prakash and Saxena 2017). The primary cell wall forms pores of cellulose, hemicellulose, and glycoproteins in which the ions can move. Cationic metal ions are attracted toward the anionic carboxylic groups and are accumulated in plasma membrane. This enhances concentration gradient across the membrane, thereby facilitating the movement of metals into the cells (Fig. 1). Few essential metals are absorbed through regulatory processes, for example, Cu uptake is achieved through ATP-dependent high-affinity biphasic system, and Zn is transported through Zn transporters (Prakash and Saxena 2017). Cr and Pb either enter by complexation with root exudates, such as uronic acid through symplast pathway (Kumar et al. 2016) or through sulfate/phosphate, or Ca transport channels through an active process (Cohen et al. 2006). Tsednee et al. (2014) revealed that excess Zn

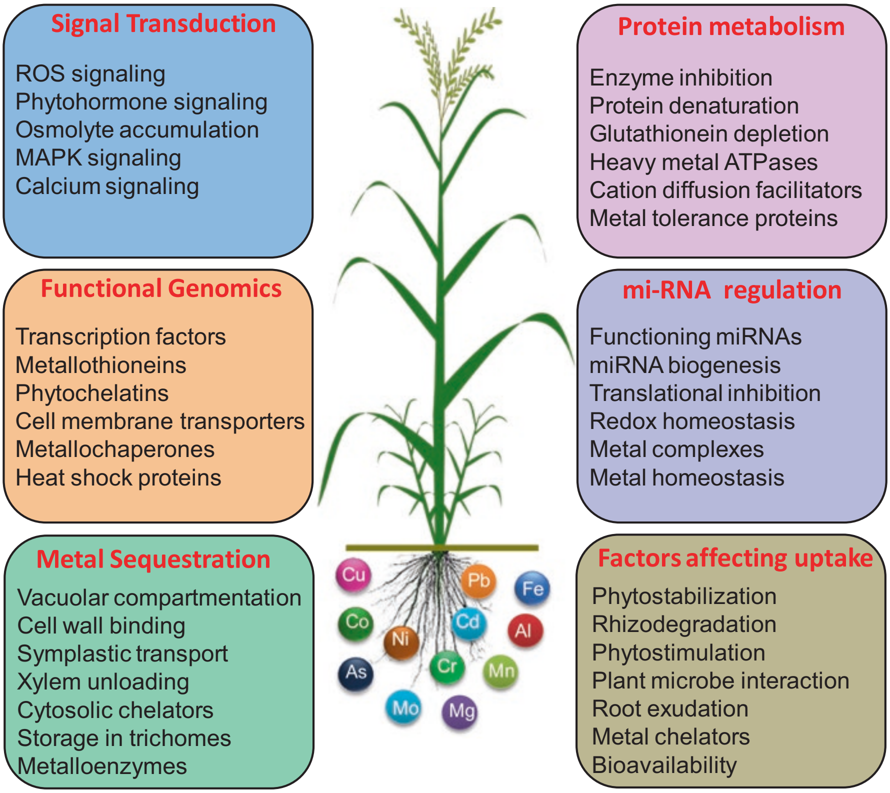

Fig. 1 Schematic diagram depicting heavy metal stress signaling response in plants

in the soil induces nicotianamine (NA) emanation from *Arabidopsis* roots, resulting in the formation of a Zn-NA chelate around the rhizosphere, which ultimately leads to its efficient uptake by roots. Metals are also absorbed by leaves in gaseous form through the stomata, such as Hg, and ionic form through cuticle (Gautam et al. 2016). However, different metals showed differential absorption rate. Cu, Zn, and Cd showed higher penetration in leaves as compared to Pb (Greger 2004).

Metal ion acquisition in the rhizosphere of higher plants is influenced by highly effective systems of root exudates and microorganisms (Rout and Panigrahi 2015). Root exudates primarily chelate metal ions such as citrate, histidine, and malate, and nicotianamine prevents the uptake of unwanted metals inside the cells (Kozhevnikova et al. 2014). In addition, cation-binding sites on the cell wall of roots allow metal exchange and their availability for uptake and diffusion (Manara 2012). Two pathways are available for metal uptake in roots, i.e., apoplastic and symplastic. The former pathway includes noncationic metal chelates, because cell walls have relatively more cation exchange capacity (Thakur et al. 2016). Heavy metal ions invade xylem by symplastic movement through electrochemical gradient across the plasma membrane (Saxena and Misra 2010). On the other hand, the later pathway includes apoplastic association of metal ions, which regulate the uptake of metals through root cells (Rout and Panigrahi 2015). In general, plants trigger various strategies under heavy metal toxicity, which incorporate immobilization, exclusion, chelation, and compartmentalization of the metal ions as well as the abundance of genes implicated in stress responses (Mustafa and Komatsu 2016). Unlike other plants, mosses absorb metals from the environment through shoot and thallus, while macroalgae absorb metal through thallus from water (Greger 2004).

Similarly, plants also possess various plasma membrane transporters such as HMA (heavy metal ATPases), CTR/COPT (copper transporter), NRAMP (natural resistance-associated macrophage protein), CDF (cation diffusion facilitators), YSL (yellow stripe-like), PDR (pleiotropic drug resistance), ABC transporters, and ZIP (ZTR/IRT-related proteins) for metal uptake and homeostasis (Ovečka and Takáč 2014; Kushwaha et al. 2015). Membrane transporters belong to a group of proteins that are embedded into the phospholipid bilayer of plasma membrane and folded to form a channel or pore to allow ion transport (DalCorso et al. 2013). NRAMP family members transport divalent cations across bacterial and eukaryotic membranes (Ovečka and Takáč 2014). The ZIP transporter family, which consists of eight transmembrane domains, amino and carboxy-terminus exposed to the apoplast, specifically uptake divalent metal ions such as Zn, Cd, Mn, Ni, and Fe (Xu et al. 2012; Milner et al. 2013). IRT1 (iron-regulated transporter 1) was initially characterized as a ZIP transporter in *A. thaliana* and expressed in root cells during Fe^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, and Cd^{2+} uptake (Vert et al. 2002; Nishida et al. 2011). Similarly, plasma membrane localized ABC transporter such as *PDR8*, and oligopeptide transporters allow uptake of HM-chelator complexes into root cells (Brunetti et al. 2015). In addition, yellow stripe 1-like (YSL) proteins allow Cd entry into the root cells (Curie et al. 2008).

It was reported earlier that low-affinity cation transporter (*LCT1*), which is a known Ca transporter in wheat, also transports Cd in yeast (Diatloff et al. 2006). Similarly, plasma membrane localized *ZntA* gene is responsible for translocation of Cd from root to shoot in *Arabidopsis* (Weerakoon 2019). However, the Cd hyperaccumulator plant *Salsola kali* metabolize Cd through low molecular weight thiols present in the stems and roots (Monferrán and Wunderlin 2013). Further, P1B-type ATPases—belonging to the HMAs transporters family—utilize energy from ATP hydrolysis to efflux both monovalent and divalent metal cations including Cu^{+}, Ag^{+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} across biological membranes (Migeon et al. 2010). For example, overexpressing *AtHMA3* improved tolerance of *Arabidopsis* against heavy metals such as Cd, Zn, Co, and Pb (Manara 2012). Oligopeptide transporters (OPTs), belonging to YSL subfamily, are involved in the uptake of Zn, Cu, Fe, Ni, Cd, and Mn (Jain et al. 2019). Recently, COPT1 was reported to transport Cu in *Arabidopsis*, which is a prime regulator of plant growth and development (Mustafa and Komatsu 2016). Metal tolerance proteins (MTPs) of CDF transporter family are divalent metal cation transporters responsible in the transport of Mn, Ni, Fe, Co, Cd, and Zn from the cytoplasm to the vacuole (Manara 2012). Whole genome analysis of *Populus trichocarpa* identified 70 metal transporter genes, higher than both *A. thaliana* and rice, pointing to a more complicated heavy metal translocation machinery in woody plant species (Migeon et al. 2010).

The next key physiological step for a plant exposed to heavy metals is improved metal xylem loading and apoplastic transport to the shoots, where these are then detoxified and stored (Choppala et al. 2014). This step involves low sequestration of metals in the xylem, which is attained from a ready efflux out of the root cell tonoplast (Kumar and Aery 2016). Metal translocation to the shoot is regulated either by transpiration or by cation exchange at the xylem vessel walls (Yamaguchi et al. 2011). In the xylem vessels, the translocation of metals occur in complexed form. For example, Zn is chelated with organic acids, Cu forms a complex with amino acids, Ni as a Ni-peptide complex, Cd as a divalent ion, and As either forms As-sulfur complexes or is chelated with cyclohexylenedinitrotetraacetic acid and nitrilotriacetic acid (Monferrán and Wunderlin 2013).

2.1 Factors Influencing Metal Uptake

Heavy metals are available in soil solution as ions, of which some such as Cd and Zn are mobile and readily available for uptake, while others, e.g., Pb, are immobile and must be mobilized before being taken up by the roots (Kumar and Aery 2016). Various factors affect the availability and mobility of such metals for uptake by roots (Fig. 1). These include light, temperature, metal interactions, external metal concentrations, root size, root exudates, microorganisms in the soil solution, soil organic matter, cation exchange capacity (CEC), and chelating agents like EDTA (Violante et al. 2010). Thus, bigger root size results in effective uptake. Similarly, increasing light intensity leads to higher heavy metal uptake in algae, lichen, and mosses (Greger 2004). Further, higher temperature improves Cd uptake in *Solanum nigrum*, *Lemna minor* and mosses, Pb and Cd uptake in *Lolium perenne,* and Mn and Zn absorption in algae (Greger 2004). Enhanced soil CEC increases root CEC and thus promotes metal uptake. Metal absorption through roots also increases with pH in hydroponic system, because of competition between metal ions and hydrogen ions. In contrast, metal uptake in soil increases with decreased pH (Zeng et al. 2011). Trivalent cations get readily taken up after forming complexes with EDTA, whereas complexes with divalent cations showed reverse effect. In addition, soil microorganisms secrete several compounds, which effectively release metals attached to soil particles, making them available for absorption by roots. However, under Fe^{3+} deficiency, roots of certain grasses release phytosiderophores (nonprotein amino acids) that bind Fe^{3+} and facilitate its absorption (Lee et al. 2009).

2.2 Sequestration of Heavy Metals and Compartmentalization

Following uptake and transport, heavy metals get accumulated inside plant cells and cause deleterious effects on the crop development (Fig. 1). Such plants show altered reactive oxygen species (ROS) and antioxidant enzyme activity, enhanced proteolysis, induced lipid peroxidation, decreased photosynthesis, chlorosis, and stunted growth, resulting in cell death (Luo et al. 2016). To deplete the toxic aftermath of heavy metal ions, plants have developed various strategies that aid in sequestration and detoxification of these harmful metals. High intracellular concentrations of metal ions are either exported to the apoplast using efflux pumps or are sequestered within the cellular compartments, i.e., Golgi apparatus, vacuoles, and cell walls (Saraswat and Rai 2011; Ovečka and Takáč 2014). Consequently, direct interaction of metal ions with enzymes and metabolites is reduced, thus preventing their inactivation. However, few of the cytosolic heavy metals, i.e, Cd, Zn, Cu, Ag, Au, Hg, and Pb, are chelated by metallothioneins (MTs), phytochelatins (PCs), and glutathione (GSH) to form LMW and/or HMW complexes and are subsequently transported into vacuoles (Joshi et al. 2015). The HMW compounds are segregated inside vacuoles at acidic pH, and the liberated heavy metals are chelated with organic compounds, i.e, malate, oxalate, citrate, and amino acids (Luo et al. 2016).

ABC transporters, particularly two subfamilies (MRP and PDR), actively transport chelated heavy metals into the vacuole. Similarly, the CDF transporter family (MTPs in plants) also plays a key role in metal ion sequestration from the cytoplasm to the vacuole, apoplast, and endoplasmic reticulum (Arbaoui et al. 2014). In addition, other compartmentalization strategies include metal ion sequestration in the specialized cell types, which include mesophyll cells, epidermal cells, and trichomes, e.g., Cd, Mn, and Pb are translocated into old leaves before leaf shedding (Thakur et al. 2016).

3 Heavy Metal-Induced Oxidative Damage in Plants

Heavy metal ions have the ability to bind protein groups and replace specific cations from binding sites (Fig. 1). This results in antioxidant enzyme deactivation and overproduction of reactive oxygen species (ROS), resulting in oxidative damage leading to altered calcium homeostasis, depletion of sulfhydryls, DNA damage, and lipid peroxidation (Rout and Panigrahi 2015). The toxicity sequence for heavy metals differs according to the taxonomical groups, i.e., Hg > Pb > Cu > Cd > Cr > Ni > Zn is seen in flowering plants such as barley, Hg > Cu > Cd > Fe > Cr > Zn > Ni > Co > Mn is seen in algae such as *Chlorella vulgaris*, and Ag > Hg > Cu > Cd > C r > Ni > Pb > Co > Zn > Fe is seen in fungi (Shaw et al. 2004). In addition, the toxic responses of both root and shoot to heavy metals vary. Under high Cu concentrations, significant increase in malondialdehyde content was observed in rice, oat, and tomato (Palma et al. 2013), enhanced lipid peroxidation was seen in *Brassica napus* and tomato (Zaheer et al. 2015), and decreased catalase and ascorbate peroxidase activity was observed in oat (Shaw et al. 2004). Further, high accumulation of malondialdehyde in *Phaseolus vulgaris* subjected to Cd and Zn (Bouazizi et al. 2010) and soybean subjected to Zn (Weisany et al. 2012) were correlated with an increase in H_2O_2 level and decline in catalase activity. Moreover, the content of thiobarbituric acid reactive metabolites and H_2O_2 was significantly enhanced in the *P. vulgaris* seedlings upon exposure to Zn (Shaw et al. 2004). A decrease in antioxidative enzymes in response to Cd treatment was reported in sunflower cotyledons (Shaw et al. 2004). Similarly, reduced GSH content was reported in *Silene cucubalus* and *Ditylum brightwellii* upon Cd exposure resulting in oxidative stress due to the accumulation of H_2O_2 and $O_2^{\bullet-}$ (He et al. 2008). Further, Cd exposure resulted in enhanced H_2O_2, nitric oxide (NO), and $O_2^{\bullet-}$ concentration in soybean roots (Pérez-Chaca et al. 2014). NO is reported to play a critical role in perception and response toward heavy metal stress (Sami et al. 2018). Its endogenous expression promotes ROS production and long-distance signaling such as the uptake of iron in roots of *S. nigrum* (Xu et al. 2010).

Heavy metals enhance ROI production by interfering with the respiratory and photosynthetic processes of plants. In maize and barley, Cu, Co, and Zn cause a significant decrease in Rubisco activity, both in vitro and in vivo (Pourrut et al. 2013). Similarly, the inhibition of chlorophyll and calvin cycle enzymes such as FDPase, aldolase, NADP and NAD-glyceraldehyde 3-P dehydrogenase, 3-PGA kinase, and Rubisco by Cd, Ni, and Cu were reported in *Phaseolus aureus*, *P. vulgaris*, and oat, leading to the accumulation of NADPH and ATP, which ultimately results in downregulation or feedback inhibition of the photosynthetic electron transport (Shaw et al. 2004). In contrast, enhanced levels of chlorophyll was observed in *Phaseolus coccineus* (runner bean) on $CuSO_4$ solution (Wianowska et al. 2004). The reduction of photosynthesis by heavy metals toxicity is because of electron transport inhibition across the electron transport chain (Rai et al. 2016).

Root-to-shoot signaling was reported to play a key role in heavy metal-induced oxidative stress, increased lipid peroxidase, or during the antioxidative defense responses, i.e., increases in glutathione reductase, superoxide dismutase, and catalase activity (Rout and Panigrahi 2015). However, increase in proline levels, which is a by-product of lipoxygenase, is linked to its ability to react with hydroxyl radicals and quench singlet oxygen, thus mitigating the damage from free radicals (Rout and Panigrahi 2015).

4 Signaling Response Under Heavy Metal Stress

Plants utilize various strategies for heavy metal detoxification and the maintenance of their cellular concentrations beneath the threshold levels of toxicity (Sharma and Dietz 2009). In general, plants adopt two defense strategies to prevent excess aggregation of metal ions in the cytoplasm, i.e., avoidance and tolerance (Thakur et al. 2016). The former defines the plants' capability to suppress surplus metal uptake using metal exclusion, complexation to various ligands, and translocation, while tolerance defines their capability to regulate accumulated metal ions (Fig. 1). The later strategy depends upon the type of metal ions, their concentration, plant species, organs, and developmental stage (Thakur et al. 2016). Essential metal ions, i.e., Mn, Cu, Zn, and Fe are involved in complex metabolic and signal transduction mechanisms such as Ca-calmodulin signaling, hormones, mitogen-activated protein kinase (MAPK) phosphorylation cascade, ROS signaling, and downregulation of stress-responsive genes (Rout and Panigrahi 2015). Transcriptional and proteomic regulation depicting numerous differentially expressed proteins also play a significant role in heavy metal detoxification and sequestration (Singh et al. 2016). Further, we discuss different strategies utilized by plants for heavy metal stress tolerance including signal transduction and transcriptional and posttranscriptional regulation.

4.1 Signal Transduction

Selection of specificity and uptake of heavy metal ions at optimal levels are vital for normal development of plants (Fig. 1; Gupta et al. 2013). In addition, plant's ability to tolerate heavy metal stress chiefly relies on their capability to discern and transduce signal through primary signaling molecules, i.e., NO, ROS, H_2S, and phytohormones, which then targets TFs (Luo et al. 2014; Islam et al. 2015). These TFs further bind to the promoter of a number of genes to regulate gene expression for any required physiological, biochemical, and molecular status (Lai et al. 2018). Heavy metals alter the concentrations of phytohormones such as salicylic acid (SA), jasmonic acid (JA), auxins, cytokinins (CKs), abscisic acid (ABA), and ethylene (ET) (Khan et al. 2018). These phytohormones mediate long-distance systemic responses in plants. Exogenous application of ABA resulted in enhanced Fe content and decreased Cd uptake by reducing IRT1 expression in *Arabidopsis* (Fan et al. 2014). Similarly, elevated ABA content was reported after high Mn treatment in *Populus cathayana* (Lei et al. 2007). Further, enhanced transcript levels were found in ABA signaling genes in the roots of rice seedlings after As treatment (Huang et al. 2012).

Cadmium was reported to affect auxin signaling resulting in decreased root growth and ROS overproduction, which was confirmed by inhibiting auxin signaling using p-chlorophenoxyisobutyric acid (Tamás et al. 2015). Zn, Pb, Cd, Cu, and Ni were also found to differentially regulate auxin concentration and distribution and the transcript abundance of genes required for the regulation of auxin metabolism in *Arabidopsis* seedlings (Wang et al. 2015). Methyl jasmonate (MeJA) alleviates Cd and Cu tolerance by enhancing PC and ABA biosynthesis and activities of CAT and APX in *Arabidopsis* and *Kandelia obovata* (Chen et al. 2014). Further, JA concentration also increases after heavy metal treatment, thereby elevating MAPK signaling and GSH biosynthesis to induce stress response in plants (Luo et al. 2016). Additionally, Cd treatment in *A. thaliana*, *Pisum sativum*, *Triticum aestivum*, *Zea mays*, *and Krigia virginica* elevates SA concentrations, whereas exogenous SA treatment decreases plant growth (Kovács et al. 2014; Liu et al. 2016). Excess Zn application resulted in enhanced SA and ABA and reduced GA3 and auxin in the leaves of *Populus tremula* × *Populus alba* (Shi et al. 2015). Similarly, in *Populus nigra*, Cd treatment increases ABA, ET, and gibberellins (GAs) concentrations and reduces cytokinin and auxin levels (Lomaglio et al. 2015).

Heavy metal stress induces H_2O_2 production through NADPH oxidase localized in plasma membrane, mitochondria, and peroxisomes and is regulated by cytoplasmic Ca^{2+}, calmodulin (CaM), protein kinases, and ethylene (Chmielowska-Bąk et al. 2014). NADPH oxidase catalyzes NADPH and transfers electrons to molecular oxygen, resulting in the formation of superoxide anions. Under heavy metal stress, NO showed long-distance signaling, which is then followed by interaction with different signaling molecules, namely, H_2S, ROS, and auxin (Fancy et al. 2017). Bharwana et al. (2014) also demonstrated that H_2S acts as a signaling molecule under heavy metal stress. They found that exogenously applied H_2S alleviates Pb-induced toxicity in *Brassica napus* and *Gossypium* by improving antioxidant system activities, reduced H_2O_2 production, and increased total GSH content and GSH to GSSG ratio.

Heavy metal-induced MAPK pathway activation is instigated through phosphatidyl inositol 3-kinase (PI3K), CDPK, ROS, NADPH oxidases, and other MAPKs in plants (Kohli et al. 2017). The MAPK cascades activate TFs, enzymes, proteins, and other signaling molecules, thus playing a pivotal role in modulating gene expression under heavy metal stress (Kohli et al. 2017). Cd exposure in *Arabidopsis* and soybean increases NO production leading to enhanced transcript abundance of MAPKs, while 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (NO scavenger) reduces MAPK activation (Fancy et al. 2017). Under heavy metal exposure, transcript abundance of MAPK and their targeted genes was upregulated in soybean (Chmielowska-Bąk et al. 2017), *Arabidopsis* (Opdenakker et al. 2012), and rice (Wang et al. 2018). In rice seedlings, MAPK signaling showed tight correlation with auxin signaling pathway as mRNA levels of OsPIN, OsARF, and OsIAA were modulated after Cd treatment (Luo et al. 2016).

4.2 Protein Metabolism

In general, plants' vulnerability to heavy metal stress involves sensing, absorption and translocation of heavy metal ions, development of PC-heavy metal complexes, and their vacuolar confiscation (Ovečka and Takáč 2014). In addition to these primary processes, other essential processes also include antioxidant-based defense reactions and the protection of membrane lipid integrity (Wagatsuma 2017). Such mechanisms are under the control of both transcriptional and posttranslational regulations. Developing plants showing tolerance to heavy metal stress require the adoption of several conventional and novel biotechnological approaches (Ovečka and Takáč 2014). These approaches, which include various genetic and molecular tools, can significantly improve heavy metal stress tolerance in plants (Fig. 1).

Another powerful tool used in the identification of processes combined with heavy metal tolerance in crops is proteomics (Hossain and Komatsu 2013). These analyses conducted in several plants have uncovered various heavy metal-responsive proteins that are essential in heavy metal tolerance. In fact, the affected proteins belong to 20 functional groups, which showed differential response. These proteins are represented mostly (67%) by those that are involved in antioxidant defense, and their abundance varies with the duration of treatment (Song et al. 2013). The group representing the second largest number of proteins upregulated upon exposure to heavy metals consists of proteins involved in protein folding, which include protein disulfide isomerases, chaperonins, and heat shock proteins (Hossain et al. 2012). Further, the third largest functional group representing pathogenesis-related proteins also demonstrate upregulation after heavy metal treatment, indicating a cross talk between the heavy metal and biotic stress responses in plants. Heavy metal treatment also leads to the upregulation of proteins involved in ion transport and lipid metabolism. This response is indicative of the contrary effects of heavy metals on lipid bilayer constitution. Among them, vacoular proton pumps represent the most abundant proteins that are known to be involved in intracellular vesicular transport between vacuoles and pre-vacuolar compartments (Sharma et al. 2016). Unexpectedly, proteins that play a role in cell wall metabolism also responded during heavy metal exposure, indicating that the cell wall plays a significant role in heavy metal stress tolerance.

4.3 Functional Genomics

The cross talk of heavy metal stress responses includes a set of molecular regulations that are needed to unravel the development of heavy metal-tolerant crops (Fig. 1). This requires information derived from omics-based approaches to define the function of several stress-responsive genes imparting tolerance to plants. Further challenge is to functionally characterize these genes to delineate the stress response process (Rout and Panigrahi 2015). Integrating genomics, transcriptomics, proteomics, phenomics, ionomics, and metabolomics enables large-scale functional analysis of genes to unravel gene interactions at cellular and whole plant level. In addition, whole genome sequencing of various plant genomes has enhanced the genetic knowledge to enlist these tools to simultaneously determine several gene functions (Joshi et al. 2016).

Numerous reports are available on metallothionein expression in crops under heavy metal stress conditions (Kumar et al. 2012). However, higher transcript abundance of class I MT genes in rice and wheat, induced by various heavy metals such as Cd, Al, Co, and Zn, is indicative of their significant role during heavy metal stress (Kumar et al. 2012). Several evidences have emerged on the antioxidant activity and ROS signaling of metallothioneins after initial demonstration by Thornalley and Vašák (1985), which demonstrated that the metal-binding Cys residues in MTs can also scavenge superoxide ($O_2^{\bullet-}$) and free hydroxyl (°OH) radicals under oxidative stress (Kohli et al. 2017). Previous reports have also pointed to the role of MTs in metal ion remobilization during senescence (Tripathi et al. 2015).

Improved tolerance toward drought and salinity, enhanced antioxidant enzymes activity, and induced expression of TFs in plants overexpressing MT genes confirmed their key role in the antioxidant pathway. We reported earlier that the ectopic expression of *OsMT1e-P* improves tolerance against multiple abiotic stresses by scavenging ROS, thus providing defense against oxidative stresses (Kumar et al. 2012). Moreover, overexpression of a salinity stress-induced *OsMT-3a* provides tolerance against salinity stress in *E. coli* cells, confirming their role in providing salinity tolerance through ROS scavenging (Mekawy et al. 2018). Further, it was reported that *PgMT2* and *PgMT3* showed early stress responsiveness under higher Cu exposure in *Prosopis glandulosa* (Michel-Lopez et al. 2017). In addition, ectopic expression of rice *OsMT2c* improved Cu stress tolerance and reduced H_2O_2 production in *Arabidopsis* by ROS scavenging (Liu et al. 2015). Similarly, heterologous expression of *MT2b* from *Iris lactea* showed enhanced root length, H_2O_2 production, and copper accumulation in *Arabidopsis* seedlings (Gu et al. 2015). Recently, it was shown that the expression of *HbMT2b* decreased significantly in latex of *Hevea brasiliensis* after tapping, wounding, and ethephon treatment, while *HbMT2* and *-3L* get upregulated after fungus infection and high-temperature stress (Huang et al. 2018b). This is correlated with HbMTs role in multiple defense response via the regulation of ROS homeostasis.

4.4 miRNA-Based Regulation

RNA interference is another promising approach for depicting gene function in heavy metal stress signaling (Fig. 1; Casacuberta et al. 2015). MicroRNAs (miRNAs) are small, single-stranded RNAs which negatively deregulate the posttranscriptional activity through targeted cleavage of the mRNA (Joshi et al. 2017). miRNAs were earlier reported as crucial regulators of adaptation to heavy metal stresses (Fig. 2; Noman and Aqeel 2017). Various reports on metal stress-responsive miRNAs and their targets are available in plants such as barley (Wu et al. 2018), *Ricinus communis* (Çelik and Akdaş 2019), *B. napus* (Zhang et al. 2018), and *O. sativa* (Ding et al. 2018). Copper-zinc superoxide dismutase (Cu-Zn SOD; CSD) is vital for dismutation of superoxide radicals during high Cu availability (Pilon 2017). miR398 regulates the induction of *CSD1* and *CSD2* mRNAs under high Cu levels. Other miRNAs such as miR395, miR397, miR408, and miR857 are also induced under Cu stress and downregulate the transcript abundance of laccase and PC genes (Jagadeeswaran et al. 2014; Paul et al. 2015). However, characterization of *Arabidopsis* squamosa promoter binding protein-like 7 (*SPL7*), a homologous gene of *Chlamydomonas reinhardtii* CRR1 (copper response regulator) and a transcriptional modulator of Cu homeostasis, confirms the tight regulation of Cu and miRNAs, e.g., miR857 (Yamasaki et al. 2009).

However, during Cd stress, downregulation of miR156, miR171, and miR396a transcripts was observed in *Brassica napus* (Zhou et al. 2012a, b). In contrast, miR393, miR171, miR319, and miR529 were found to be highly expressed, while miR166 was downregulated during Cd stress in *Medicago* (Zhou et al. 2008). Microarray analysis of Cd-treated rice seedlings showed 19 miRNAs to be differentially regulated after 24 h treatment (Ding et al. 2011). However, during Cd stress,

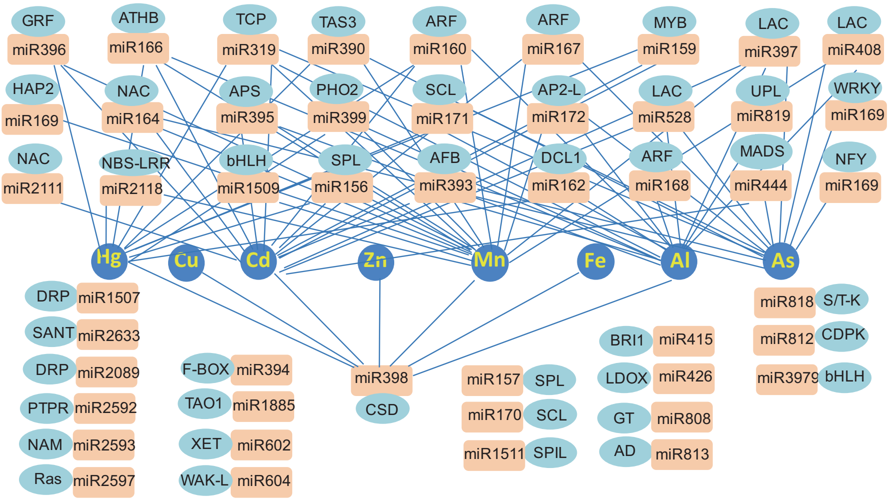

Fig. 2 Schematic diagram representing various miRNAs showing differential expression and their target families in various plant species. Upper half shows differential response of miRNAs and the cross talk between different metal responses. The lower half shows differentially regulated miRNAs specific to the metals

miR393 induced the expression of *TIR1* (transport inhibitor response 1), which further regulates auxin signaling by accumulating AUX/IAA proteins (Noman and Aqeel 2017). Similarly, overexpressing miRNA395 in *B. napus* showed tolerance against Cd-induced oxidative stress (Huang et al. 2010). miRNAs such as miR529, miR393, miR319, and miR171 showed higher expression, while miR166 gets downregulated, and miR319, miR160, and miR395 remained unaffected under Hg stress in plants (Noman and Aqeel 2017).

Eighteen novel miRNAs were reported to be differentially expressed in *O. sativa* (Yu et al. 2012), while 69 miRNAs showed differential regulation in *Brassica juncea* during As stress (Srivastava et al. 2012). Other than Cu^{2+}-binding proteins (CBPs), miR528 reduces expression of IAR1 protein that regulates conjugated auxin levels to protect *O. sativa* seedlings from As injury (Li et al. 2010). Similarly, *OPT1* (oligopeptide transporter) was also reported as a putative target of miR159 during As stress (Srivastava et al. 2012). In addition, during Al stress miRNAs were also identified in *Medicago*, and miR396, miR390, miR319, miR160, and miR159 were found to be downregulated (Chen et al. 2012). Moreover, 30 aluminum-responsive miRNAs were characterized in soybean, where miR396 and miR390 were found to be upregulated (Zeng et al. 2012). Six miRNAs were found to be upregulated, while 13 miRNAs were downregulated during Al stress (Lima et al. 2011), where high abundance of miR528 showed its function in SCF-mediated protein accumulation to tackle metal toxicity. Further, under Al toxicity in rice, miR808 targets fucosyl transferase enzyme leading to cell wall structural modifications (Lima et al. 2011).

5 Conclusion and Future Outlook

The heavy metal contamination of water and soil poses a vital threat to mankind and food safety and is currently emerging as a prime health hazard to humans and crops. This has become more accentuated and prominent with human-made disturbances of biological resources of the planet. Oxidative damage due to heavy metals poses a serious threat to the photosynthetic machinery in plants. Thus, there is an urgent need to intensify research against phytotoxicity and the machinery used by plant species to combat heavy metal stress. Plants play a crucial role in the remediation of metal-enriched soils. Plants demonstrate higher accumulation of osmolytes, stress-related proteins, transcription factors, and signaling molecules associated with heavy metal tolerance, suggesting complex stress tolerance network for crop survival and yield under stressed conditions. Huge amount of data is now available at both genome and proteome levels on plant's response to heavy metal stress imparting enormously to our knowledge on metal detoxification and maintaining ionic homeostasis in the cells. Commendable advances have been made in the identification of key signaling cascades for plant heavy metal stress tolerance such as metal transporters, phytochelatins, and metallothioneins. However, an intriguing cross talk was reported during heavy metal stress signaling, i.e., *cis*-acting and *trans*-acting regulatory elements, miRNA-based posttranslational and transcriptional regulation, and epigenetic regulation of plant adaptation toward metal(loid)-rich conditions. Further advancement is required for subcellular proteomic analyses of plant tissues exposed to heavy metal stress and identification of metal transporters having role in uptake, translocation, and vacuolar sequestration to decipher intricate regulation of metal homeostasis in plants. In addition, genome sequencing and in silico analysis of metal hyperaccumulator species are required to decipher complex stress regulatory networks and their downstream genes, which can be orchestrated through both genetic engineering and genome-assisted breeding for sustainable agriculture.

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Heavy Metal Toxicity and Plant Productivity: Role of Metal Scavengers

P. L. Gratão, L. R. Alves, and L. W. Lima

1 Heavy Metal Toxicity: An Oxidative Challenge

Among the numerous sources of abiotic stress, the heavy metal contamination in agricultural soil is a worldwide concern for crop production, while the metal mining, smelting, and subsequent industrial processes are important sources of this environmental pollution. The presence of heavy metals, specifically in cultivated soil, represents a great concern due to the high toxicity and persistence in the environment and may be related to the excessive use of phosphate fertilizers, which lead to the metal uptake by crops and consequent food chain bioaccumulation, representing a major threat to living organisms (Xu et al. 2013).

Different morphological and physiological mechanisms are used by plants in order to survive the wide range of environmental conditions. Entire populations can adapt to genetic changes and natural selection for many generations, while an individual plant can momentarily change its physiology to survive under different abiotic stresses in a process called "acclimation." Furthermore, few species have developed and adapted in such a way to deal with heavy metals that they can be called tolerant plants, showing an incredibly high capacity to accumulate or exclude large amounts of heavy metals and strongly overcome the possible induced stress.

Plants species are chemically diverse due to different mechanisms evolved to overcome environmental changes and different abiotic stresses. Heavy metal contamination can directly cause a strong oxidative stress due to an imbalance between the antioxidant defense system and the overproduction of reactive oxygen species (ROS) in different cellular compartments, leading to membrane disruption

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(lipid peroxidation) as well as proteins and nucleic acids denaturation, which can negatively affect important metabolic processes such as photosynthesis and carbon fixation.

These reactive molecules are partially reduced and thus unstable forms of the atmospheric oxygen (O_2). They occur as a natural result of the aerobic metabolism in mitochondria or in plastids, due to the higher oxidized environment (Cuypers et al. 2010), by the transfer of one electron (superoxide radical, $O_2^{-\bullet}$), two electrons (hydrogen peroxide, H_2O_2), or three electrons (hydroxyl radical, $OH^\bullet$) to the O_2 molecule. These ROS are able to oxidize (take electrons) and thus denature proteins and lipids from membranes in different organelles (Fig. 1) and oxidize other molecules due to the unpaired number of electrons in the outer atom shell, leading to

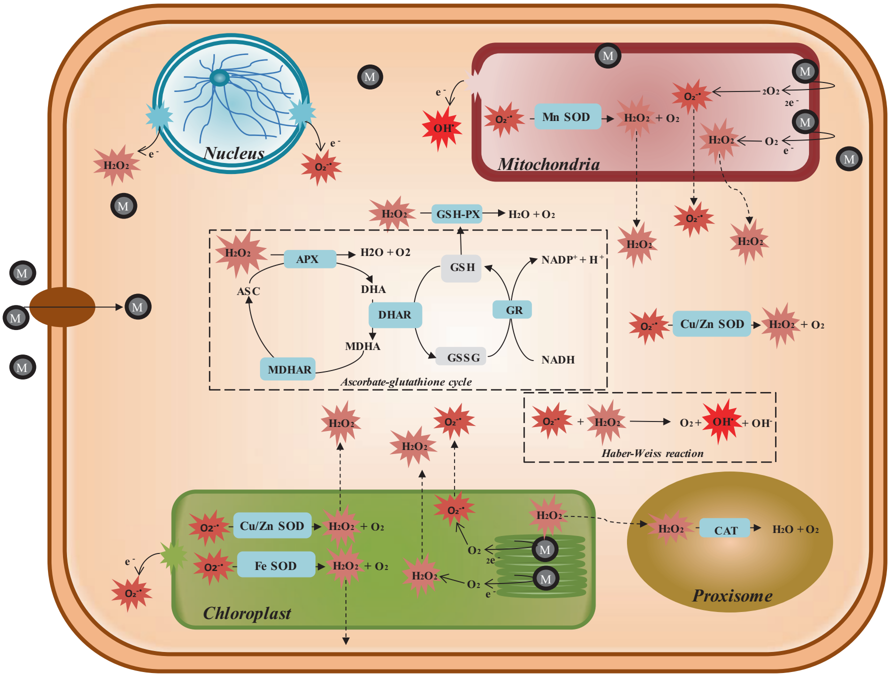

Fig. 1 Induced oxidative stress by heavy metal. Enzymatic reaction of superoxide dismutase (SOD): dismutation of the $O_2^{-\bullet}$, forming O_2 (cell oxygen) and H_2O_2 (hydrogen peroxide) in chloroplast (Cu/Zn and Fe SOD), mitochondria (Mn SOD), and cytosol (Cu/Zn SOD). H_2O_2 degradation catalyzed by the catalase (CAT) enzyme in peroxisomes, resulting in H_2O (water) and O2 (oxygen) formation. Glutathione peroxidase (GSH-Px) activity: H_2O_2 reduction concomitantly with the oxidation of the specific substrate reduced glutathione (GSH). Ascorbate-glutathione cycle enzymes: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR). Other compounds: hydrogen peroxide (H_2O_2), water (H_2O), ascorbate (ASC), dehydroascorbate (DHA), monodehydroascorbate (MDHA), glutathione (GSH), and oxidized glutathione (GSSG). Haber-Weiss reaction: hydroxyl radical ($OH^\bullet$) formation from the combination of $O_2^{-\bullet}$ and H_2O_2. The lipid peroxidation is also shown in the image, where the reactive molecules H_2O_2, $O_2^{-\bullet}$ and $OH^\bullet$ reacts with membrane lipids and disrupts the structure of chloroplast, nucleus, and mitochondria

organelles and cell destruction and thus to the death of tissues and organs such as roots, leaves, and fruits.

ROS can also cause the polyunsaturated fatty acid oxidation in different membranes in the cell, in a process called "lipid peroxidation," changing the membrane structure and its function and damaging its structure (Bino et al. 2005). This process can be used as an oxidative stress indicator through the analysis of malondialdehyde (MDA) concentration in tissues, formed from the decomposition of by-products of the lipid peroxidation (Cho and Seo 2005; Dewir et al. 2006).

The cellular defense mechanisms evolved by plants correspond to enzymatic and non-enzymatic antioxidant responses capable of breaking down the ROS molecules in order to reestablish the regular cell homeostasis. This system is also responsible for the heavy metal sequestration in order to neutralize the cascade of events that could lead to another series of oxidative reactions (Fig. 1).

Among the enzymatic antioxidant responses, the superoxide dismutase (SOD, EC 1.15.1.1) catalyzes the dismutation of the $O_2^{-\bullet}$ to form O_2 and H_2O_2. This process is important to avoid the combination of $O_2^{-\bullet}$ and H_2O_2 in a reaction called Haber-Weiss, responsible to produce the hydroxyl radical ($OH^{\bullet}$) (Fig. 1) which is a highly reactive and not a breakable molecule (Cuypers et al. 2010).

This enzyme can be found in chloroplasts, mitochondrial matrix, and the cytoplasm of cells, and three different isoforms are known. The most abundant in plants is the copper (Cu) and zinc (Zn) SOD, found in the stroma of chloroplasts and in the cytosol. Another important SOD isoform has manganese (Mn) in the active site and can be found in the mitochondrial matrix. The Fe (iron) SOD is rarely found in plants but can be associated with chloroplasts (Kumar et al. 2014).

The H_2O_2 concentration generally becomes higher within the cell due to a direct effect of SOD activity, which can induce the oxidative damage of different molecules and membranes. Consequently, the H_2O_2 need to be quickly converted into H_2O and O_2 by specific enzymes such as catalase (CAT, EC 1.11.1.6) and other peroxidases in different cellular compartments (Roychoudhury et al. 2012). The CAT can be found in all living organisms and is the main route of H_2O_2 degradation. Three different isoenzymes of CAT are known. The CAT1 is responsible for 80% of the total H_2O_2 (formed during the photosynthetic reactions in chloroplasts) degradation within peroxisomes. Meanwhile, CAT2 is primary found in vascular tissues, and CAT3 is located within the mesophyll of leaves (Mhamdi et al. 2010).

Other enzymes are also responsible for the H_2O_2 degradation in the cell environment. Peroxidases are hemeproteins responsible for the H_2O_2 molecule reduction concomitantly with the oxidation of a specific substrate, the ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione peroxidase (GSH-Px, EC 1.11.1.7), for example (Fig. 1). These peroxidases participate in many essential metabolic processes such as the cell growth regulation, lignification, phenolic oxidation, defense against pathogens, while the most impressive is their role in the antioxidant defense.

The mechanisms related to the induced oxidative stress in plants can be the same for different types of heavy metals, however, few exceptions are known. Cadmium (Cd) is considered one of the most toxic among all heavy metals (Xu et al. 2013), due to its effortless assimilation by plants and the higher accumulation within the food chain. This can be explained by the protonated form of Cd (Cd^{2+}) present in soil, which can be easily assimilated by plants via $Zn^{2+}$and other cation transporters in roots (Qin et al. 2013).

Cadmium is very stable and cannot oxidize, or donate electrons, to O_2 and produce ROS directly; however, Cd reacts with sulfur (S) in the thiol groups of different amino acids, such as cysteine; replaces other metals, such as Cu^{2+}, Zn^{2+} or Mg^{2+}, in the active site of most enzymes, including SOD and peroxidases; reduces their concentration or activity in the cell; and indirectly causes a strong oxidative stress (Zhou et al. 2013; Cuypers et al. 2010). The reaction of Cd with thiols groups, mainly glutathione (GSH), reduces its abundance in the cellular environment, which can lead to the oxidative stress (Cuypers et al. 2010). The increase in Fe concentration in the cellular environment, due to its replacement by Cd in different proteins, can also induce the overproduction of $OH^{\bullet}$ radicals, highly reactive and resistant to antioxidant mechanisms of cellular defense, resulting in a severe oxidative stress (Cuypers et al. 2010).

Foliar chlorosis is a clear symptom of the heavy metal, especially Cd, stress in which the cellular photosynthetic apparatus is severely affected. The energy from light photons is the driving force to oxidize water and different pigments, within photosystem I and II, and protein molecules in order to generate an electron flow in the thylakoid membrane to produce NADPH and ATP. Different membrane proteins are involved in the electron transport chain in chloroplasts, and different metals such iron (Fe) and cupper (Cu) are responsible for the oxido-reduction reactions in the system.

The molecular structure of chlorophyll is characterized by the presence of a central magnesium (Mg) atom, and the replacement of this metal by Cd can change the molecular stability of this pigment, impairing the absorption of light energy, electron transfer, and the consequent production of carbohydrates (Chenn et al. 2011; Xu et al. 2013). Chlorosis can also be explained by the decrease in chloroplast concentration within the cell; decrease in cell replication, phosphorus (P), and manganese (Mn) deficiency; and a negative influence on enzymes related to chlorophyll (Chenn et al. 2011; Xu et al. 2013).

2 Non-protein Thiols: Key Metabolites in Heavy Metal Scavenging

The non-enzymatic mechanisms for cellular detoxification against ROS are important and work side by side with the enzymatic antioxidant system in order to preserve the cellular redox state. The molecules taking part in this process include phytochelatins (PCs), proline, flavonoids, alkaloids, and carotenoids, among others (Foyer and Noctor 2012) (Fig. 2). It is important to mention that phytochelatins are formed from the reduced glutathione (GSH) and are the most important molecules against heavy metals. Phytochelatins can bind metals to their thiol groups,

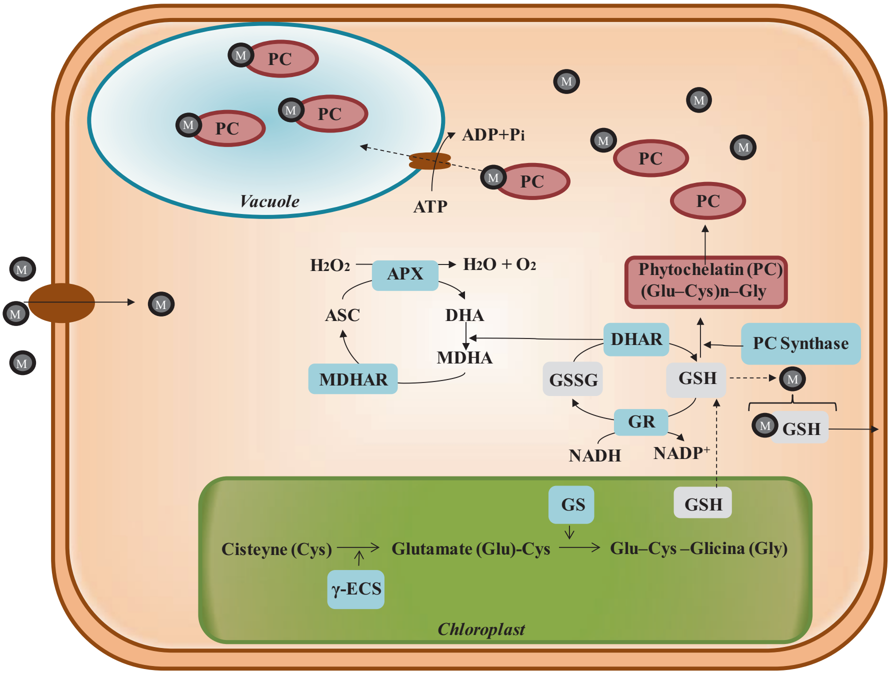

Fig. 2 Ascorbate-glutathione cycle, GSH, and phytochelatins synthesis in plants. Enzymes: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR). Other compounds: hydrogen peroxide (H_2O_2), water (H_2O), ascorbate (ASC), dehydroascorbate (DHA), monodehydroascorbate (MDHA), glutathione (GSH), and oxidized glutathione (GSSG)

inactivate, and store them into vacuoles within the cell, especially in the roots (Lefèvre et al. 2016).

Glutathione (γ-glutamylcysteinyl glycine) is a tripeptide formed by the amino acids glutamate, cysteine, and glycine. It is a major component of the non-protein thiols (NPTs) group and plays an important role in the enzymatic antioxidant defense against ROS, acting along with the enzyme ascorbate peroxidase (APX) in the ascorbate-glutathione cycle. This metabolite can be found in plants under the oxidized form (GSSG) or also in the reduced form (GSH), which is important for non-enzymatic antioxidant defense. The system always prioritizes an increased concentration of GSH over the GSSG in the cellular environment (Hernández et al. 2015).

The enzyme glutathione reductase (GR, EC 1.6.4.2) converts the GSSG into GSH using NADPH as an electron donor. The formed GSH corresponds to the substrate used by the enzyme dehydroascorbate reductase (DHAR, EC 1.8.5.1) in order to form the ascorbate (ASC), which is used by the APX enzyme within the antioxidant defense (Hernández et al. 2015) (Figs. 1 and 2). The enzyme GR is almost of universal occurrence, from eukaryotes to prokaryotes and from heterotrophic and photosynthetic bacteria to higher plants. This enzyme has the prosthetic group FAD (flavin adenine dinucleotide) responsible for catalyzing the electron transfer reaction from NADPH to GSSG in order to form GSH (Voet and Voet 1995).

The GSH plays an essential role in both the enzymatic antioxidant defense against ROS and the PCs formation, and hence, the consumption of GSH increases several fold under heavy metal stress (Roychoudhury et al. 2012). The first step in the GSH synthesis pathway happens mainly in the chloroplast, where the amino acids glutamate and cysteine are bound together by the enzyme γ-glutamylcysteine synthetase (γ-ECS); thus, this is a key and rate-limiting step to the entire metal scavenging process (Zechman 2014) (Fig. 1). Zhu et al. (1999) demonstrated, in one of the primary studies in this area, that overexpression of γ-ECS increased the concentration of NTPs such as GSH, Cys (cysteine), and PCs in seedlings of *Brassica juncea*, under Cd stress.

In the second step, amino acid Gly (glycine) binds to the first molecule in a reaction catalyzed by glutathione synthetase (GS, EC 6.3.2.3) (Noctor et al. 2011). The *GSH2* gene translates the GS, where the mRNA transcript can form two different peptides with two different targets in the cell (Hernández et al. 2015). The largest transcript protein is targeted to the plastid and shortest to the cytosol. The PCs are synthesized from the GSH by the enzyme glutathione-γglutamylcysteinyl transferase or PC synthase, which transfers a γ-Glu-Cys group to GSH in the cytosol. PCs have the general chemical form of (γ-Glu-Cys)n-Glu, where n corresponds to 2–11 (Anjum et al. 2015) (Fig. 2). The thiol group of PCs binds to the heavy metal and neutralizes its reduced state to avoid the oxidation of other molecules, while the metal-PC complex is then stored in the vacuole through ATP-Binding Cassette type transporters like ABCC family transporters (Hernández et al. 2015).

3 The Role of Proline and Glycine Betaine in Improving Heavy Metal Stress Tolerance in Plants

There are numerous mechanisms acting together to avoid negative effects of environmental stresses. Among them, accumulation of compatible osmolytes, such as proline, is an important phenomenon under heavy metal stress. This amino acid is essential to plant metabolism; it is involved in the storage and transfer of energy and intracellular NAD(P)H/NAD(P)+redox balance (Szabados and Savoure 2010; Sharma et al. 2011; Giberti et al. 2014). Moreover, strong evidences connect the pivotal role of proline in stress adaptation responses. For instance, proline acts as a potent non-enzymatic antioxidant, which contributes to the stabilization of proteins, membranes, and DNA and helps in the scavenging of ROS, which can ameliorate the phytotoxicity of heavy metals. In fact, many plants increase synthesis and decline degradation of proline during a stress condition (Anjum et al. 2016), which reinforce its benefit to plants. Therefore, proline biosynthesis, degradation, and regulation must be well-defined to enable studies aiming the manipulation of proline metabolism to improve stress tolerance.

Proline is synthesized by both ornithine and the glutamate precursors. In ornithine pathway, ornithine is transaminated to pyrroline-5-carboxylate (P5C) by ornithine-δ-aminotransferase (OAT; EC 2.6.1.13) with an intermediate product, L-glutamate γ-semialdehyde (GSA), and then it is reduced to proline by δ1-pyrroline-5-carboxylate reductase (P5CR; EC 1.5.1.2). The ornithine pathway operates preferably under high nitrogen supply in mitochondria. In glutamate pathway, the precursor glutamate is reduced twice catalyzed by pyrroline-5-carboxylate synthetase (P5CS; EC 2.7.2.11) and P5C reductase (P5CR). The labile intermediate γ-glutamyl phosphate is reduced into GSA by δ-pyrroline-5-carboxylate (P5C) (Kaur and Asthir 2015). This pathway is predominating during osmotic stress and nitrogen limitation (Sánchez et al. 2001). In both pathways, the activity of P5CS can be stimulated by abiotic stresses (Kaur and Asthir 2015).

Proline degradation occurs predominantly in mitochondria, where proline is converted to P5C by proline dehydrogenase (ProDH) or proline oxidase (POX). Afterwards, P5C dehydrogenase (P5CDH) converts P5C to glutamate. The ProDH and P5CDH use FAD and NAD as electron acceptors and provide electrons for mitochondrial respiration (Deuschle et al. 2004).

The regulation of proline biosynthesis may be influenced by endogenous signals, including light (Arora and Saradhi 2002), nitrogen availability (Kishor et al. 1995), and abiotic stresses. Whereas, proline degradation is activated in the dark or after stress (Hayashi et al. 2000). The increase of ROS production by abiotic stresses also plays an important role in the regulation of proline synthesis and degradation (Rejeb et al. 2014). Moreover, plant hormones may contribute to proline regulation. For instance, a study conducted with hormonal mutants of *Solanum lycopersicum* indicated that auxin and ethylene may control proline accumulation (Alves et al. 2017). And, an inhibitory effect of sucrose on abscisic acid induced proline accumulation (Verslues and Bray 2006).

Smirnoff and Cumbes (1989) reported that proline acts in hydroxyl radical scavenging. Afterward, other studies reaffirmed the effectiveness of proline in detoxification of singlet oxygen (Alia et al. 2001), ROS (Matysik et al. 2002), and free radicals (Kaul et al. 2008). Nonetheless, the mechanism of how proline acts in plants under stress conditions was not fully defined.

Recently, to verify the role of proline in ROS scavenging, a density functional theory coupled with polarizable continuum model (DFT/PCM) was used. Signorelli et al. (2014) proposed the proline-proline cycle, where proline captures hydroxyl radical by H-abstraction followed by the second H-abstraction which also captures another hydroxyl radical producing P5C, which is then regenerated to proline by the action of the enzyme P5CR and directly promotes ROS scavenging.

Besides, proline plays a fundamental role in antioxidant defense system, controlling the negative effects of ROS accumulation. Several studies attribute proline-mediated enhancement of the activity of antioxidant enzymes and consequently amelioration of heavy metal stress. For instance, exogenous proline application increased the activity of antioxidant enzymes, including SOD in *Solanum nigrum* under Cd stress (Xu et al. 2009). Moreover, exogenous proline alleviated the oxidative damage induced by Cd accumulation by enhanced antioxidant enzymes activities

(SOD, CAT, and GPX) and established better levels of plant growth, water status, and photosynthetic activity in proline-treated *Phoenix dactylifera* (Zouari et al. 2016a; b). In olive plants, similar responses were observed, where plants under Cd stress treated with proline showed an increase of SOD, CAT, GPX, and APX activities, photosynthetic activity, nutritional status, plant growth, and oil content of olive fruit (Zouari et al. 2016c). In tobacco, application of proline declined lipid peroxidation and increased SOD and CAT activities without reducing Cd contents under Cd stress (Islam et al. 2009).

From these data, it is clear that proline plays fundamental role in the regulation of ROS scavenging or/and as signaling molecule able to regulate antioxidant responses during heavy metal stress.

In addition to proline, glycinebetaine (GB) accumulation may contribute in heavy metal stress alleviation by amelioration of toxic effects of oxidative stress. GB can decrease ROS levels under stress and maintain membrane stability under non-physiological conditions (Chen and Murata 2002). GB acts by enhancing the activity of ROS-scavenging enzymes and induces the expression of specific genes (Einset et al. 2007; Bharwana et al. 2014). GB is known to accumulate in response to stress in many crop plants. It has been observed that tolerant genotypes usually accumulate more GB when compared to sensitive genotypes in response to stress. For this reason, it is fundamental to understand the synthesis and regulation of GB to connect with its role in heavy metal tolerance and study strategies to enhance GB action.

GB is a quaternary ammonium compound, mainly abundant in chloroplast where it plays a crucial role in adjustment and protection of thylakoid membrane, therefore maintaining photosynthetic efficiency (Genard et al. 1991). GB is synthesized from serine in chloroplast. In this organelle, choline is converted to betaine aldehyde, by choline monooxygenase (CMO), which is then transformed to GB by betaine aldehyde dehydrogenase (BADH) (Hanson and Scott 1980; Rhodes and Hanson 1993). Although several studies report that the GB accumulation improves the responses against heavy metal stress, the benefits of GB in stress mitigation and its specific role in modulating signal transduction remain to be elucidated. In fact, GB mitigates the damaging effects of oxidative stress by unknown mechanisms; certainly, GB is not involved directly in ROS scavenging (Chen and Murata 2008). Some studies have argued that the increase in GB concentrations under stress is a product of stress and not an adaptive response to stress. For instance, although the GB levels increased during Zn stress in lettuce, plants exhibited a strong biomass reduction. With these results, Paradisone et al. (2015) concluded that GB is not a tolerance mechanism to toxicity and it was a Zn stress symptom.

On the other way, several studies affirm that exogenous GB application may alleviate the oxidative damage by heavy metal stress. As evidenced in cotton (*Gossypium L.*), plants treated with 1 mM of GB improved responses against toxic effects of Pb, by enhancing the chlorophyll synthesis, photosynthetic activities, and antioxidant enzyme activities, and reduced the electrolytic leakage, MDA, and H_2O_2 levels (Bharwana et al. 2014). During Cr stress, the foliar application of GB successfully alleviated the toxic effects on mung bean (Jabeen et al. 2016) and wheat roots, shoots, and grains (Ali et al. 2015) by increased plant growth, biomass, proteins, and chlorophyll contents, enhanced activities of antioxidant enzymes in both shoots and roots, and decreased Cr accumulation and electrolyte leakage. Several studies report the beneficial effects of GB application against Cd stress.

For instance, the application of GB in cotton decreased Cd concentration in different plant parts, reversing the reduction in plant growth and biomass observed in the presence of Cd. GB application enhanced photosynthetic pigments, protein contents, antioxidant enzymes and decreased electrolyte leakage production, H_2O_2, and MDA contents (Farooq et al. 2016). GB foliar application significantly alleviated Cd stress in maize plants by reversing growth inhibition, improving photosynthetic performance and CAT activity, and reducing MDA content and Cd accumulation in the shoot. GB application also enhanced levels of Zn, Cu, and Fe (Li et al. 2016). In perennial ryegrass, 20 mM GB application increased SOD, CAT, and POD at enzyme and gene expression levels, which reduced Cd content in shoots and improved cell membrane stability by reducing oxidation of membrane lipids (Lou et al. 2015).

It is clear that proline and GB have pivotal role in plant defense against heavy metal stress. Therefore, it is extremely important to perform studies involving the synthesis and regulation of these osmolytes to use them as an alternative strategy to improve plant stress tolerance. Manipulation of osmolytes synthesis by genetic engineering and exogenous application of such compounds by soil treatment, seed priming, or foliar application are interesting strategies to explore these areas.

4 Concluding Comments

Plants must adapt themselves to the prevailing conditions for their survival, resulting in the acquisition of a wide range of metal tolerance mechanisms. It is understood that the antioxidant systems have the capacity to interrupt the cascades of uncontrolled oxidation, some of which have been discussed in this chapter. Measurements of antioxidants as stress markers will remain an essential aspect in assessing stress responses in plants, but specific aspects related to plant species must be considered. Understanding the responses the responses of plants to heavy metal stress and the regulation of the enzymes and other compounds involved in stress tolerance will lead to indications as to their usefulness in detoxification programs. Therefore, heavy metal stress cannot be understood without establishing the integrated processes involving other metabolic pathways that have not been mentioned in this chapter. In conclusion, the information available in the literature aims to improve our understanding of the basic events of the phytotoxicity caused by heavy metal contamination, the induction of oxidative stress in plants, and the integration of multiple systems to overcome contaminated environmental conditions.

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Cadmium (Cd): An Emerging Regulatory Metal with Critical Role in Cell Signalling and Plant Morphogenesis

Renata Bączek-Kwinta

1 Cadmium in Nature

Cadmium ($_{48}$Cd) is the element belonging to the transition metal group of the periodic table. It is one of the most toxic metals in the environment (Wang et al. 2009). The impact of Cd on the human health was described in many papers, and several reviews can be found, e.g. Szczygłowska et al. (2014).

In nature, Cd does not exist in the free state but is present mainly in the coal and sulphide ores of zinc, copper and lead (Bettinelli et al. 1988; Scoullos 2001). Environmental pollution with this metal results from human activities, because Cd is used for various kinds of industry including the production of nickel-cadmium batteries, pigments, stabilisers, plastics and electroplating coatings, solders and alloys, cadmium rods, fireworks, fluorescent paints, as well as car tyres and cement (Pál et al. 2006). Fertilisers, mainly superphosphates, are also the important sources of Cd in the environment, because they are contaminated with Cd in the amount of 10–100 mg/kg (Hsu and Kao 2004; Wang et al. 2009). Moreover, contamination of agricultural soils with Cd may occur as a result of the use of sewage sludge, but the neutral or basic pH of the soil limits Cd bioavailability (Chen et al. 2011).

The sources of contamination are also car exhausts and fossil fuels, e.g. coal. During combustion, Cd is oxidised to CdO and released into the atmosphere. The highest concentration of Cd in the air has been recorded in industrial districts and large cities. During rainfall, metal can easily enter the soil and water bodies, as its compounds are characterised by a very good solubility in water (Das et al. 1997). Cd content in natural water bodies is low and does not exceed 1 μg/dm^3. However, nowadays the increasing contamination of water has been observed. The quantities of Cd found in rivers are larger than in the seas. Cd accumulates mainly in the

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sediments but can enter the tissues of organisms living in water reservoirs (Soltan and Rashed 2003; Wang et al. 2009). Terrestrial plants take Cd from the soil, and this allows Cd to enter into the subsequent links of the food chain, posing a real threat to animal and human health (Murakami et al. 2007; Wang et al. 2009; Chen et al. 2011). Cadmium contamination of plants can be sometimes asymptomatic; however, it changes various aspects of plant metabolism, which will be discussed in this chapter.

2 The Uptake and Distribution of Cadmium in Plants

2.1 Cd in Plant Organs

The amount of Cd in a plant depends on the species, Cd concentration in the soil, and its bioavailability as well (Wang et al. 2006; Mendoza-Cózatl et al. 2008). Moreover, the uptake of Cd relies on the specificity of root secretions, occurrence of mycorrhiza, presence of organic matter, concentration of other elements, soil pH and its redox potential and, last but not least, temperature (Das et al. 1997; Pál et al. 2006; Morel et al. 2009; Gao et al. 2016).

Plants with the fibrous root system comprising large number of thin roots covering a large area accumulate more Cd than plants creating taproot system (Lux et al. 2011). However, regardless of the root architecture, Cd accumulation is higher in roots than in the above-ground parts, while in the case of aerial parts, there is more Cd in older than in younger leaves (Das et al. 1997).

2.2 Cd Transporters, Mechanisms of Translocation and Final Distribution

Cd is translocated through the cell membrane and between organelles by different protein transporters and cation channels specific for divalent cations such as Ca, Cu, Fe, Mg and Zn. They belong to ZIP family transporters, iron-regulated transporters (IRT1), depolarisation-activated and hyperpolarisation-activated calcium channels (DACC and HACC, respectively), voltage-insensitive cation channel (VICC), yellow stripe 1-like protein (YSL), as well as to natural resistance-associated macrophage proteins 1 (NRAMP); (Kerkeb et al. 2008; Oomen et al. 2009; Guerinot 2010; Sasaki et al. 2012; Yoneyama et al. 2015). The common entrance route is important in the case of plant nutrition and biofortification with Fe and Zn, which, in contrast to Cd, are the nutrients essential to plants and their consumers (Yoneyama et al. 2015; Gao et al. 2016). Moreover, as some transport proteins are common for Cd, Fe and Zn, the interrelationship between Cd and the other two metals at the physiological level occurs. In addition, some NRAMP proteins can be induced during leaf senescence (Takahashi et al. 2011), corroborating the idea of a subtle and complex Cd interaction with plant metabolism, which can be discussed later.

The loading of Cd into the xylem and its transport to the aerial parts are promoted by heavy metal ATPases (HMA2 and HMA4), while the heavy metal ATPase3 (HMA3) located at the tonoplast of the root cells counteracts such translocation via compartmentalisation of Cd in the vacuole (Verret et al. 2005; Haydon and Cobbett 2007; Morel et al. 2009). The role of the compounds containing thiol groups (-SH) such as phytochelatins (PCs) in Cd binding and neutralisation is well known (Hassan and Aarts 2011). Interestingly, Cd attached to PCs can be transported into the phloem; hence PCs are involved in Cd binding for long-distance transport, too (Mendoza-Cózatl et al. 2008).

3 Toxicity to Plants

3.1 The Impact on Plant Anatomy, Morphogenesis and Development

The toxicity of Cd can be noticed from germination to senescence. Cd not only inhibits the growth of seedlings but also interferes with their development (Pál et al. 2006). Plants grown on soil contaminated with Cd are considerably smaller (stunted). In addition, they produce small leaves with a low assimilatory surface and sometimes have shortened petioles (Rivera-Becerril et al. 2002; Haouari et al. 2012; Kusznierewicz et al. 2012). The cause of the toxic effects of Cd is the disturbance of various physiological processes crucial for proper growth and development of a plant, such as cell division and elongation, uptake and transport of water and macronutrients and micronutrients, gas exchange (photosynthesis, transpiration, mitochondrial respiration) and nitrogen fixation (Hsu and Kao 2004; Barbosa et al. 2010; Chen et al. 2011).

Growth and development of roots is inhibited as a result of the disturbance of cell division and the synthesis of cell wall components by Cd. Roots become smaller and produce less root hairs, which leads to a reduction in the absorption area and thus negatively affecting water uptake by plants. Moreover, the diameter of vessel is decreased by lignification (Elobeid et al. 2012). At the same time, the increased share of cortex and the ingrowth of hypodermis occur (Vázquez et al. 1992). Visual root disorders caused by an excess of Cd include deformation and blackening (Rivera-Becerril et al. 2002; Pál et al. 2006; Lux et al. 2011; Haouari et al. 2012). The reason is probably the genotoxicity of Cd (Barbosa et al. 2010; Parween et al. 2011). Cd can also negatively affect the uptake of macronutrients and micronutrients, namely, iron (Fe), nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), copper (Cu) and sodium (Na) (Pál et al. 2006). Moreover, by interaction with proteins and lipids, it affects membrane fluidity, which disturbs different redox processes (Pál et al. 2006; Sandalio et al. 2001; Romero-Puertas et al. 2002).

Cd affects photosynthesis mainly through the inhibition of the chlorophyll (Chl) biosynthesis (Wang et al. 2009), although some impairment in thylakoid formation can also occur (Ouzounidou et al. 1997; Pál et al. 2006). Inhibition of Chl occurs by inhibiting the synthesis of ALA dehydratase and protochlorophyllide reductase and the reduction of Mg^{2+} and Fe^{2+} uptake (Horváth et al. 1996; Wang et al. 2009). The lack of Fe, and its altered ratio to Zn, triggers some characteristic symptoms such as the so-called iron chlorosis of leaves, which is visible as yellow interveinal spaces with darker veins, blackening of the edges of leaves and downward bending of leaves called epinasty (Kabata-Pendias and Pendias 1992; Das et al. 1997). Cd may also interact with some compounds of photosynthetic machinery, e.g. oxygen-evolving complex (Zhu et al. 2008). Besides, it contributes to degradation of membrane lipids in thylakoid affecting the antennae of LHCII and participates in inhibition of PSI and PSII (Joshi and Mohanty 2004). As Cd has high affinity to the sulfhydryl groups of proteins, this involves inhibition of different enzymatic reactions, e.g. the photosynthetic CO_2 binding by the key enzyme Rubisco (Das et al. 1997; Rivera-Becerril et al. 2002).

Cd affects chlorophyll ratio, but in different way in mature and young leaves (Ebbs and Uchil 2008). While mature leaves lose their chlorophyll *b* fast due to the conversion into chlorophyll *a*, the young ones are initially greener due to the large amount of chlorophyll *b*, then the chlorosis occurs, and the *b* form is lost faster than the *a* one. The reason is the interplay with the senescence signalling resulting in the interconversion of chlorophyll *b* into *a* before degradation (Ebbs and Uchil 2008).

The intensity of the symptoms caused to plants by Cd depends on several factors, namely, the species and its variety/cultivar, stage of development, time of the exposure to the metal, its dose, either used or naturally present, as well as physical and chemical properties of the soil (Bączek-Kwinta et al. 2011; Kusznierewicz et al. 2012; Haouari et al. 2012). Cd accumulation can lead to the premature ageing of different organelles and organs (McCarthy et al. 2001; Balestrasse et al. 2004; Vollenweider et al. 2006). As the senescence/ageing process is regulated by phytohormones, it is logical to assume that the interaction of Cd with phytohormones exists, which has been experimentally confirmed and will be discussed later in this chapter. This is not the only physiological interaction, and any of such relationships is important to metabolism and, consequently, growth and development of plants. However, some plants are resistant to Cd and even accumulate it in large amounts (Poschenrieder et al. 2006; Liu et al. 2008; Liu et al. 2016; Tang et al. 2016), which implies further questions about their specific adaptive responses.

3.2 Cd/Zn and Cd/Fe Interactions

Cd and Zn belong to the same group IIB transition elements and have similar chemical properties, while their biological significance is different. Cd is considered non-essential and highly toxic, while Zn is definitely a micronutrient necessary for, e.g. auxin synthesis, DNA replication and antioxidative response, although it can be toxic, too, when its amount in the soil is too high and/or a plant is continuously exposed to it (Reichman 2000; Bidar et al. 2008).

Zn and Cd as well as Cd and Fe often co-exist in the soil, and their combination results in antagonistic effect of Cd on Zn or Fe mobility and uptake by plants (Gupta and Potalia 1990), although it depends on the soil type and the amounts of clays, oxides and organic matter (Lee and Dolittle 2006; Sarwar et al. 2010). However, at the biochemical level, the reason for such antagonism lies, e.g. in the competition for some membrane transporters specific to divalent cations. In the case of zinc, there are ZIP proteins but also AtHMA4, known as *Arabidopsis* heavy metal pumps belonging to the P1B type ATPase. Hence, Cd surplus leads to the shortage of Zn and the toxicity of Cd (Verret et al. 2004; Bækgaard et al. 2010). Amino acid histidine and non-proteinaceous amino acid nicotianamine, known mostly as Ni chelator, can also bind both Zn and Cd (Hassan and Aarts 2011; Anjum et al. 2015). In the case of Fe, some proteins belonging to the NRAMP (natural resistance-associated macrophage protein family) mediate translocation of both Cd and Fe (Takahashi et al. 2011).

On the other side, when present in surplus, Fe and Zn enhance oxidative processes, for example, the overproduction of oxygen-free radicals (O_2^-*) via the Fenton reaction. Strong oxidative stress triggers oxidative injury to membranes through lipid peroxidation by O_2^-* and other reactive oxygen species (ROS), protein damage when carbonyl groups are formed and activation or inhibition of antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POX) and catalase (CAT) (Sandalio et al. 2001; Romero-Puertas et al. 2002; Wua et al. 2003). Cd is not involved in Fenton reaction, although it can indirectly produce radicals by displacing Fe from its cellular sites (Gupta and Potalia 1990; Romero-Puertas et al. 2004). It is noteworthy that SOD comprises both Fe and Cu/Zn isoforms. However, the research on the impact of Cd on these isoforms has not given a clear answer whether Cd can substitute Fe or Zn, although the effect was obtained on the animal, namely, neural cells (Huang et al. 2006). Probably the effect of Cd depends on its concentration, plant species, its growth stage, the organ and even the kind of tissue and organellum (Romero-Puertas et al. 2004; Pawlak et al. 2009). The explanation of such differences may be that Cd triggers some changes in the signalling, which will be described later in the Part 4.

An interesting example of Cd/Zn antagonism is the substitution of Zn by Cd in Zn-finger transcription factors and, in consequence, an impairment in transcription mechanisms (di Toppi and Gabbrielli 1999). Last but not least, Zn is a co-factor of the enzyme carbonic anhydrase (CA) which catalyses the interconversion of CO_2 into HCO_3^- and plays a key role in photosynthetic CO_2 fixation in C4 plants, while in the C3 ones, it probably facilitates the diffusion of CO_2 across the chloroplast (Badger and Price 1994; Sasaki et al. 1998; Hacisalihoglu et al. 2003). Cd may partially cover for Zn in CA in some hyperaccumulating plants, which will be described in the later part of the chapter (Table 1).

Table 1 The impact of Cd on different processes regulated by Zn

The kind of Cd/Zn interaction	The reason	Literature data
Zn mobility	Sharing of membrane transporters such as ZIP proteins or P1B ATPase	Verret et al. (2004), and Bækgaard et al. (2010)
Zn chelation	Binding to histidine and nicotianamine	Hassan and Aarts (2011), Anjum et al. (2015)
DNA transcription	Substitution in zinc fingers	di Toppi and Gabbrielli (1999)
CO_2 binding in photosynthesis	Substitution in carbonic anhydrase (CA)	Liu et al. (2016), and Tang et al. (2016)

3.3 Can Cd be advantageous to Plants?

Cd is widely regarded as highly toxic to plants, because it causes a number of physiological disorders not only leading to deterioration of plant growth and development but also to their death (Benavides et al. 2005). However, for some Cd-hyperaccumulating and Cd-tolerant plants, its mode of action can be termed advantageous in terms of protection from pathogens and herbivores (Poschenrieder et al. 2006; Liu et al. 2008). For example, European Cd hyperaccumulator *Thlaspi caerulescens* (Brassicaceae) is protected from the thrip *Frankliniella occidentalis* by Cd (Jiang et al. 2005). Cd can be more toxic to pathogen or herbivore than to the host plant, can hamper the virulence of the pathogenic biota and/or can increase plant resistance to them (Poschenrieder et al. 2006). An increased synthesis of antimicrobial glucosinolates in Cd-treated white cabbage belonging to Brassicaceae, too, was obtained (Kusznierewicz et al. 2012). The impact of Cd on photosynthesis of its hyperaccumulators seems to be intriguing, and it is related to the presence of carbonic anhydrase (CA; EC 4.2.2.1.). As it was mentioned earlier, CA catalyses the reversible bicarbonate (HCO_3^-) formation from water and CO_2. CA exists in different isoforms, and for the first time, the positive mode of action of Cd was noticed in the case of marine diatom *Thalassiosira weissflogii*, possessing two CA isoforms. One of them had Zn in its active site, but due to the low Zn concentration in water, the diatoms were used to substitute it with Cd without any negative side effects (Lane and Morel 2000; Lane et al. 2005). The fact that the CA plays an important role in the binding of CO_2 during photosynthesis in cyanobacteria, algae and higher plants (Badger and Price 1994) became the basis for the research on the Cd/CA/photosynthesis relationship and growth in Cd-hyperaccumulating plants.

The study conducted on the Chinese species *Picris divaricata* Vant., the first known Cd/Zn hyperaccumulator from the Asteraceae family (Liu et al. 2016), allowed us to understand the physiological role of Cd in such plants. A hydroponic experiment revealed that plant growth was enhanced by some concentrations of Cd, and CA activity was significantly increased. In addition, two bands of purified CA protein appeared on the gel electrophoresis, indicating CA isomers. Moreover, trace amounts of Cd in purified CA significantly increased with the supplied Cd concentration. The conclusion was that Cd may play a biological role by enhancing the activities and forming the active Cd-specific CA in *P. divaricata* (Liu et al. 2016). These results are consistent with the research on another Asian Cd-hyperaccumulating plant, *Sedum alfredii* (Crassulaceae) (Tang et al. 2016), which revealed an upregulation of CA gene by Cd as well as by its natural coenzyme, Zn. In addition, other chloroplast genes involved in transcription and translation (*rps18* and *rps14*), electron transport and ATP synthesis (*atpF* and *ccsA*), Photosystem I (of PSA and LHCA), Photosystem II (PSBs and LHCBs), as well as the genes involved in chloroplast development and metabolism were upregulated. Plant biomass and some photosynthetic parameters were at the level of those supplemented with Zn (Tang et al. 2016).

The results of the study on both Cd/Zn hyperaccumulating plant species indicated that Zn may be substituted by Cd in their tissues with the same metabolic impact. The research on other species will allow to answer the question whether the phenomenon is universal for all Zn/Cd hyperaccumulators and if we can term Cd a specific, conditionally essential element. One of the best known hyperaccumulators of Cd is the *Thlaspi caerulescens* (Roosens et al. 2003), already mentioned in this part. Plants of this species accumulate large amounts of Cd in their tissues, reaching the value of the order approx. 3600 mg Cd kg^{-1} DW of a leaf, which is several times greater than the dose lethal to most plant species. In the case of the hydroponics, the amount of Cd can be even much higher and reach about 10,000 mg Cd kg^{-1} DW of a leaf. Interestingly, despite a large total intake of Cd, the plants did not show any adverse physiological effects, although the tolerance threshold depends on genetic (or epigenetic) and environmental factors (Liu et al. 2008). It is very interesting whether Cd can also become an integral part of CA in this species. On the other hand, the mechanisms of physiological changes caused by Cd, discussed in the following parts of the chapter, will aim to clarify the regulatory mechanism of Cd on other aspects of metabolism than photosynthesis.

4 Cadmium Signalling

4.1 ROS, NO and Hormones

As mentioned earlier, ROS generation is enhanced during Cd stress. As ROS, mainly H_2O_2, are known as signalling molecules affecting the redox regulating molecular hub (Foyer and Noctor 2012), such signalling can be affected by Cd. On the other hand, the treatment of leaves from Cd-grown plants with different effectors and inhibitors revealed that ROS production was regulated by different processes such as protein phosphorylation, Ca^{2+} channels and cGMP (Romero-Puertas et al. 2004). To make matter more complex, it has to be mentioned that ROS interact with nitric oxide (NO) resulting in a specific protective response (Herrera-Vásquez et al. 2015). In terms of Cd, endogenous NO applied on rice seedlings enhanced its tolerance by increasing pectin and hemicelluloses contents in the root cell wall, which allowed the plants to bind and immobilise the metal within the cell wall structure (Xiong et al. 2009).

The other way of Cd mode of action is the interaction with different phytohormones, too, and some phytohormones trigger NO signalling (Xu et al. 2010). It is noteworthy to mention that such interaction with the phytohormones like jasmonic acid (JA) and ethylene and ROS as well leads to accelerated senescence (Balestrasse et al. 2004; Rodriguez-Serrano et al. 2009). However, this may involve the activation of protective mechanisms allowing a plant to survive in Cd-spiked environment, too. The examples and possible mechanisms are described below and summarised graphically in Figs. 1 and 2.

4.2 Ethylene

Cd increases the activity of one of the key enzymes necessary for ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) (Chmielowska-Bąk et al. 2013; Schellingen et al. 2014). The involvement of ethylene biosynthesis and signalling in Cd signalling were revealed by the use of ethylene-insensitive mutants (*etr1-1*, *ein2-1*, *ein3-1*) (Schellingen et al. 2015). It is important in terms of the sequestration of Cd by glutathione (GSH) and its polymer phytochelatins, because stress-mediated ethylene signalling is known to alter GSH biosynthesis (Hassan and Aarts 2011; Cao et al. 2009). Hence, the direct protective mechanism is involved allowing plants to neutralise, more or less efficiently, the toxic metal.

Both Cd and ethylene precursor ACC activate some kinases, the enzymes that phosphorylate various substrates in different cellular compartments. Phosphorylation by mitogen-activated protein kinases (MAPKKK, MAPKK and MAPK) is the way of transducing signals as a phosphorylation cascade from upstream kinases to downstream targets, e.g. enzymes. Interestingly, Cd activates stress-inducible MAP Kinase (SIMK), stress-activated MAP kinase (SAMK) and two MAP kinases MMK2 and MMK3, which are involved in the response to pathogen-associated stimuli (elicitors) (Cardinale et al. 2002). As some of these kinases are activated by ACC (Ouaked et al. 2003), the other way of ethylene-mediated plant response to Cd is likely, and, besides the direct response towards the metal may enhance the resistance to microbial attack.

4.3 Jasmonic Acid

It should be added that the plant responds to other biotic stresses also. Insect herbivores depend on another phytohormone, jasmonate (JA), known mostly as the "wounding hormone" (Yan et al. 2013). Interestingly, the stable forms of jasmonate are formed in different plants as a result of Cd stress (Maksymiec et al. 2005). It is worthy to note that water stress-induced JA is a signal leading to the regulation of ascorbate and glutathione metabolism and has important role for acquisition of the tolerance to water stress, which is often coincided with cadmium stress (Shan and

Liang 2010). Methyl jasmonate (MeJA) can be effective in terms of protection of photosynthetic apparatus from Cd, provided sulphur is available to plants allowing them for GSH synthesis (Per et al. 2016). As already mentioned, GSH can bind Cd and participate in mitigation of oxidative stress. In the paper of Per et al. (2016), GSH protected chloroplast ultrastructure, reduced H_2O_2 and lipid peroxidation and enhanced net photosynthesis, chlorophyll content and Rubisco activity. The application of MeJA significantly reduced the translocation/accumulation of Cd in both the shoots and roots of Cd hyperaccumulator, wild European plant *Solanum nigrum* L., and restored antioxidant enzyme (SOD and CAT) activities in its leaves (Yan et al. 2015). Similar antioxidative effect of MeJA, together with the expression of genes encoding metallothioneins, the cystein-rich proteins involved in metal detoxification, was obtained for a mangrove *Kandelia obovata* (Chen et al. 2014).

4.4 Salicylic Acid

JA cross talks with salicylate (SA), another phytohormone involved sin stress response and protective mechanism activation. In terms of Cd toxicity and signalling, some experiments proved its preventive role. In the paper of Metwally et al. (2003), Cd increased SA content in *Hordeum vulgare* roots, and the experiment on the wild-type and SA-deficient genotype of *Arabidopsis* revealed that after 5 days of Cd treatment, the chlorophyll content decreased, and lipid peroxidation was prevented in plants sensitive to SA (Zawoznik et al. 2007). On the other hand, Cd-induced phytotoxicity in *Arabidopsis* was intensified by elevated endogenous SA, while SA deficiency in transgenic line *hang* decreased growth inhibition, and SA signalling blockage in *npr1-1* mutant had some protective impact (Tao et al. 2013). Moreover, the results of Gondor et al. on *Zea mays* L. (2016) implied that SA itself and its salt form, NaSA, act in a different way, because some antioxidant enzyme activities were increased by SA, while GSH-related redox changes were influenced mostly by NaSA. The effect of SA and NaSA on the distribution of Cd also differed, as SA facilitated the transport of Cd to the leaves, while NaSA increased the PC level in the roots. Moreover, the response of plants was dependent on SA or NaSA applied prior to or together with Cd. A NaSA application preceding the Cd treatment provided the best protection from Cd toxicity (Gondor et al. 2016). Hence, understanding the role of SA in plant response to Cd seems to be far from a simple mechanistic model.

4.5 Brassinosteroids

Brassinosteroids (BRs) are plant hormones comprising almost 70 polyhydroxylated sterol derivatives. BRs are structurally similar to animal steroid hormones and regulate multiple physiological functions, from embryogenesis and seed germination to leaf senescence (Clouse and Sasse 1998). The transcriptome-wide analysis and the targeted study of the reporter genes performed by Villiers et al. 2012 on *Arabidopsis* revealed that Cd stress condition triggers the activation of BRs signalling pathway. Moreover, artificially elevated BR contents in plants lead to hypersensitivity to Cd, and this further initiates some adaptive mechanism. They may depend on BR signal itself or to be typical to Cd-triggered signal (in the latter case, e.g. phytochelatin synthesis or root/shoot partition) (Villiers et al. 2012). The results, together with the data taken from biomedical papers aiming the impact of Cd on steroid receptors in mammalian cells and its further consequences including tumour formation (Garcia-Morales et al. 1994; Höfer et al. 2010), lead to the conclusion that Cd is an universal metallohormone, either of toxic or protective properties. Hence, it would be discussed whether an increasing amount of Cd in the environment due to the human activity affects life on Earth by different ways.

4.6 Abscisic Acid

Cd affects abscisic acid (ABA) synthesis which results in the expression of ABA-regulated genes. Interestingly, ABA regulates cysteine biosynthesis, and thus the synthesis of GSH and phytochelatins can be increased, resulting in enhanced Cd accumulation in *Solanum* species (Stroiński et al. 2010; Stroiński et al. 2013; Wang et al. 2016). It is noteworthy that some of *Solanum* species and ecotypes are efficient Cd hyperaccumulators, which implies the possibility of the use of ABA for further improvement of hyperaccumulating properties in the case of, e.g. *Solanum photeinocarpum* (Wang et al. 2016) (Fig. 1).

Interestingly, in Cd-hypersensitive woody species *Populus euphratica,* exogenous ABA restricts Cd influx resulting in alleviated toxicity (Han et al. 2016). The possible explanation is linked to the Cd^{2+}-permeable channels activated by endogenous H_2O_2. As Cd-triggered ABA enhancement upregulates antioxidant activity, the entry of Cd to the plant tissue is restricted (Han et al. 2016) (Fig. 1).

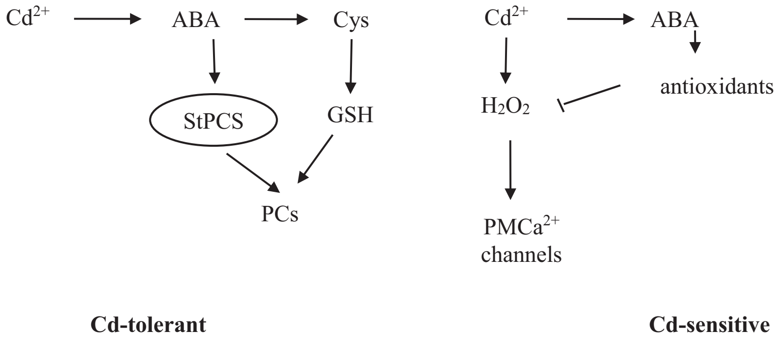

Fig. 1 The comparison of the Cd-triggered ABA regulation of metabolism resulting in differentiated response (*Solanum tuberosum*) and Cd-sensitive plant (*Populus euphratica*). ABA abscisic acid, StPCS phytochelatin synthase, PCS phytochelatin, Cys cysteine, GSH glutathione, PM plasma membrane. (Based on Stroiński et al. (2013) and Han et al. (2016))

4.7 *microRNAs*

microRNAs (miRNAs) control various processes via downregulation of transcript formation or inhibition of translatory machinery in many cases (Bartel 2004). Hence, the question arises concerning their impact during heavy metal stress, too. Indeed, in the late 2000s, some papers indicated the role of mi319, mi317, miR390 and miR395 in Cd stress in *Arabidopsis*, *Brassica napus*, *Medicago truncatula*, *Oryza sativa* and *Nicotiana tabacum* (Xie et al. 2007; Zhou et al. 2008; Huang et al. 2010; Ding et al. 2011; He et al. 2016). The mode of action of these, and maybe others, miRNAs, is complexation of Cd, maintaining cell growth and ionic balance, triggering defence against ROS and hormonal signal transduction ending with the impact on some specific transcription factors (He et al. 2016). Taken together, it is tempting to hope that elucidation of the role of miRNAs during metal stress will answer various scientific questions, allow to ask the new ones and bring some practical advantages.

5 Conclusions

Cadmium is an extremely dangerous pollutant due to its increasing amount in the environment, good solubility in water and easy uptake and translocation within the cell and between cells. In larger amounts, it is very toxic to plants, but the tolerant and hyperaccumulating species or phenotypes are equipped with different cellular mechanisms allowing them to sequestrate it and to mitigate the impact of stress. The protective response is mediated by the ROS/NOS interactions, phytohormonal cross talk and microRNA synthesis (Fig. 2). On the other hand, the presence of Cd

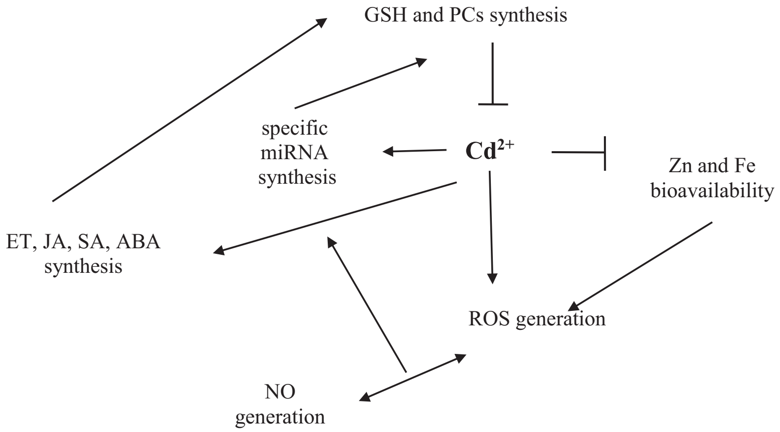

Fig. 2 A summing-up overview of the impact of Cd on basic metabolic and signalling routes leading to various alterations in plant response. GSH glutathione, PCs phytochelatins, miRNA microRNA, ET ethylene, JA jasmonic acid, SA salicylic acid, ABA abscisic acid, ROS reactive oxygen species, NO nitric oxide

in the active site of the photosynthetic enzyme, carbonic anhydrase and Cd mode of action mimicking hormones shed a new light on this element as a conditionally essential and a metallohormone, too.

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Heavy Metal Hyperaccumulator Plants: The Resource to Understand the Extreme Adaptations of Plants Towards Heavy Metals

Manoj Shrivastava, Ashish Khandelwal, and Sudhakar Srivastava

1 Introduction

Heavy metals are natural components of the earth crust, but their levels have reached to toxic levels due to different anthropogenic activities. Fifty-three elements fall in the category of heavy metals. Excess quantity of metals in the environment leads to contamination. The annual worldwide release of heavy metals is 22,000 t for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc (Singh et al. 2003; Sarma 2011). The phytoremediation of metal-contaminated soils often also referred to as bioremediation, botanical bioremediation and green remediation is a low-cost method for soil remediation, and some extracted metals may be recycled for value. The concept of using metal hyperaccumulator plants to selectively remove and recycle excessive soil metals was introduced by Chaney (1983). It gained public exposure in 1990 (Anonymous 1990) and has been increasingly examined as a potential practical and more cost-effective technology than the conventional technologies, viz. soil replacement, solidification and washing (Chaney et al. 1997). Phytoextraction of toxic heavy metals by plants offers great promise for commercial development. The hypertolerance of metals is the key plant characteristic required for hyperaccumulation; vacuolar compartmentalization appears to be the source of hypertolerance of natural hyperaccumulator plants (Chaney et al. 1997).

Hyperaccumulators are the plants that contain in their foliar tissue on dry weight basis >100 μg g^{-1} cadmium, thallium or selenium; >300 μg g^{-1} of cobalt, copper or chromium; >1000 μg g^{-1} of nickel, arsenic, lead or rare earth elements (REEs); and

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> 10,000 µg g^{-1} of zinc and manganese, when growing in their natural habitat (Baker and Brooks 1989; Reeves 2006; van der Ent et al. 2013). Important characteristics of hyperaccumulators plants are given below (Chaney et al. 1997):

- The plant must be able to tolerate high levels of the element in root and shoot cells: hypertolerance is the key property that makes hyperaccumulation possible. Such tolerance is mainly due to vacuolar compartmentalization and chelation of metal.
- A plant must have the ability to translocate an element from roots to shoots at high rates.
- There must be a rapid uptake rate for the element at levels that occur in soil solution.

For most elements the threshold concentration is 1000 µg g^{-1} (0.1%) dry mass, except for zinc and manganese (10,000 µg g^{-1}), gold (1 µg g^{-1}) and cadmium (100 µg g^{-1}). Some of the metal hyperaccumulator plant species are presented in Table 1. *Thlaspi caerulescens* has been identified as a Zn and Cd hyperaccumulator (Reeves and Brooks 1983). Baker et al. (1994) reported that the natural populations of *T. caerulescens* in the UK contained up to 21,000 mg Zn kg^{-1} and 160 mg Cd kg^{-1}

Table 1 Metal concentrations in some major hyperaccumulator plant species

Sr. no.	Plant	Metal	Metal concentration (µg g^{-1})	Mode of study	References
1	*Asparagus racemosus*	Se	14,900	Field (naturally contaminated soil)	Beath et al. (1937)
2	*Typha domingensis*	Se	30	Hydroponics	Carvalho and Martin (2001)
3	*Berkheya coddii*	Ni	5500	Pot	Robinson et al. (1997)
4	*Alyssum bertolonii*	Ni	10,900	Pot	Li et al. (2003)
5	*Alyssum caricum*	Ni	12,500	Pot	Li et al. (2003)
6	*Alyssum corsicum*	Ni	18,100	Pot	Li et al. (2003)
7	*Alyssum heldreichii*	Ni	11,800	Field (naturally contaminated soil)	Bani et al. (2010)
8	*Alyssum markgrafii*	Ni	19,100	Field (naturally contaminated soil)	Bani et al. (2010)
9	*Alternanthera sessilis*	Cr	1017	Hydroponics	Sinha et al. 2002
10	*Pteris vittata*	Cr	20,675	Hydroponics	Kalve et al. (2011)
11	*Eleocharis acicularis*	As	1470	Field (naturally contaminated soil)	Sakakibara et al. (2011)
12	*Pteris ryukyuensis*	As	3647	Pot in greenhouse (spiked)	Srivastava et al. (2006)

(continued)

Table 1 (continued)

Sr. no.	Plant	Metal	Metal concentration (μg g^{-1})	Mode of study	References
13	*Pteris vittata*	As	8331	Hydroponics	Kalve et al. (2011)
14	*Hydrilla verticillata*	As	388	Hydroponics	Srivastava et al. (2011)
15	*Haumaniastrum robertii*	Co	10,200 μg g^{-1}	Field (naturally contaminated)	Chaney et al. (2010)
16	*Potamogeton crispus*	Cd	49.09	Hydroponics	Sivaci et al. (2008)
17	*Rorippa globosa* (Turcz.)	Cd	218.9	Pot	Sun et al. (2010)
18	*Thlaspi praecox* Wulfen	Cd	>1000	Pot	Vogel-Mikus et al. (2010)
19	*Atriplex halimus* subsp. Schweinfurthii	Cd	606.51	Hydroponics	Nedjimi and Daoud (2009)
20	*A. halimus* L.	Cd	830	Hydroponics	Lutts et al. (2004)
21	*Solanum photeinocarpum*	Cd	158	Pot	Zhang et al. (2011)
22	*Sedum alfredii*	Zn	13,799 μg g^{-1}	Hydroponics	Jin et al. (2009)
23	*Crucianella maritima*	Zn	390 μg g^{-1}	Mine tailings	Conesa et al. (2011)
24	*Thlaspi caerulescens*	Zn	39,600	Mine tailings and contaminated river gravels	Chaney et al. (2010)
25	*Thlaspi caerulescens*	Zn	51,600 μg g^{-1}	Field (contaminated)	Cunningham and Ow (1996)
26	*Arthrocnemum macrostachyum*	Pb	620 μg g^{-1}	Mine spoils	Conesa et al. (2011)
27	*Paspalum conjugatum* L.	Pb	150 μg g^{-1}	Plastic bags (spiked)	Paz-Alberto et al. (2007)
28	*Aeolanthus biformifolius*	Cu	13,700 μg g^{-1}	Field (mineralized)	Chaney et al. (2010)
29	*Eleocharis acicularis*	Cu	20,200 μg g^{-1}	Field (naturally contaminated soil)	Sakakibara et al. (2011)
30	*Festuca rubra*	Hg	3.17 μg g^{-1}	Field (polluted soil)	Luis et al. (2003)
31	*Silene vulgaris*	Hg	4.25 μg g^{-1}	Pot	Araceli et al. (2012)
32	*Brassica juncea*	Au	10 mg kg^{-1}	Field (contaminated site)	Harris et al. (2009)
33	*Alyxia rubricaulis*	Mn	11,500 mg kg^{-1}	Field (mineralized)	Chaney et al. (2010)
34	*Eleocharis acicularis*	Zn	11,200 mg kg^{-1}	Field (naturally contaminated soil)	Sakakibara et al. (2011)

dry matter (DM) in the shoots. The ability to hyperaccumulate heavy metals from soils is one of the most important criteria in the selection of plant species for phytoremediation purposes. So far 721 hyperaccumulator species from 52 families and 130 genera are included in the global data base of hyperaccumulator plants which mainly belong to Phyllanthaceae (59 species) or Brassicaceae (83 species) family (Reeves et al. 2017). Adaptive response of plants in terms of their physiological, molecular, genetic and ecological trait to heavy metal-contaminated environment makes plant to sustain in the contaminated environment; hence, plant acts as a hyperaccumulator. In general, hyperaccumulation is also affected by soil type (pH, organic matter, cation-exchange capacity) and type of heavy metal salt present in the environment (Barman et al. 2001; Espinoza-Quinones et al. 2005; Xian and Shokohifard 1989; Otte et al. 1993). Essential elements such as iron and copper may also become toxic which depends upon oxidation state, complex form, dose and mode of exposure (Beyersmann and Hartwig 2008). Many plants/crops can accumulate toxic metals without hindering the yield potential. This uptake mainly depends upon soil type, chemical specification and habitat characteristic of the plants, i.e. terrestrial, aquatic, etc. The adaptive response and characteristic features such as wide distributions, high adaptability, large biomasses and short growth cycles of ornamental plants can also be used as to clean up metal-contaminated soil and water to attain more economical and high practical value. The potential for application of hyperaccumulators in bioremediation is limited by the following factors (Cunningham et al. 1995):

- Hyperaccumulators often accumulate only a specific element and have not been found for all elements of interest.
- Most hyperaccumulators grow slowly and have small biomass.
- Little is known about their agronomic characteristics, pest management, breeding potential and physiology.
- The plants are often rare and grow in remote regions; in certain cases, their habitat is threatened by mining, development and other activities. Using wild plants as a seed source is, therefore, also unreliable.

Given these limitations, efforts are being made to develop traditional crop plants with ‘hyperaccumulator tendencies’. One proposed strategy may be to cross hyperaccumulators with high biomass relatives.

2 Response of Hyperaccumulator to Metals

The response of metals towards heavy metals is highly variable, and it differs within genotypes of same varieties and also among different varieties. Under metal stress condition, plants respond with several mechanisms at the same time. Heavy metals particularly accumulate in plasmatic compartment of cytosol and chloroplast stroma (Brune et al. 1995), and plants may act in different ways by production of amino acids, sugars, metabolites, phytochelatins, etc. Two basic strategies by which plants respond to elevated concentrations of heavy metals in the environment include exclusion mechanisms, whereby plants avoid excessive uptake and transport of metals, and accumulation and sequestration mechanisms, whereby large amounts of metals are taken up and transported to the plant shoots (Baker 1981). Few ornamental plants, *Celosia* L., *Malva* L. and *Althaea* L., showed increased tolerance to heavy metal stress and also effectively accumulate these metals (Liu et al. 2008, 2014; Wu et al. 2017; Zhang et al. 2010). Wu et al. (2018) demonstrated high Cd bioconcentration factor (1.41–3.31) and Cd translocation factor (1.03–7.37) in *Malva rotundifolia* as a model hyperaccumulator. Manganese hyperaccumulator *Phytolacca acinosa* showed adaptation to protect chloroplast structure and photosynthesis at high Mn (8 mM) concentration (Weng et al. 2013). Many hyperaccumulation phenotypes naturally serve as a defence strategy against pathogens and herbivores (Boyd and Martens 1994; Martens and Boyd 1994; Boyd et al. 2002; Hanson et al. 2003; Jhee et al. 2005; Küpper and Kroneck 2007).

Phytoextracting or hyperaccumulator plants acquire 'soil-bound' heavy metals by mobilizing them in the soil solution. The mobilization of 'soil-bound' metal can be accomplished in a number of ways (Raskin et al. 1997):

(i) By secretion of metal-chelating molecules like phytosiderophores, low molecular weight organic acids, etc. into the rhizosphere to chelate and solubilize 'soil-bound' metal. Phytosiderophores like deoxymugeneic acids, avenic acid, etc. are released in response to iron deficiency and can, in principle, mobilize other heavy metals like Cd, Pb, Cu, Zn, Mn, etc. from soil. Metal-chelating proteins, related to metallothioneins (Robinson et al. 1993) or phytochelatins (Rauser 1995), may also function as siderophores in plants. Ni-hyperaccumulator, *Alyssum lesbiacum*, may use histidine, an excellent Ni chelator, to acquire and transport Ni (Kramer et al. 1996a, b).

(ii) Roots can reduce 'soil-bound' metal ions by specific plasma membrane-bound metal reductases, which may increase metal availability. Pea plants deficient in Fe or Cu have an increased ability to reduce Fe^{3+} and Cu^{2+}, which is coupled with an increased uptake of Cu, Mn, Fe and Mn (Welch et al. 1993).

(iii) Plant roots can solubilize soil-bound toxic metals by acidifying their soil environment with protons extruded from the roots.

(iv) Roots can employ rhizospheric organisms (mycorrhizal fungi or root-colonizing bacteria) to increase the bioavailability of metals.

Mobilized metals are taken up by plant roots from the soil solution and exported to the shoots. Theoretically, toxic metals may compete for the same transmembrane carriers as those used essential metals like by Ca and Mg; however, the high concentrations of these ions in soil solution make this unlikely. Most environmentally hazardous metals are too insoluble to move freely in the vascular system of the plant. Many form sulphate, carbonate or phosphate precipitates immobilizing these metals in apoplastic and symplastic compartments. Apoplastic transport of these metals is further limited by the high cation-exchange capacity of cell walls, unless the metal ion is transported as a non-cationic metal chelate. In hyperaccumulating plant species, some toxic metals may be transported to the shoot as complexed to organic acids, mainly citrate (Baker and Brooks 1989; Senden et al. 1992). *Brassica juncea*, a good Cd accumulator, showed that, in roots, Cd was present as a CdS_4 complex, which may contain phytochelatins (Salt et al. 1995). In the xylem sap, Cd was coordinated predominantly with oxygen or nitrogen ligands, consistent with the involvement of organic acids (Salt et al. 1995). In the leaves, Cd preferentially accumulated in trichomes.

Three major principles likely to apply in plants to avoid metal toxicity (Briat and Lebrun 1999):

(i) Regulation of metal ion uptake at the root level, as well as long-distance and intracellular partitioning to control overloading
(ii) Maintaining intracellular metal ions in nontoxic forms at various subcellular compartments by various molecules bearing affinity for metal ions
(iii) Metal-dependent activation of antioxidant mechanisms when metal ions are in excess to protect plant integrity in case of transport and storage mechanisms is overloaded

2.1 Metal Uptake by Roots of Hyperaccumulator Plants

Apoplast plays an important role in the transport and distribution of ions between tissues and cells and in cellular responses to environmental stresses (Dietz 1996). Apoplastic binding of metallic cations can contribute significantly to the total metal content of the roots and may also serve as a transient metal storage pool (Van Cutsem and Cillet 1982). Selectivity for cation binding in the cell wall can also account for the different metal toxicity tolerance by plants. (Wang et al. 1992). Cation uptake selectivity from the soil solution is dependent upon specific sites located in the plasma membrane of individual cells (Marschner 1995). Both low- and high-affinity metal transporters are responsible to transport for different heavy metal cations (Cu^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+}) into plant root cells (Clarkson and Liittge 1989). Graminaceous species synthesize phytosiderophores, belonging to the mugineic acid family, and secrete them into the rhizosphere where they can bind to Fe(III). Phytosiderophores released upon Fe deficiency conditions efficiently chelate and mobilize not only Fe(III) but also zinc (Treeby et al. 1989).

2.2 Metal Accumulation in Hyperaccumulator Plants

Once inside plant cells, metals in excess, and unused for metabolism requirements, need to be stored to prevent their toxicity. This is achieved by various specific proteins and peptides and by various small organic molecules.

2.2.1 Metal-Binding Proteins

Two major proteins are responsible for metal ion storage and detoxification: the ferritins and the metallothioneins. The ferritins are a class of ubiquitous multimeric iron-storage proteins able to sequester several thousand iron atoms per molecule (Harrison and Arosio 1996). In plants, these proteins are coded by a small gene family and are located in the plastids (Briat and Lobreaux 1997). Their expression is developmentally regulated and can be activated by various environmental signals among which Fe excess is a common signal. Concerning the response to Fe signal, two cellular pathways have been implicated in the differential regulation of various plant ferritin genes: abscisic acid dependent and abscisic acid independent. These are modulated by antioxidants and serine/threonine phosphatase inhibitors (Briat and Lobreaux 1997; Savtno et al. 1997). Plant ferritins are, therefore, likely to constitute an important component of the oxidative stress response in plants and probably participate in the protection of plastids against oxidative stress by storing excess free Fe. In addition to an increase in ferritin concentration, Fe excess also increases the concentration of dehydroascorbate and decreases the ascorbate concentration (Kanpfenkel et al. 1995). Therefore, Fe-mediated oxidative stress leads to both ferritin accumulation and increased Fe uptake by the protein.

Metallothioneins are small proteins (MW of 6000–7000), which have been identified in various organisms. They sequester excess amounts of certain metal ions, most commonly Zn^{2+}, Cu^{+} and Cd^{2+} by coordination of these metals with cysteine residues organized as CysXCys or CysXXCys repeats (Hamer 1986; Robinson et al. 1993). Metallothionein synthesis is transcriptionally activated by metal ions (Fürst et al. 1988). Plants also contain functional metallothionein homologs (Zhou and Goldsbrough 1994). Metallothioneins (MTs) are cysteine-rich proteins required for heavy metal tolerance in animals and fungi. The proteins encoded by these genes can be divided into two groups (MT1 and MT2) based on the presence or absence of a central domain separating two cysteine-rich domains. Four of the MT genes (MT1a,MT1c,MT2a and MT2b) are transcribed in Arabidopsis and the fifth gene, MT1b, is inactive (Zhou and Goldsbrough 1995).

Proline is synthesized from glutamate and ornithine in plants under metal-stressed conditions. Consumption of NDAPH and ATP involved during enzymatic activities such as conversion of glutamate to proline via Δ^1-pyrroline-5-carboxylate reductase (PCR) proves the role of proline in electron sink mechanism. The proline accumulation differs according to metal type as well as genotype of plants. Proline accumulation at equimolar concentration in nutrient media was found to be in the following order Cu > Cd > Zn, whereas at equal toxic strength, proline accumulation was Cd > Zn > Cu (Schat et al. 1997). In general, it is often found that metal content in leaves is restricted due to more accumulation of proline content in shoot as compared to roots where proline restricts movements of heavy metals from shoot to leaves. Increase in induction factor (IF) indicates amount of proline generated through increase in transcript level. The increase IF = 6 at 25 μM and IF = 31 at

50 μM for Pb showed strong transcript regulation for Δ^1-pyrroline-5-carboxylate synthetase (P5CS1) in *Arabidopsis thaliana* roots. However, weak up- and downregulation was observed in leaves with IF = 1.4 for Δ^2-pyrroline-5-carboxylate synthetase (P5CS2). Proline synthesis is mediated mainly through three enzymatic activities, namely, Δ^1-γ-glutamyl kinase activity of Δ^1-pyrroline-5-carboxylate synthetase (P5CS1), glutamic-γ-semialdehyde dehydrogenase activity of Δ^1-pyrroline-5-carboxylate synthetase (PCS) and two isogenes of Δ^1-pyrroline-5-carboxylate reductase (PCR). The metal-tolerant species such as *Armeria maritima*, *Deschampsia cespitosa* and *Silene vulgaris* found to contain high proline content in all plant parts as compared to their wild non-tolerant varieties (Farago and Mullen 1979). Similarly, Cu induced ABA-mediated proline accumulation also observed in environmental stress conditions (Chen et al. 2001; Arora and Saradhi 1995).

Approximately 75% of metal accumulators are Ni accumulators. A large and proportionate increase in the histidine concentration in xylem sap of Ni-hyperaccumulator plants indicates more affinity of histidine for Ni, which was also confirmed by extended X-ray absorption fine structure (EXAFS) in *Alyssum lesbiacum* (Kramer et al. 1996a, b). The increased root concentration is also observed in Ni-hyperaccumulator *Thlaspi goesingense* as compared to Ni-non-hyperaccumulator *T. arvense* (Persans et al. 1999). The transgenic plant showed ten times higher biomass and two times more histidine level as compared to wild-type plants in the presence of toxic Ni concentration which suggest mechanistic link between histidine and Ni tolerance in the plants (Wycisk et al. 2004).

2.2.2 Phytochelatins

Phytochelatins, also called class III metallothioneins, are peptides of general structure $(\gamma\text{-Glu-Cys})_n(\text{Gly})$ with n = 2–11; these peptides are, therefore, not primary gene products (Rauser 1995; Grill et al. 1991; Zenk 1996). Variants around this general structure have also been identified, such as γ-Glu-Cys-β-Ala in certain legumes, $(\text{y-Glu-CyS})_n$ and $(\gamma\text{-Glu-CyS})_n(\text{Glu})$ in maize and $(\gamma\text{-Glu-CyS})_n(\text{Ser})$ in some species of the Poaceae family. These structures of phytochelatins arise from chain extension of glutathione (or homoglutathione in some legumes) (Briat and Lebrun 1999). The increase in phytochelatins is concomitant to the disappearance of glutathione. Phytochelatin synthesis is absent or severely affected in plant mutants deficient in glutathione synthetase or γ-glutamylcysteine synthetase, the two key enzymes of the glutathione biosynthetic pathway. Phytochelatin synthesis in plants is activated in the presence of many different metals (Cd, Cu, Zn, Ag, Sn, Sb, Te, W, Au, Hg, Pb and Bi). Although the enzyme involved, phytochelatin synthase (γ-glutamylcysteine dipeptidyltranspeptidase), is constitutively expressed, it is a self-regulating enzyme as the product of the reaction chelates the enzyme-activating metals. At the cellular level, phytochelatins have been shown to be synthesized in the cytosol and transported to the vacuoles (Briat and Lebrun 1999).

2.2.3 Metal Chelation by Small Molecules

In addition to proteins and peptides, a very large number of low molecular weight compounds play an important role in the handling of metals in plants. Among them, some organic acids, amino acids and phosphate derivatives are of particular interest. Citrate has a high capacity to chelate metal ions. Metals such as Fe(II), Ni(II), Cd(II) and Pu(IV) also have a strong affinity for citrate (Cataldo et al. 1988). Although to a lesser extent than citrate, other organic acids such as malate and oxaloacetate are also able to bind metals (Cataldo et al. 1988). Some amino acids and their derivatives are also major metal chelators, and this property could confer, in some cases, plant resistance to toxic levels of metals. An increased concentration of free histidine is likely to be responsible for Ni tolerance (Kramer et al. 1996a, b). Nicotianamine (NA) is an aminocarboxylate occurring in all plants and resulting from the condensation of three S-adenosyl methionine molecules (Shojima et al. 1990). This amino acid derivative has affinity for Fe, Cu and Zn and can be accumulated in the vacuole (Stephan et al. 1996; Pich et al. 1997). Specific to grasses, the mugineic acids are synthesized by NA deamination followed by reduction and hydroxylation (Shojima et al. 1990). These molecules are also able to bind Zn^{2+}, Cu^{2+} and Fe^{3+} (Treeby et al. 1989).

Phytate (myo-inositol hexakisphosphate) is an essential phosphorus store in the seeds, and it is also known to chelate Ca^{2+}, Mg^{2+}, K^{+}, Fe^{2+}, Zn^{2+}, Mn^{2+}, etc. (Hocking and Pate 1977; Mikus et al. 1991). Hence, phytate plays an important role in detoxification of heavy metals in plants. Rhizoderm is known to play a key role in mineral nutrient uptake (Grunwald et al. 1979), and endoderm regulates the active transport of minerals to the central stele (Marschner 1995). A carefully controlled synthesis or mobilization of phytate in these specialized cell layers might therefore play an important role in the control of metal loading of the shoots.

3 Long-Distance Transport of Metal Ions in Hyperaccumulator Plants

Metals, once taken up by roots, are then loaded in the xylem sap and translocated to the aerial parts of plants through the transpiration stream. Organic acids, and especially citrate, are the main metal chelators in the xylem (Cataldo et al. 1988). Amino acids in the xylem sap are also potential metal chelators for Ni and Cd (Cataldo et al. 1988). Free histidine in the xylem sap enhanced translocation of this metal to the shoots in some plants (Kramer et al. 1996a, b). Nicotianamine, a methionine derivative, could also be a Cu(II) shuttle in the xylem (Pich et al. 1994; Pich and Scholz 1996). Phloem sap contains Fe, Cu, Zn and Mn (Hocking and Pate 1977; Stephan et al. 1994). The sole molecule identified as a potential phloem metal transporter is nicotianamine (Stephan and Scholz 1993), which has been found in a stoichiometry of 1.25 with four metal ions (Fe, Cu, Zn and Mn) in the phloem sap

(Stephan et al. 1994, Schmidke and Stephan 1995). Nicotianamine could therefore transport metals for a long distance once they have been loaded in the phloem, but this loading could require another chelator.

4 Intracellular Partitioning and Subcellular Transport of Metals in Plants

Possible mechanisms of metal inactivation in plants may involve metal binding to cell walls, reduced transport across cell membranes, active efflux, intracellular compartmentalization and chelation (Tomsett and Thurman 1988). Metal accumulation can increase up to poisoning concentration. This cytoplasmic toxicity can be decreased by binding compounds such as phytochelatin for plant cells (Rauser 1990; Zenk 1996), while prokaryotes rather prevent accumulation of metals like Cd, Zn, Co and Ni by active cation efflux (Nies 1992). In most plant cells, the vacuole comprises more than 80–90% of the cell volume and acts as a central storage compartment for ions (Wink 1993). Tonoplast functions as an effective and selective metal diffusion barrier. Plant cell vacuoles are the major site for phytochelatin-Cd complex accumulation (Vögeli-Lange and Wagner 1990). Martinoia et al. (1993) have shown that vacuolar membranes from a wide range of plant species can mediate MgATP-dependent, uncoupler-insensitive, transport of glutathione S-conjugates. Two transporters from *Arabidopsis thaliana* AtMRPI and AtMRP2, which belong to the ATP-binding cassette (ABC) transporter family, showed a high capacity for the transport of various glutathionated compounds (Lu et al. 1994, 1998). Vacuolar transport of As is mediated in uncomplexed arsenite [As(III)] form by ACR3 in As hyperaccumulator plant, *Pteris vittata* (Indriolo et al. 2010), and after complexation with phytochelatins by ABCC1 and ABCC2 in rice (Song et al. 2014).

5 Resistance Mechanisms of Hyperaccumulator Plants to Toxic Concentration of Metals

Metal ion overload can lead to intracellular concentrations beyond the storage and detoxification capacities of plant cells. Because of the physico-chemical properties of metal ions, their excess displaces the cellular redox balance towards a pro-oxidant state, following the activation reactions. As a consequence metal toxicity should normally activate antioxidant defences. High Fe concentration in *Nicotiana plumbaginifolia* doubled ascorbate peroxidase and catalase activities in the leaves (Kampfenkel et al. 1995). Abnormally high levels of Fe and Cu in plants increase oxygen radical production and activation of antioxidants enzymes (Pich et al. 1994). Among these enzymes, cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase are predominant (Herbick et al. 1996). Also, induction of plant Fe- and

Mn-superoxide dismutases by different metals has been reported (del Rio et al. 1991).

Hyperaccumulator plants developed a number of strategies to resist this toxicity, including active efflux, sequestration and binding of heavy metals inside the cells by strong ligands, and their tolerance differs from metal to metal. For example, the cadmium/zinc hyperaccumulator *Thlaspi caerulescens* is sensitive towards copper (Cu) toxicity, which is a problem for phytoremediation of soils with mixed contamination (Mijovilovich et al. 2009). *Thlaspi caerulescens*, which is considered as a model hyperaccumulator species, reported an enhanced uptake of metals into the root symplasm and a reduced sequestration into the root vacuoles which is associated with the higher root-to-shoot metal translocation efficiency (Shen et al. 1997; Lasat et al. 1998). This may be due to elevated expression of xylem-loading transporters in the roots (Papoyan and Kochian 2004; Weber et al. 2004). Parameswaran et al. (2007) identified a protein Cd/Zn-pumping P_{1b}-type ATPase TcHMA4, which showed Cd and Zn transport affinity in the submicromolar range. In most of the hyperaccumulators, the metal is sequestered preferentially into compartments mainly in the epidermal vacuoles where it does no harm to the metabolism (Küpper et al. 1999, 2001; Frey et al. 2000; Bidwell et al. 2004; Broadhurst et al. 2004). This showed that hyperaccumulation must be mediated by active pumping of heavy metals into their storage sites, which was achieved by an extremely increased expression of metal transport proteins in leaves of hyperaccumulators compared with non-accumulators (Pence et al. 2000; Assunção et al. 2001; Becher et al. 2004; Papoyan and Kochian 2004; Küpper et al. 2007). Küpper et al. (2004) reported that phytochelatins have not much relevance for Cd detoxification in the Cd hyperaccumulator *T. caerulescens*; therefore, the main detoxification strategy in hyperaccumulators is sequestration of the hyperaccumulated heavy metals and clearly not binding to strong ligands. However, the nonproteogenic amino acid nicotianamine (NA) seems to play an important role in metal homeostasis of plants as it binds iron, Zn and Cu, mainly for long-distance transport in the vascular bundle (von Wiren et al. 1999; Liao et al. 2000), and NA synthase has been shown to be highly overexpressed in hyperaccumulators compared with non-accumulator plants (Becher et al. 2004; Weber et al. 2004; van de Mortel et al. 2006, 2008).

6 Ecological Adaptation of Hyperaccumulator to Metal-Contaminated Soil

Various soils are detrimental to the growth of plants owing to elevated metal ions, either due to natural processes or man's activities. Many plants have evolved ecotypes that are able to grow more or less normally on these soils. Mechanisms of metal tolerance by plants are in accordance with Levitt's general stress concept, which is that adaptation can be either achieved by avoiding the metal stress, by tolerating it or both (Levitt 1980). Avoidance by exclusion is the most common mechanism of plant adaptation to metal toxicity and relies on different kinds of reduced metal uptake: (i) at the plasma membrane level, by deposition on cell wall components, or (ii) by chelate secretion (Meharg 1993). Tolerance to metal stress relies on plant capacity to detoxify metals having entered the cells. The postulated mechanisms involve biochemical detoxification and compartmentalization of the metal within the cell (Tomsett and Thurman 1988). An extreme case of metal accumulation inside tolerant plants is observed with hyperaccumulating plants. These plants can accumulate a thousandfold more metal than normal plants without phytotoxic symptoms. Leaf tissue metal content is generally in the range of 0.1 to several percent. These plants are distributed all over the world and comprise many various genera among approximately 400 taxa (Baker 1989). Some plants behave as polymetallic hyperaccumulators such as *Thlaspi caerulescens* which accumulate zinc, cadmium, nickel and other metals, though to a lesser extent (Baker et al. 1994). The highest metal content ever determined in any plant concerns nickel accumulation in *Sebertia acuminata latex*, an endemic tree from New Caledonia (Jaffré et al. 1976). *Sebertia acuminata* contains 25% of nickel with citric acid as the counter ion for 40% of the metal present (Sagner et al. 1998). Further, hyperaccumulating plants may be an inadvertent consequence of an efficient mineral (possibly zinc and calcium) nutrient uptake system (Boyd and Martens 1998). Genetic control of metal tolerance by one dominant gene has been observed in many occurrences both in crops and adapted populations of wild plants. For example, copper tolerances in *Mimulus guttatus*, arsenic tolerance in *Agrostis capillaris*, etc. have been reported (Macnair 1993). It has to be kept in mind that the 'Tolerance Index' (TI) has been widely used to measure the degree of metal tolerance reached by a certain plant (Baker 1987). An exploitation of this natural genetic variation of metal tolerance traits is certainly amenable to QTL analysis, enabling a possible molecular analysis of these quantitative traits in the future (Koornneeff et al. 1997).

7 Conclusions and the Way Forwards

Hyperaccumulating plants are the preferred candidates for phytoextraction of heavy metals from metal-polluted soil. An efficient metal extraction requires several factors, viz. (i) a high aerial biomass combined with a high exploratory capacity of the root system, (ii) a high root uptake capacity for the desired metal, (iii) a high level of translocation from roots to shoots which are easily harvested and (iv) a high capacity for metal accumulation, hence a high level of resistance to toxic metal concentrations. Appropriate breeding strategies may be adopted to increase the combination of yield and shoot metal concentration. Therefore, a need exits to characterize the mechanisms used by hyperaccumulators to accumulate, translocate and tolerate metals. The cloning of responsible genes may be done to convert high biomass agronomic plants into special phytoremediation cultivars. Genetic control of metal tolerance by one dominant gene has been observed in many cases. An exploitation of this natural genetic variation of metal tolerance traits is certainly amenable to QTL analysis owing to the development of genetic tools such as marker technology. Further, the recycling of shoot metals in commerce may provide value for the ash from metal hyperaccumulators, such that there is no need to pay for safe disposal. Biomass ash containing 20–40% metals may proved to be rich ore. Increasing the yield of a crop could give a linear increase in phytoremediation capacity with increasing yield. But increasing from ‘normal’ tolerance to ‘hypertolerance’ and hyperaccumulation increases the potential annual removal of the soil contaminant severalfold. Improved hyperaccumulator plants and agronomic technology, to increase the annual rate of phytoextraction and to allow recycling of toxic soil metals accumulated in plant biomass, are very likely to support economical viable environmental remediation.

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Halophytes and Heavy Metals: Interesting Partnerships

G. C. Nikalje, N. Saini, and Penna Suprasanna

1 Introduction

The continuous progress in industrial, urban, and agricultural aspects of human activities in recent years has resulted in chemical pollution of the environment (Gavrilescu et al. 2015). Presence of toxic metallic elements in soil and water has increased to a level that endangers human health (Kelishadi et al. 2014). In order to combat this pollution, wide range of techniques mainly for removal of heavy metal has been developed. Conventional physicochemical remediation being expensive, labor-intensive, and detrimental to both microbiological and soil ecosystem, is now replaced by modern, alternative techniques (Fasenko and Edwards 2014; Singh and Santal 2015). Modern techniques involve microorganisms (bioremediation) and plants (phytoremediation) to transform or remove toxic elements from the environment (Kang 2014; Mani and Kumar 2014; Gavrilescu et al. 2015). In recent times, phytoremediation has emerged as the most viable and useful technology for soil cleanup in numerous heavy metal-polluted locations. Phytoremediation as the method of choice holds certain advantages such that it is less expensive, promotes biodiversity, reduces erosion, and provides significant reduction of the volume of contaminated material for disposal (Lee 2013; Wan et al. 2016). Several plant species have been studied as the candidates of choice for phytoremediation.

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Halophytes are able to survive even at higher concentrations of sodium and chloride ions that would be toxic to most of the crop species (Nikalje et al. 2017a, b). Mechanisms of adaptation that allow halophytes to survive high salt concentrations are not exclusive to sodium and chloride but also confer tolerance to other toxic ions, including heavy metal ions. It has been recently shown that halophytes have the ability to transform heavy metals into nontoxic compounds making some halophytes excellent candidates for phytoextraction and phytostabilization (Liu et al. 2018; Nikalje and Suprasanna 2018; Nikalje et al. 2019b).

2 Heavy Metals and Toxicity

Heavy metals are those metals with relatively high densities, ranging from 3.5 g/cm^3 to above 7 g/cm^3. These metals are generally non-biodegradable, have long-term persistence in soil, and easily get accumulated in living bodies. Above certain concentrations and over a narrow range, the heavy metals turn out to be toxic. For example, Cd content in plants ranges between 0.2 and 0.8 mg kg^{-1} and becomes toxic when concentrations reach 5–30 mg kg^{-1}. Even at lower concentrations, they are capable of causing various diseases and disorders (Alloway 2013). The most common heavy metal contaminants are of As, Cd, Cr, Cu, Hg, Pb, etc. The toxicity caused by metal ions is categorized into three processes: (1) generation of reactive oxygen species, which further results in oxidative stress, (2) direct interaction with proteins, and (3) displacement of essential cations in specific binding sites (Sharma and Dietz 2009). Heavy metals form OH^- from H_2O_2 through the Haber–Weiss and Fenton reactions. These radicals are capable of damaging cells through lipid peroxidation (Sharma and Dietz 2009). The oxidized lipids interfere with protein function through uncontrolled hydrophobic interactions (Farmer and Mueller 2013). The accumulation of heavy metals in plants causes wide range of negative effects on their growth and development. The photosynthetic machinery of plants is highly sensitive to metal toxicity. Cadmium is found to be severely affecting the chlorophyll content, photosynthetic rate and intracellular CO_2 concentration of plants (Dong et al. 2005). Other heavy metals like copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn) decrease the photosynthetic efficiency of PS II in *Elodea densa* and *Thalassia hemprichii*, resulting in poor chlorophyll content of plants (Maleva et al. 2012; Li et al. 2012). Metal toxicity results in the reduction of chlorophyll pigments, photosynthesis rate, PS II quantum yield, stomatal conductance, and CO_2 assimilation, further causing changes at cellular and tissue levels (Singh et al. 2016). Nickel (Ni) is found to be affecting several enzyme activities such as amylase, protease, and ribonuclease, thus retarding seed germination and growth of many crops. Toxic concentration of Ni in soil causes other detrimental effects on plants such as reduction in plant height, root length, fresh and dry weight, chlorophyll content, and enzymatic activity of carbonic anhydrase and increased malondialdehyde content (MDA) and electrolyte leakage (Siddiqui et al. 2011). Lead (Pb) toxicity has strong effect on seed morphology and physiology. It affects seed germination and development, root elongation, transpiration rate, chlorophyll content, and amount of water and proteins present in plant, causing alternated chloroplast, obstructed electron transport chain, and inhibition of calvin cycle enzymes, impaired uptake elements like Mg and Fe, and poor stomatal efficiency (Pourrut et al. 2011). Arsenic toxicity in plants results in oxidative stress by generating reactive oxygen species (ROS) and inhibiting antioxidant defense system of tissues (Hartley-Whitaker et al. 2001).

Nitrogen metabolism plays a crucial role in plant growth and development and is affected by metal toxicity. The metals induce protease activity and thus reduce the activity of nitrate metabolizing enzymes such as nitrate reductase, nitrite reductase, and ammonia assimilation enzymes such as glutamine synthetase, glutamine oxoglutarate aminotransferase, and glutamate dehydrogenase (Chaffei et al. 2003). The nitrate metabolism is severely affected by cadmium, inhibiting the uptake of nitrate and its transportation (Lea and Miflin 2004) ultimately leading to altered primary nitrogen assimilation pattern.

At cellular level, chromium hampers cell cycle, inhibits cell division, and thereby reduces root growth of plants (Sundaramoorthy et al. 2010). The expression of a cyclin-dependent kinase (CDK) is found to be reduced due to Cd toxicity which further results into altered transition of G1 to S phase and progression of cell cycle (Pena et al. 2012). Copper (Cu) alters distribution of auxin by modulating PIN1 proteins, resulting in inhibition of primary root elongation (Peto et al. 2011). Cd toxicity resulted in overexpression of Gpx (a thioredoxin-dependent enzyme in plants) and reduced activity of glutathione reductase (GR), thus modulating the level of thiol during the germination (Smiri et al. 2011). Further, high concentration of Cu ions resulted in oxidative stress by up-regulating antioxidant and stress-related proteins like glyoxalase I, peroxiredoxin, aldose reductase, and regulatory proteins like DnaK-type molecular chaperone, UlpI protease, and receptor-like kinase in plants (Shethy and Ghosh 2013).

3 Halophytes: Responses to Heavy Metals

Halophytic plants are able to cope up with several abiotic constraints occurring simultaneously in their natural environment. These plants are naturally present in the environment and are characterized by an excess accumulation of toxic ions, mainly sodium and chloride ions. Studies revealed that halophytes are able to tolerate other stresses including heavy metal toxicity. The tolerance to salt and heavy metals is partly based on common physiological mechanisms (Przymusinski et al. 2004). It is assumed that halophytes and heavy metal-tolerant plants possess both specific and general functioning mechanisms of tolerance towards numerous abiotic factors (Shevyakova et al. 2003a, b). One such study on the halophyte *Mesembryanthemum crystallinum* suggested that salinity stress overlaps with copper toxicity to some extent, as several integrated mechanical and chemical signals are responsible for stress-related responses (Thomas et al. 1998).

The halophytic plants have developed different strategies to survive and complete their life cycles in highly saline conditions. This includes controlled uptake of Na^+ and Cl^- ions, their compartmentalization into vacuoles, and protection of sensitive organelles such as nuclei or chloroplasts by production of different stress proteins. Production of osmolytes such as proline, glycine, betaine, and carbohydrates is another important feature for salinity tolerance (Shevyakova et al. 2003a, b). Proline plays significant functions during metal stress by performing three major actions, namely, metal binding, antioxidant defense, and signaling (Sharma and Dietz 2006). It is also accumulated in response to Cd, Cu, and other heavy metals (Shevyakova et al. 2003a, b; Lefevre et al. 2009; Nedjimi and Daoud 2009). Presence of cadmium triggered the oversynthesis of glycinebetaine, which is an efficient osmoprotectant synthesized in Chenopodiaceae family (Lefevre et al. 2009). Heavy metal stress induces both secondary water stress and oxidative damage to cellular structures, and the ability of halophytes to synthesize these osmoprotectants is involved in coping up with heavy metals (Lefevre et al. 2009; Shah et al. 2001; Verma and Dubey 2003; Nedjimi and Daoud 2009). These osmolytes play a crucial role in protecting macromolecular subcellular structures and mitigate oxidative damage caused by free radicals produced in response to the stress (Szabados et al. 2011). Under control conditions, the amount of osmolytes is high in halophytes as compared to glycophytes, which makes halophytes well prepared during stress condition and more tolerant than glycophytes (Slama et al. 2015; Nikalje et al. 2017c).

Antioxidant system enables halophytes to cope with heavy metal stress better than other common plants (Shah et al. 2001; Verma and Dubey 2003). A facultative halophyte *Mesembryanthemum crystallinum* responds to cadmium stress by activation of the peroxidase system, which decreases the damaging effect of reactive oxygen species (Shevyakova et al. 2003a, b). Besides tolerance mechanisms, halophytes have evolved number of secondary mechanisms to handle excess salt as well as toxic ions (Table 1). At the leaf level, they have developed salt glands, salt bladders, trichomes, or succulent tissues to remove the excess of deleterious toxic ions from photosynthetically active tissues and regulate plant tissue ion concentration (Lefevre et al. 2009). Leaves of *Tamarix smyrnensis* are found to be covered with salt glands, which accumulate and excrete Cd and Pb on the surface of the leaves suggesting that this salt-tolerant plant uses its salt excretion mechanism to

Table 1 Example of halophytes and their modified structures for tolerance

Halophytic plant	Modification	References
Mesembryanthemum crystallinum	Bladder cells	Agarie et al. (2007)
Limonium bicolor	Secretory cells	Feng et al. (2014)
Aeluropus littoralis	Cuticular chamber	Barhoumi et al. (2008)
Chloridoid grasses	Bicellular salt gland	Amarasinghe and Watson (1998)
Suaeda salsa	Succulent leaves	Song and Wang (2015)
Limonium bicolor	Salt glands	Yuan et al. (2016)
Porteresia coarctata	Secretory hairs	Dassanayake and Larkin (2017)

excrete excess metals on its leaf surface as a possible detoxification mechanism (Manousaki et al. 2008; Kadukova et al. 2008).

Metal excretion through trichomes has been observed in a number of other estuarine and salt marshy halophytes such as the *Armeria maritima* (Neumann et al. 1995), *Avicennia marina* (MacFarlane and Burchett 1999, 2000) *Avicennia germinans* (Sobrado and Greaves 2000), and *Spartina alterniflora* (Windham et al. 2001; Weis and Weis 2004).

4 Mechanism of Metal Tolerance

Halophytes have evolved a number of tolerance mechanisms against heavy metal ion toxicity, which include (a) avoidance or exclusion that minimizes the cellular accumulation of metals; (b) excretion of toxic ions through specialized structures and tolerance, which allow plants to survive; and (c) accumulating high concentrations of metals in vacuoles (Nikalje et al. 2018).

Based on the above mechanism, these plants are categorized into three groups: (a) metal excluders, (b) excretors, and (c) accumulators or hyperaccumulators (Dahmani-Muller et al. 2000).

4.1 Metal Excluders

The halophytes that effectively limit the levels of heavy metal translocation within their system and maintain relatively low levels in their shoot over a wide range of soil levels are referred to as metal excluders (Baker and Walker 1990). Heavy metal excluders are the plants which have high levels of heavy metals in the roots but have shoot/root quotient which is less than 1 (Boularbah et al. 2006). These metal excluders can survive on highly contaminated soil, and their uptake of heavy metals is quite low even at higher concentration of heavy metals (Wenzel et al. 2003). In the presence of toxic metal Cu and Cd, growth performance of propagules and 6-month-old seedlings of *Bruguiera gymnorhiza* was examined, and it was found that older mangrove seedlings showed more tolerance because of their more efficient exclusion mechanism (Wang et al. 2013). Several adaptation mechanisms including avoiding the uptake of metals actively and exclusion of ions resulted in tolerant *A. marina* against Pb toxicity (Burchett et al. 2003). In soil contaminated with heavy metal ions, *Atriplex* showed increased concentration of heavy metal ion in shoots and roots, suggesting an exclusion strategy for metal tolerance (Senock et al. 1991; Kachout et al. 2012). The metal transporters like ATPases, cation diffusion facilitator, multidrug and toxin efflux, natural resistance-associated macrophage proteins, and zinc–iron permease play an important role in metal transport (Williams et al. 2000). Transformation of a protein, ACHMA1, isolated from *Atriplex canescens* showed enhanced tolerance to copper and other abiotic stresses in yeast (Sun et al. 2014).

The metal hyperaccumulator *T. caerulescens* shows increased expression of FDR3 which is a member of MATE family protein under metal stress in roots (Kramer et al. 2007). Metal hyperaccumulation has also been shown to be mediated by 200 times enhanced expression of metal transporter genes in hyperaccumulators compared to related non-accumulator plants (Verbruggen et al. 2009; Leitenmaier and Küpper 2013).

4.2 Excretion

Few halophytes use excretion as one of the methods for the removal of excess salt ions from their tissues. The modification in morphological and physiological features of plant, especially of roots and glandular tissue, is crucial for metal accumulation, transport, partitioning, and excretion in halophytes during metal stress (MacFarlane et al. 2007; Chen et al. 2016). Along with removal of Na^+ and Cl^- ions, other toxic ions such as Cd, Zn, Pb, or Cu are accumulated and excreted by salt glands or trichomes on the surface of the leaves through a process known as "phytoexcretion" (Manousaki and Kalogerakis 2011). Less than 5 % of toxic metal ions are flushed out from plant system by using this mechanism.

Excess of Cd and Pb is excreted out on the leaf surface of the *T. smyrnensis*, though its salt gland showed that it uses its salt excretion mechanism to excrete excess metals (Hagemeyer and Waisel 1988; Burke et al. 2000; Lefevre et al. 2009). Similarly, in *Limoniastrum monopetalum*, leaves excreted Cd and Pb from salt glands (Manousaki et al. 2014). Several other halophytes (*Atriplex halimus*, *Tamarix aphylla*, *T. smyrnensis*, *Armeria maritime*, and *A. marina*) showed the release of toxic elements such as Cd, Pb, or Zn through their salt glands or trichomes (Ruiz-Mirazo and Robles 2011; Lokhande and Suprasanna 2012).

4.3 Accumulation

Some of the plants, referred to as accumulator plants, have the ability to grow on highly contaminated soils, accumulating higher amounts of toxic metals in their tissues without exhibiting any symptoms of toxicity (Baker et al. 1991). Ion accumulators are often known as hyperaccumulators, as they have the capacity to uptake higher concentrations of ions from the soil as an adaptation mechanism. According to definition, a hyperaccumulator must accumulate at least 100 mg g^{-1} (0.01% dry wt.) of Cd, As, and some other trace metals; 1000 mg g^{-1} (0.1 dry wt.) of Co, Cu, Cr, Ni, and Pb; and 10,000 mgg^{-1} (1% dry wt.) of Mn and Ni (Reeves and Baker 2000). Accumulation of toxic ions in plants depends upon the ability to store accumulated excess metals in organs or subcellular compartments where no sensitive metabolic activities take place. The central vacuole is one of the most suitable storage reservoirs for the accumulation of metals in plants (Oosten and Maggio 2015).

Sesuvium portulacastrum accumulated Cs content in leaves (536.10 µg.g^{-1}) than in stem (413.74 µg.g^{-1}) and roots (284.69 µg.g^{-1}) (Nikalje et al. 2019a). Bioaccumulation factor (BAF) is generally used to determine the plant's ability to accumulate metals from soils, defined as the ratio of metal concentration in plants to that in soil (Govindasamy Agoramoorthy et al. 2008; Qiu et al. 2011). Chelation of the metal cation by ligands and sequestration of metals away from sites of metabolism in the cytoplasm are two crucial mechanisms needed for plants to be a hyperaccumulator. Till date, about 400 plant species are found to be metal hyperaccumulators, and some of these are now used in field applications (Zaier et al. 2010; Mariem et al. 2015).

5 Metal Uptake and Mechanisms of Transport

Plants take up ions from soils in response to concentration gradients induced by selective uptake of ions by roots or by diffusion of elements in the soil. The level of accumulation of elements differs varies on the plant species, the age and growth stage of the plant, seasonal variations, metal speciation and bioavailability in the environment, and metal characteristics (Cacador et al. 2000).

5.1 Forms of Metal Uptake and Root Exudates

The oxidation state of metal ions and coordination of these ions with the environment directly affect their absorption, translocation, and detoxification in root tissues (Salt et al. 2002). For example, mobility of inorganic arsenic in the form of arsenite (As III) is lesser than that of inorganic arsenate (As V); however, the former is more toxic to the environment (Jose et al. 2009).

Few plants release different soluble organic substances from their roots, including both low-molecular-weight (LMW) organic acids and high-molecular-weight (HMW) polysaccharides and other organic substances which form complexes with heavy metals in the soil (Bertin et al. 2003). The compound thus formed affects the availability of the heavy metals (Bertin et al. 2003). Root exudates like oxalate and malonate from halophyte *Juncus maritimus* form complex with heavy metals (Pb, Cr, Cu, Zn, Ni, and Cd). The complex so formed is able to increase metal bioavailability in polluted estuarine environments (Mucha et al. 2005). Application of citric acid around the rhizosphere of *Halimione portulacoides* plants showed enhanced Cd uptake and decreased Ni uptake (Duarte et al. 2007).

Thus, the complexes, either organic or inorganic, have the ability to affect the metal uptake by plants. Metal complex having hydrophilic ligands has the ability to enter the xylem vessels through an apoplastic pathway from the tips of the root where the Casparian bands are absent or poorly developed (Lutts and Lefevre 2015). Depletion of free metal ions from the rhizosphere results in the dissociation of metal complexes in this area, further causing an enhanced diffusion flux and metal uptake (Degryse et al. 2006). Thus, these dissolved complexes have the ability to increase metal uptake; however, the magnitude of this increase depends on the concentration and liability of the complexes so formed.

Few plant roots are found to be associated with arbuscular mycorrhizal fungi which showed interference with heavy metal absorption (Liao et al. 2003). Halophytic plants like *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa* showed reduced plant uptake of toxic metals especially Pb, whereas in *Aster tripolium* increased Cd and Cu accumulation was observed (Carrasco et al. 2006; Carvalho et al. 2006). Presence of anoxic conditions in wetlands causes dominance of a reduced form of heavy metals in soil which is scarcely available to the plants (Weis and Weis 2004).

5.2 Heavy Metal Movement Across Roots

Plants uptake and mobilize As (V) by phosphate transport channels (Tripathi et al. 2007). As (V) being similar to phosphate ion competes with it for root uptake and often interferes in different metabolic processes like ATP synthesis and oxidative phosphorylation (Tripathi et al. 2007). Chromium ion enters into the plant system either by reduction or by formation of complex with root exudates, which increase its solubility and mobility through the root xylem (Bluskov et al. 2005). Zayed and Terry (2003) reported that Cr(III) enters in the plant system by passive mechanism, whereas uptake of Cr(VI) is inhibited by SO_4^{2-} and Ca^{2+} ions. In halophytic plant *Suaeda salsa*, uptake of Cd ion is regulated by Ca ion transporters or channels present in root cell plasma membranes (Li et al. 2012).

5.3 Transport and Accumulation of Heavy Metals

Accumulation of heavy metals in plant system is the final step of metal absorption, which is directly influenced by the transpiration rate and shoot relative growth rate (Lutts and Lefevre 2015). Cr ions, poorly translocated to aerial parts, are mobilized and accumulated inside tissues depending on its chemical form (James and Barlett 1983). Bioaccumulation factor (BF) is defined as the ratio of the metal concentration present in plant tissues to that in the soil. This parameter is generally used to determine the efficiency of metal accumulation in plants. The transfer factor (TF) stands for the ratio of metal concentration in shoot to that of roots (Sousa et al. 2008). Halophytic plant *Arthrocnemum macrostachyum* accumulates Cd at high concentration with a BF exceeding the critical value of 1 and lower TF value. The values for BF and TF decrease with high external concentration of pollutant (Redondo-Gomez et al. 2010).

Studies using x-ray absorption spectroscopy on tumbleweed showed that Cd ion binds to oxygen in the roots, oxygen and sulfur groups in the shoots, suggesting the role of small organic acids in Cd transport (de la Rosa et al. 2004). These organic acids act differently on different heavy metal ions. Presence of citric acid improves the translocation of Cd ion but drastically reduces translocation of Ni in the halophyte *Halimonie vulgaris* (Duarte et al. 2007). Halophytic plant *Sesuvium portulacastrum* showed higher amount of Pb translocation from the root to the shoot in comparison to glycophyte *Brassica juncea* in presence of citric acid (Ghnaya et al. 2013).

The presence of chloride influences heavy metal ion mobility in the soil as well as within the plant system. Presence of Cl^- in soil facilitated the translocation of Cd to the shoot by enhancing the Cd flow into the xylem (Wali et al. 2014). External application of NaCl into the medium containing Cd–lipid complexes promotes the release of bounded Cd from lipids resulting in the formation of soluble Cd–Cl complexes in the medium (Girault et al. 1998).

In few halophytic plants, significant proportion of Na^+ gets accumulated in the mucilage (Ghanem et al. 2010). Generally, heavy metal gets concentrated in compartments exhibiting low metabolic activity, such as cell walls or vacuoles as an efficient mechanism of tolerance (Carrier et al. 2003). Halophytic plant *Halimione portulacoides* had more than 65% of the absorbed heavy metals accumulated in the root cell wall, and almost 50% of metals, which are accumulated in the leaves, are retained by cell wall polymers only (Sousa et al. 2008). Heavy metals are preferentially accumulated in the epidermis and in vascular bundle of collenchyma in hyperaccumulating species *Noccaea* (*Thlaspi*) *praecox* (Vogel-Mikus et al. 2008).

The chemical nature of heavy metal sometimes influences its redistribution in tissues. *N. praecox* showed reallocation of essential mesophyll cations (Fe, Mn and Zn) with increased Cd concentrations in the environment (Pongrac et al. 2010). *Zygophyllum fabago* was able to protect its photosynthetically active tissues against Cd or Zn toxicity through the accumulation of Cd or Zn ions in less metabolically active tissues by reallocating few essential elements such as K (Lefevre et al. 2014).

6 Cross-Tolerance and Augmenting Uptake of Heavy Metals Through Salinity

Halophytic plants have the ability to withstand a number of stress conditions by undergoing various adaptations including the development of cross-tolerance (Dhar et al. 2013). When a plant is exposed to any stress condition, it activates a number of responses in order to withstand that stress. Cross-tolerance is defined as a biological phenomenon by the virtue of which a plant, which is resistant to one stress, is able to develop tolerance to another form of stress (Foyer et al. 2016; Nikalje et al. 2019b). During cross-tolerance, two or more different types of pathways activate a signaling cascade. These different signaling pathways may operate independent of each other giving same kind of response in the end or interact with each other to give a final response (Knight and Knight, 2001). This could be either additive regulatory pathway, negative pathway, or competitive pathway (Capiati et al. 2006). The common mechanism of salt and metal tolerance of halophytes is shown in Fig. 1. Elements like stress sensors, calcium channels, CDPKs, MAPKs cascade, and transcription factors are included in cross talks (Chinnusamy et al. 2004). Besides these, hormones, oxidants and antioxidants are also involved (Munne-Bosch et al. 2013).

Halophytic plant *Thellungiella salsuginea* withstands both high salinity and oxidative stress, which has provided information on plant's ability for cross talk between combined stresses (Taji et al. 2004). Further, the gene *ThCBL* encoding for calcineurin B-like protein, gene *ThC4PT1* encoding for cyclophilin, and gene *ThZF1* encoding for Cys-2/His-2 transcription factor are involved in cross talk against different stresses in *Thellungiella salsuginea* (Amtmann 2009). *Kandelia obovata* is highly tolerant to cadmium. Under Cd stress, *KoFSD2* and *KoCSD3* genes were significantly expressed in roots of *Kandelia*, and overexpression of KoFSD2 improved cadmium tolerance in transgenic tobacco by maintaining lower O^{2-} and H_2O_2 levels (Pan et al. 2019).

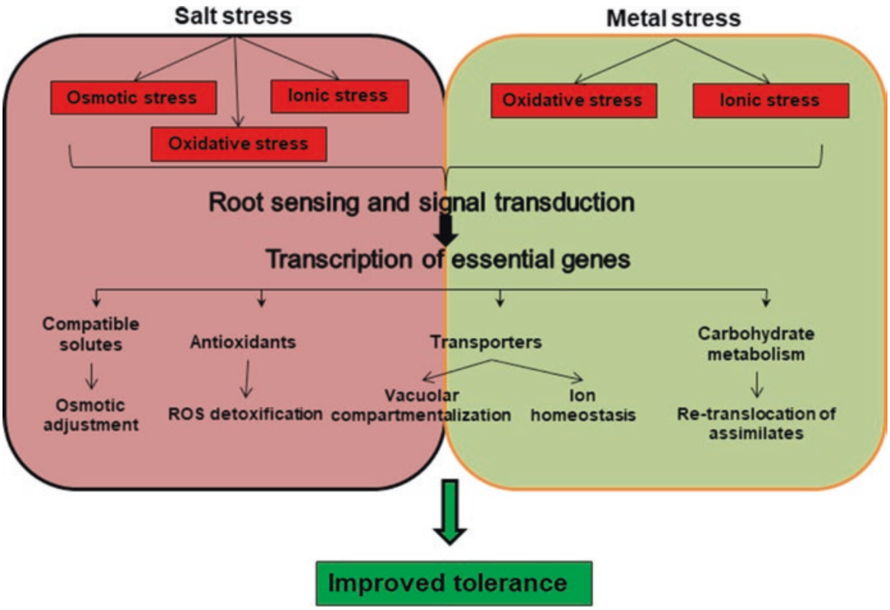

Fig. 1 Mechanism of salt and metal tolerance in halophytes: salt and metal toxicity both induce oxidative and ionic stress in halophytes. Being the first organ which comes in contact with stress-causing factors, the roots sense and induce cascade of signal transduction events. This leads to induction of ion transporters, antioxidant molecules, compatible solutes, carbohydrate metabolism, etc. The compatible solute carries out osmotic adjustment; antioxidants detoxify ROS by scavenging of free radicals. Transporters help in vacuolar compartmentalization and ion homeostasis, while carbohydrate metabolism helps in re-translocation of assimilates. The cumulative effect of all these stress responsive factors improves tolerance to both salt and metal tolerance in halophytes

7 Applications of Halophytes in Restoration of Metal-Contaminated Soils

Halophytes can grow in land with poor quality, which enables their use for phytoremediation of soils with poor fertility resulting in lower operational costs. Selection of plants suitable for restoration of contaminated soil is one of the crucial factors in phytoremediation, keeping in mind that plant selected should not translocate the metals into their aerial parts, provide sufficient cover of vegetation that stabilizes low levels of metals in soils, and prevent metals from mobilizing or leaching into groundwater. The applications of halophytes in restoration of metal-contaminated soil are shown in Fig. 2 and Table 2.

Halophytes like *Atriplex halimus*, *Atriplex nummularia*, *Mesembryanthemum crystallinum*, *Sesuvium portulacastrum*, *Tamarix smyrnensis*, and *Salicornia* sp. have shown their potential in phytoremediation and their value-added products (Lutts and Lefevre 2015; Muchate et al. 2016; Nikalje et al., 2018; Nikalje et al. 2019a,c). Annual halophyte like *Chenopodium botrys* is found to be effective in removing heavy metals, especially Cd, removing six times more than Cd removed by the hyperaccumulator *Noccaea caerulescens* (Mazharia and Homaeed 2012). Leaves of halophytic plant *Tamarix gallica* showed accumulation of As, mostly in its polysaccharidic fraction of cell wall components (Sghaier et al. 2016). Xerohalophytic plant *Atriplex atacamensis* has the ability to absorb and accumulate high concentration of As mainly in its roots. The absorbed As(V) gets quickly

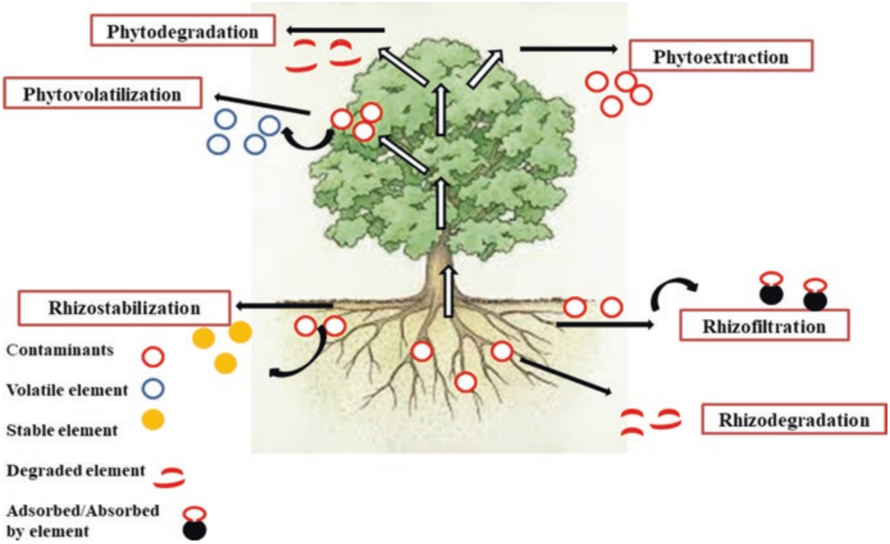

Fig. 2 Applications of halophytes in restoration of metal-contaminated soils: halophytes imply different strategies to cope up with toxic metals, namely, rhizodegradation, rhizofiltration, rhizostabilization, phytoextraction, phytodegradation, and phytostabilization. These strategies can be utilized for restoration of saline and metal-contaminated soils

Table 2 Examples of halophytes and heavy metal accumulation

Halophytic plant	Heavy metal	References
Halimione portulacoides	Cu, Cd, and Pb	Reboreda and Caçador (2008)
Atriplex halimus	Cd and Zn	Lefèvre et al. (2010)
Atriplex hortensis and *Atriplex rosea*	Cu, Pb, Ni, and Zn	Kachout et al. (2012)
Atriplex atacamensis	As	Vromman et al. (2011)
Sesuvium portulacastrum	Cd and Pb	Ghnaya et al. (2007)
Sesuvium portulacastrum	As	Lokhande et al. (2011)
Sesuvium portulacastrum	Cs	Nikalje et al. (2019a, b, c)
Mesembryanthemum crystallinum	Cu	Shevyakova et al. (2003a, b)
Tamarix aphylla	Cu and Zn	Lutts et al. (2004)

reduced to As(III) either enzymatically by arsenic reductase or non-enzymatically by glutathione, which further reacts with thiols as a strategy of detoxification (Zhang et al. 2012; Smith et al. 2010; Verbruggen et al. 2009; Zhao et al. 2010).

Often the plants that accumulate toxic metals in aerial parts when harvested, make the soil devoid of or lessened with soil contaminants and this conditioning allows cultivation of other crops. Halophytic plant *Sesuvium portulacastrum* has the ability to accumulate salts and heavy metal ions (chromium, cadmium, copper, zinc) from tannery effluent. From ternary samples *Sesuvium* accumulated high amount of Cr (49.82 mg), Cd (22.10 mg), Cu (35.10 mg), and Zn (70.10 mg) per gram dry weight of leaf samples (Ayyappan et al. 2016). Rabhi et al. (2010) assessed potential of *Sesuvium portulacastrum* for desalination of saline soil. Their results showed that *Sesuvium* has the ability to reduce both salinity and sodicity of saline soil, it can accumulate high amount of Na^+ ions (872 mg per plant), and the phytodesalinized soil can be utilized for cultivation of *Hordeum vulgare* crop. Some organic pollutants also concomitantly contaminate some of heavy metal-contaminated sites. Halophytes like *Spartina alterniflora* has been found to increase bioremediation of oil-contaminated salt marshy areas (Tate et al. 2012). Muchate et al. (2016) evaluates the potential of *S. portulacastrum* for desalinization purpose and found considerable lower soil electrical conductivity (2.2 dS m−1) and increased biomass in 90 days. Increased biomass can offer scope for production of value-added products. Halophytes hold an additional advantage for phytoextraction applications. Since they can be cultivated with saline water easily, this is a desirable feature because of lack of high-quality irrigation water needed for cultivation in arid regions. The plant *Tamarix smyrnensis* showed total Cd removal from soil increased from 9.4 μg in the absence of salt to 19.7 μg at 0.5% NaCl (Manousaki et al. 2008). These salt-tolerant plants are viewed as promising candidates for the immobilization or the removal of heavy metals not only from regular soils but also from saline soils, suggesting their application in decontaminating saline soils polluted with metals (Manousaki et al. 2008; Manousaki and Kalogerakis 2009; Nedjimi and Daoud 2009) and mine tailings in semiarid areas (Lutts and Lefèvre 2015).

8 Conclusions

Heavy metal accumlation in soils has become a mjor environmental issue and strategies are in place for remediation of such soils. Being endowed with natural saline habitat, halophytes have shown remarkable metal tolerance ability. The basic strategies against salt and metal toxicity are exclusion, excretion, and accumulation of metal/salt ions.This has been shown to be based on their common physiological and molecular responses to both salt and metal stress. In addition, accumulation of compatible solutes, osmolytes, induction of antioxidant enzymes helps plant in maintaining osmotic balance. In some halophytes, it is proved that supplementation salt improves metal tolerance. This may be because of an efficient cross-tolerance mechanism and augmentation of uptake of heavy metals through salinity. This phenomenon is reported from only few halophytes like *Sesuvium portulacastrum*. There is a need to direct more concerted efforts on screening more halophyte species to prove such an observation. The ability of halophytes to combat with both metal and salt ions is becoming successful in environmental cleanup and restoration of contaminated soils. Further research needs to be directed on optimization of growth conditions and tolerance level of candidate halophyte species to metal and salt at field level.

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Metabolome Modulation During Arsenic Stress in Plants

Preeti Tripathi and Rudra Deo Tripathi

1 Introduction

Progressively increasing contamination of arsenic (As) is a dreadful situation these days, which adversely influences all components of the environment. Arsenic is introduced in the environment through natural processes (volcanic eruptions, weathering of rocks, minerals, and fossil fuels) and anthropogenic activities (metal mining and smelting, agrochemicals, industrial effluents, and phosphate fertilizers), with its high level for the most part of India, Bangladesh, and China (Mondal et al. 2006, Tripathi et al. 2012a, b). The worst conditions of As contamination came across in Bangladesh and West Bengal (India) due to natural processes (Smith et al. 2000, Nordstrom 2002, Brammer and Ravenscroft 2009).

The subsurface mobilization of As is caused by the combination of chemical, physical and microbial factors, and various theories have been proposed to explain the mechanism of As mobilization (Mondal et al. 2006; Drewniak et al. 2012). Of these, the important theories are the pyrite oxidation and oxyhydroxide reduction (Hossain 2006). Flooding induces reducing (anaerobic) conditions in soils; hence, adsorbed arsenate [As(V)] is reduced to arsenite [As(III)] by anaerobic metal-reducing bacteria released by mediating reductive dissolution of As-rich Fe(III) oxyhydroxides (Islam et al. 2004). Besides natural processes, the global input of As

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to soil by human activities was estimated about 52,000 to 1,12,000 tons per year in earlier decade (Nriagu and Pacyna 1988).

Inorganic arsenic (iAs) forms such as As(V) and As(III) are class 1 carcinogens (IARC 2004). It is often implicit that organic forms, dimethylarsonic acid [DMA(V)] and monomethylarsonic acid [MMA(V)], are lesser toxic to humans than iAs (Schoof et al. 1999). However, reduced trivalent intermediate species of DMA and MMA are actually more cytotoxic and genotoxic than iAs (Thomas et al. 2001). There is a widespread chronic iAs poisoning in regions of South and Southeast Asia, South America, and elsewhere, due to the consumption of drinking water extracted from shallow underground aquifers with geogenically elevated iAs (Naujokas et al. 2013). According to the World Health Organization (WHO), the permissible limit of As in drinking water is 10 μg L^{-1}. Besides As-contaminated drinking water, plant-based foods are also recognized as an important source of iAs (Williams et al. 2005; Meharg et al. 2009).

Rice is specifically a problem regarding the entry of As into the food chain, owing to a combination of the anaerobic growing conditions and specific plant physiological characteristics. Nearly half the world's population relies on rice for subsistence diet. Rice contributes about 50% iAs intake to the populations exposed to high level of iAs in drinking water in South Asia (Meharg et al. 2009, Tripathi et al. 2012a, b). However, the excessive use of methylated arsenical pesticides in the USA has led to As buildup in soil as well as in rice grains (Williams et al. 2005). Besides, As stress also affects the productivity of the crop plants with high As accumulation.

In the view of past and recent researches, the studies of As uptake and metabolism have been taken into consideration in this review. Further, As toxicity and tolerance mechanisms are discussed for better understanding of the affected biological pathways and responsible metabolites insides the plants.

2 Arsenic Acquisition and Translocation in the Plant

To understand and control the As hazard from contaminated soils, it is crucial to know how various species of As are taken up by plants. Iron plays a pivotal role in the biogeochemical cycle of As, with Fe oxyhydroxides on soil particulate surfaces, or root surfaces of wetland plants as iron plaque, serving as strong adsorbent for As. Reductive dissolution of Fe oxyhydroxides under reducing environment releases the adsorbed As, leading to enhanced As availability to plants. There are significant varietal differences in the amount of iron plaque formation and, consequently, As sequestration in the iron plaque (Tripathi et al. 2014). Varieties that sequester more As in iron plaque translocate less As to aboveground tissues (Liu et al. 2004). Rice cultivars with high porosities tended to possess higher rates of radial oxygen loss and had higher capacities for limiting the transfer of As to aboveground tissues due to formation of more Fe plaques (Wu et al. 2012). Nonetheless, there are various factors other than the amount of iron plaque that may affect the uptake of As, e.g., the plant species, ionic species and their concentrations, pH of the rhizosphere, or the presence of other elements.

The mobility of As in the plants varies with As speciation as well as physicochemical characteristics of the soil (Marin et al. 1992). The most available and predominant forms of As are As(III), As(V), MMA, and DMA for plant uptake. The rate of uptake of different As species varies in the order of As(III) > As(V) > MMA(V) > DMA(V) in most of the plant, but exceptionally in rice the order is As(III) > MMA(V) > As(V) > DMA(V) (Raab et al. 2007). Differential uptake pathways responsible for the uptake of inorganic and organic species of As are described below.

2.1 Transport of Inorganic Arsenic

Arsenic mobility is dependent on the soil pH. Plant roots assimilate As(V) via phosphate transporters. The chemical analogs of inorganic phosphate (Pi) ions, such as $H_2AsO_4^{-1}$, gradually increase at pH range of 3–6; while, $H_2AsO_4^{-1}$ and $HAsO_4^{-2}$ species are significantly present at the pH between 7 and 8. Above pH 8 and below 11, the most predominant chemical form is $HAsO_4^{-2}$.

Plants have both low- and high-affinity Pi transport systems. High-affinity transport is mediated by PHT (phosphate transport) 1 proteins. Competition of As(V) with Pi for entry into the cell through both of these transport systems has been demonstrated in numerous plants, monocots and dicots, and both As-hyperaccumulators and non-hyperaccumulators (Ullrich-Eberius et al. 1989, Meharg and Macnair 1990, Tu and Ma 2003). Research validated that phosphate reduces As(V) uptake in plants (Ullrich-Eberius et al. 1989). The presence of minimum 13 and 9 members of phosphate transporter (*Pht1*) family was reported in rice and *Arabidopsis*, respectively (Zhao et al. 2010, Tripathi et al. 2012a, b). Due to sharing of same transport system, the toxicity of As(V) is lower under high P conditions (Tripathi et al. 2012a, b). Thus, Pi fertilization can protect plants, including the hyperaccumulator *P. vittata*, from As(V) toxicity (Tu and Ma 2003). In roots, the major part of As(V) is reduced to As(III), and the rest is further loaded into the xylem vessels by PHT proteins (Zhao et al. 2010). Therefore, some amount of As(V) is also present in shoots along with As(III) and other organic arsenicals (Zhao et al. 2010).

Recent studies have shown that a number of plant aquaporins belonging to the NIP (nodulin26-like intrinsic protein) subfamily are permeable to As(III), which is present predominantly as the undissociated neutral molecule (arsenous acid) at pH < 8 (Ma et al. 2008). NIP aquaporins also mediate transport of a range of small neutral molecules including ammonia, urea, boric acid, and silicic acid (Wallace et al. 2006). In yeast, a variety of proteins like hexose permeases are also involved majorly for As(III) transport (Finnegan and Chen 2012). While in plants, the role of yeast hexose permeases like proteins is still unknown if they provide a path for As(III) entry into plant cells. However, various plasma intrinsic proteins (PIPs) like OsPIP2;4, OsPIP2;6, and OsPIP2;7 are also suggested to transport As(III) in plants (Mosa et al. 2012). In rice, two silicon (Si) transporters, *Lsi1* (OsNIP2;1 aquaporin) an influx transporter and *Lsi2* (an efflux transporter), play a major role in the uptake of As(III) (Ma et al. 2008). The localization of *OsLsi2* to the proximal side of epidermal and endodermal cells (Ma et al. 2007) and OsLsi1 to the distal side of the same cells (Ma et al. 2006) is an elegant example of the heterogeneous distribution of proteins in a membrane. Thus, usage of highly efficient Si uptake pathway for passage of As(III) might be explaining why rice is efficient in accumulation of As than other cereals (Williams et al. 2005). Thus, Si fertilization may be an effective strategy to decrease As accumulation in rice grown in As-contaminated soil (Tripathi et al. 2013b). *Lsi2* plays an important role in the root to shoot transport of As(III) and its accumulation in the rice grain. *Lsi2* has a low degree of homology (18%) with the As(III) efflux transporter *ArsB* in *Escherichia coli* (Ma et al. 2007). Mutants of rice defective in silicon efflux transporter (*Lsi2*) showed significantly decreased transportation of As(III) to the xylem and accumulation in shoots and grains transport (Zhao et al. 2010). In most plant species analyzed, As(III) dominates in the xylem sap, also even when As(V) is supplied to plant roots due to high capacity of roots for As(V) reduction.

2.2 Transport of Organic Arsenic

The mobility of organic As MMA(V) and DMA(V) within the plant appears to be substantially greater than that of As(III) or As(V) (Raab et al. 2007, Li et al. 2009). Interestingly, *Lsi1* also contributes to the uptake of undissociated pentavalent MMA and DMA (Li et al. 2009); the rice *lsi1* mutant has lost 80% and 50% of the uptake capacity for MMA and DMA, respectively, compared with the wild-type rice. MMA and DMA have lower dissociation constants than As(III). In contrast to As(III), the rice *Lsi2* is not involved in the efflux of MMA or DMA toward the stele, possibly because most MMA and DMA are dissociated at the cytoplasmic pH (Li et al. 2009). Inadequate information is available about phloem loading and unloading of As. Using rice panicles excised below the flag leaf node, Carey et al. (2010) found that DMA was transported to the immature grain approximately 30 times more efficiently than As(III). To ensure the As(III) and DMA loading in grain through phloem, stem girding was applied and further tenfold and 50% decreased was obtained for As(III) and DMA transport, respectively. The outcome implies that As(III) is transported to rice grain primarily through the phloem, while both the phloem and xylem are contributed equally for DMA transport to grain.

3 Arsenic Detoxification Mechanism in Plants

Plants are able to detoxify As by adopting a range of detoxification strategies. The first step involves As reduction, synthesis of metal binding peptides such as metallothioneins (MTs) and phytochelatins (PCs) to make complex with As and their compartmentalization/vacuolar sequestration to keep the free As concentration at its minimum level (Zhao et al. 2010, Tripathi et al. 2012a, b). The second step is the activation of antioxidant defense system to counteract the As-induced oxidative stress (Zhao et al. 2010).

Arsenic is mainly taken by terrestrial plants as As(V) or As(III) (Meharg and Hartley-Whitaker 2002), followed by rapid reduction of As(V) to As(III) through arsenate reductase (AR) enzyme or via reducing agents such as GSH and ascorbic acid (Meharg and Hartley-Whitaker 2002). Both roots and shoots of rice exhibit As(V) reduction activities (Duan et al. 2007), but roots may be quantitatively more important because As(III) is the main form found in the xylem sap of a number of plant species (Zhao et al. 2003). PCs have a high affinity for As(III), but not for As(V). PCs are thought to have a fundamental role in As detoxification in plants (Schmöger et al. 2000). Arsenic is a strong inducer of PC synthesis and combines rapidly with sulfhydryl-rich protective molecules like GSH and PC (Schmöger et al. 2000). They are synthesized posttranslationally from GSH by the transpeptidation of γ-glutamyl-cysteinyl dipeptides through the action of the constitutive enzyme PC synthase (Grill et al. 2006). The PC–As(III) complexes are likely to be stored in vacuoles. The yeast vacuolar transporter Ycf1p, a member of the ATP-binding cassette (ABC) superfamily, confers As(III) resistance by transporting the conjugated As(III)–$(GS)_3$ complex into the vacuole (Ghosh et al. 1999). In *Arabidopsis*, this transport occurs via the ABC transporters MRP1/ABCC1 and MRP2/ABCC2 (Song et al. 2010). Homologs of *Arabidopsis* ABCC1 and ABCC2 are found throughout the plant kingdom (Mendoza-Cózatl et al. 2011). A homolog of ABCC2 was among several ABC transporters to be upregulated at the transcript level in response to As in rice (Norton et al. 2008a, b). Thus, in many plants, As(III) present in root cells is rapidly complexed to PC and sequestered in the vacuole, severely restricting the transport of As from the root (Mendoza-Cózatl et al. 2011) and preventing its interaction with cellular metabolism.

Another probable constitutive mechanism of As detoxification is the efflux of As(III) to the external medium. Recently, Indriolo et al. (2010) characterized two genes from *P. vittata* (*ACR3* and *ACR3;1*), which encode proteins similar to the ACR3 As(III) effluxer of yeast. In another study, examining heterologous expression of the yeast As(III) efflux system, *ACR3* was cloned from yeast and transformed into wild-type and nip7;1 *Arabidopsis* (Ali et al. 2012). At the cellular level, all transgenic lines showed increase tolerance to As(III) and As(V) and a greater capacity for As(V) efflux. Expressing *Saccharomyces cerevisiae ScACR3* in rice enhanced As(III) efflux and also reduced As accumulation in rice grains (Duan et al. 2012). Rice transgenics overexpressing As(III)-S-adenosyl methyltransferase (*arsM*) has been found to methylate As and gave tenfold higher volatile arsenical, maintaining low As levels in rice seed along with MMA(V) and DMA(V) in the roots and shoots of transgenic rice (Meng et al. 2011).

4 Consequences of Arsenic Toxicity on Plant Metabolism

The mode of toxicity differs involving As species; As(V) interferes with phosphate metabolism such as phosphorylation and ATP synthesis, whereas As(III) binds to vicinal sulfhydryl (-SH) groups of proteins affecting their structures or catalytic functions (Hughes 2002). In plant tissue, As(V) is rapidly reduced to As(III) and normally itself not have high enough cytoplasmic concentrations to exert toxicity (Bertolero et al. 1987). As chemical analog of phosphate, As(V) can disrupt several cellular biochemical reactions which used phosphate as a substrate (Gresser, 1981). Arsenate can be translocated through cellular membranes by PHT proteins, creating imbalances in phosphate supply. Possible As(V)-susceptible biological reactions include central to cellular metabolism, i.e., glycolysis, oxidative phosphorylation, biosynthesis of phospholipid, nucleic acids retrieval, and cellular signaling including protein phosphorylation/dephosphorylation.

The mode of action of As(III) differs substantially from that of As(V). As(III) is a thiol-reactive compound that can bind up to sulfhydryl groups such as glutathione (GSH) or phytochelatin (PC), thiol-containing proteins, and cofactors. The binding of As(III) to proteins can have profound effects on their folding (Cline et al. 2003). In various systems, proteins that are known to bind As(III) include transcription factors, signal transduction proteins, proteolytic proteins, metabolic enzymes, redox regulatory enzymes, and structural proteins. Compared to As(III), MMA(III) is a more potent inhibitor of various enzymes (Styblo et al. 1997). MMA(III) and DMA(III) both (low to mid micromolar concentration) were able to displace Zn^{2+} from a zinc-finger protein (Schwerdtle et al. 2003), an important class of proteins involved in gene expression and DNA repair.

A number of hydroponic studies infer that As phytotoxicity depends on the chemical species supplied to the plant, but the most phytotoxic form of As was still unidentified (Abbas and Meharg 2008). The inconsistent order of phytotoxicity of different As species might be a sign that As reacts in a different way with the offered nutrients or responds to plant species-specific manner.

At higher concentrations, As interferes with plant metabolic processes and can inhibit growth, often leading to death (Jiang and Singh 1994). Arsenic exposure could hamper normal growth of plants with toxicity symptoms such as biomass reduction, loss of yield and fruit production, and morphological changes (Mokgalaka-Matlala et al. 2008, Srivastava et al. 2009). Consequences of arsenic toxicity have been depicted in Fig. 1 which showed the alteration on growth, physiology, and plant metabolism. The severity of straight head disease (sterility of the florets/spikelets) was increased in rice grown in As-spiked soil solution (Zhao et al. 2010).

Interveinal necrotic (Singh et al. 2006) and whitish chlorotic symptoms (Shaibur et al. 2008) have been reported in As-stressed plants. Arsenic damages the chloroplast membrane and disorganizes the functions of integral photosynthetic process (Stoeva and Bineva 2003). Rate of CO_2 fixation and functional activity of photosystem II (PSII) were reduced in oat plants growing under As stress (Stoeva and Bineva 2003). As a consequence leaf gas exchange, the chlorophyll fluorescence ratio Fv/Fm, leaf photosynthesis activity, carotenoid content, and the functional activity of PSII decreased (Georgieva and Yordanov 1993, Stoeva and Bineva 2003).

High concentrations of As may interfere with plant metabolism and membrane stability index, impair macro- and micronutrient uptake, or may simply compete with essential plant nutrients (Meharg and Macnair 1990); for instance, As is known to compete with plant P uptake (Mokgalaka-Matlala et al. 2008).

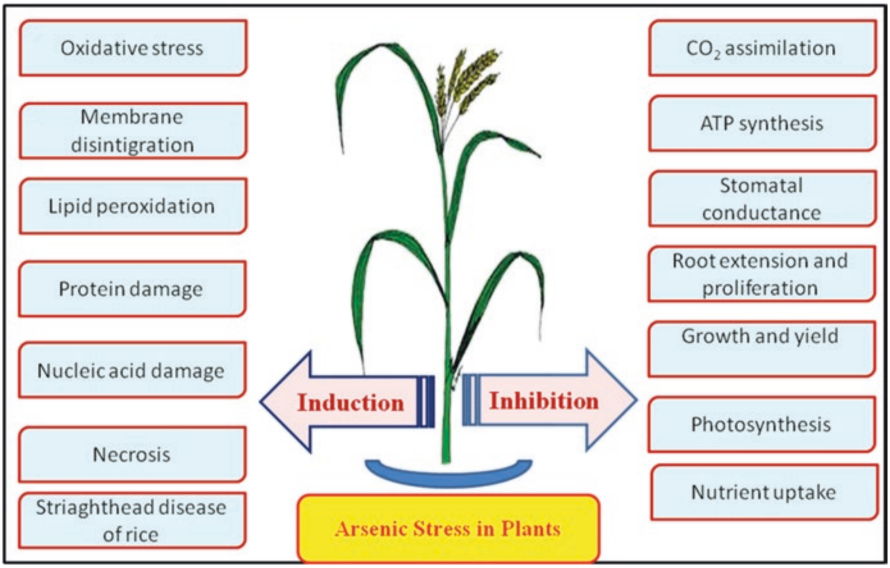

Fig. 1 Consequences of arsenic toxicity in plants

Arsenic exposure generates reactive oxygen species (ROS) in plant tissues and induces oxidative stress such as lipid peroxidation of biomolecules, ultimately resulting in death of the plants (Ahsan et al. 2008). Based on the chemical and physical properties, As causes toxicity in three different ways: (a) enhanced production of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$), (b) blocking of essential functional groups in biomolecules, and (c) displacement of essential metal ions from biomolecules. Arsenic stimulates generation of ROS, either by direct electron transfer or as a consequence of metal mediated inhibition of metabolic reactions (Zhao et al. 2010).

Under normal cellular conditions, ROS homeostasis is precisely balanced. At low level of environmental stresses such as temperature and light, minute ROS imbalances occur that can act as signals of cellular status and are easily managed by pre-existing antioxidant defense mechanisms (Van Breusegem and Dat 2006). However, under stronger stresses, such as As exposure, where ROS generation increases, these defense mechanisms may be harassed, leading to cellular damage and death (Van Breusegem and Dat 2006).

ROS are capable of causing considerable cellular damage through oxidation of lipids, proteins, amino acids, and nucleic acids (Møller et al. 2007). Exposure of protein to $OH^{\bullet}$ radical produces dimers, trimers, and tetramers by covalent cross-linking due to formation of intermolecular bityrosine. Exposure to $OH^{\bullet}$, $O_2^{\bullet-}$, and O_2 further leads to fragmentation or aggregation of protein. Arsenic may also affect the protein synthesizing machinery of the cells by their effect on enzymes of nitrogen metabolism (Schmidt et al. 2005). Lipid peroxidation not only compromises cellular function but leads to the production of lipid-derived radicals

(Van Breusegem and Dat 2006, Møller et al. 2007). The molecular targets that are most sensitive to the ROS produced by As exposure are not yet clear, although there are many candidates.

These insights provide the facts that As can affect growth and productivity due to excess of morphological, physiological, biochemical, and molecular alterations inside the plants. However, tolerant plant species adapt an array of detoxification strategies, viz., genomic, proteomic, and metabolomic approaches to overcome the As stress. A lot of reviews on As stress in plants reflect the genomic and proteomic modulation in detail. Thus, in the next section we summarize the role of various metabolites modulated during As stress in plants.

5 Metabolome Modulation During Arsenic Stress and Tolerance in Plants

Metabolomics refers to the identification and quantification of all low-molecular-weight metabolites required by the organisms during developmental stages (Arbona et al. 2013), and some metabolites have been reported to be involved under heavy metal stress tolerance strategies. In the following section, we discuss the role of metabolomics under arsenic stress. Despite the fact that transcriptomic approaches provide almost complete coverage and proteomics approaches are now capable of detecting most of the cellular protein complement, metabolomics is currently capable of determining only a small fraction of the metabolites found in any one cell. The more challenging aspect of metabolomic technologies is the refined analysis of quantitative dynamics in biological systems. For metabolomics, gas and liquid chromatographies coupled with mass spectrometry are well suited for analysis of high sample numbers in reliable measurement times with respect to both technical accuracy and the identification and quantification of small-molecular-weight metabolites. However, to the best of our knowledge, a very limited study has been performed to recognize the modulation of differential metabolomic pathway during As stress. Thus, an account about the metabolites involved in antioxidant systems, thiol metabolism, amino acid pool, carbohydrate metabolism, hormonal balance, and others has been particularly given below during As stress (Fig. 2).

5.1 Thiolic Content and Related Enzymes

The central role played by the binding of As(III) to sulfhydryl groups in GSH and PC in the detoxification of the metalloid indicates a critical importance for sulfur metabolism in determining plant survival in As-contaminated soils. The biosynthesis of GSH and PC that is typically induced by As exposure requires adequate supplies of the GSH building blocks glutamic acid (Glu), cysteine (Cys), and glycine (Gly). In both shoots and roots of wild-type *Arabidopsis*, the mass ratios of free Glu/Gly/Cys

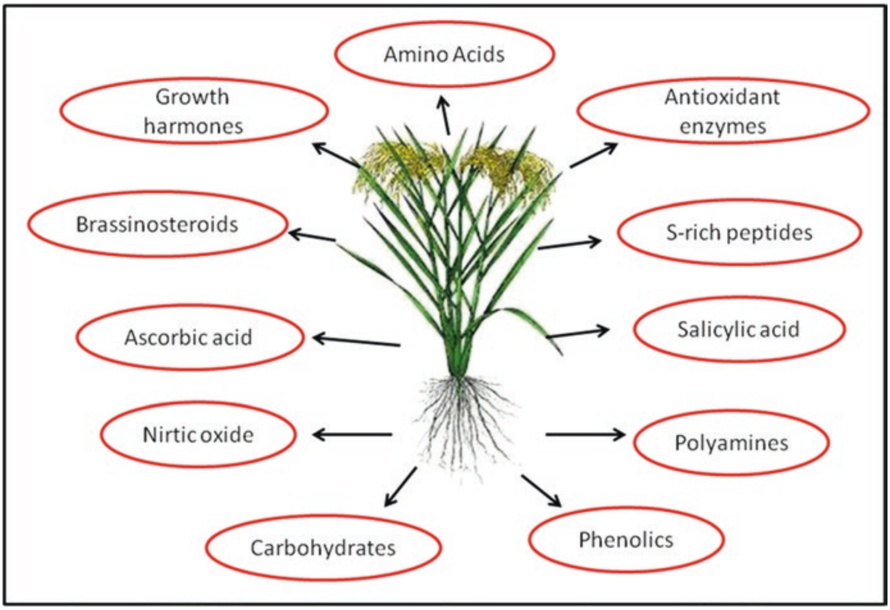

Fig. 2 Different metabolites modulated during As tolerance and detoxification

were about 20:3:1 (Muñoz-Bertomeu et al. 2009). Thus, at least in *Arabidopsis*, Cys is by far the limiting substrate for GSH biosynthesis. However, other studies indicate that As(V) exposure can decrease cellular Cys pools (Sung et al. 2009) and that under some growth conditions, it is possible that the synthesis of PC can deplete GSH pools, decreasing the antioxidant capacity of the cell (De Vos et al. 1992). The Cys content increased in some aquatic plants such as *H. verticillata*, rootless plant *C. demersum*, and crop plant *B. juncea* during As stress (Srivastava et al. 2007, 2009, Mishra et al. 2008, Tripathi et al. 2012a, b). Before sulfate acquired from the soil can be used for the biosynthesis of Cys, and thus the biosynthesis of GSH and PC, it must be reduced via sulfite to sulfide (Takahashi et al. 2011). In a sulfur metabolome study, high-resolution Fourier transform mass spectrometric approach in combination with stable isotope labeling was used in *Arabidopsis thaliana* (Gläser et al. 2014), and about approximately 140 sulfur metabolites that have not been assigned to the databases to date were identified. Further, unraveling of unidentified sulfur metabolite during As stress is of prime importance in plants.

Once sulfate is reduced to sulfide, the sulfide is combined with O-acetylserine to form Cys in a reaction catalyzed by O-acetylserine (thiol)-lyase (OAS-TL), also known as Cys synthase. The O-acetylserine is formed from Ser in a reaction catalyzed by Ser acetyltransferase. OAS-TL exists either as a free active homodimer or in association with Ser acetyltransferase as an inactive subunit of the Cys synthase complex (Takahashi et al. 2011). It appears that As(V) and As(III) exposure may cause a downregulation of OAS-TL in As-sensitive plants (Requejo and Tena 2006, Srivastava et al. 2009). Thus, it seems likely that Cys biosynthesis, and, therefore, As detoxification through GSH and PC would be compromised. Indeed, the Cys and GSH levels in an As-sensitive variety of *B. juncea* were lower upon As exposure than in the untreated control (Srivastava et al. 2009). In contrast, a *B. juncea* variety with increased tolerance to As showed a more general induction of the sulfate assimilation and GSH biosynthetic pathways (Srivastava et al. 2009), similar to that seen in yeast (Thorsen et al. 2007). A list of As-modulated antioxidants are given in Table 1. In the As-tolerant variety, there was an induction in Cys synthase activity, as well as in the activities of Ser acetyltransferase and γ-ECS, the penultimate enzyme in the biosynthesis of GSH. These increases in enzyme activity were

Table 1 Arsenic affected thiolic enzymes in different plant species

Thiolic enzyme	Plant species	Remarks	References
5′-Adenylylsulfate reductase (APS) activity	*A. thaliana*, *H. verticillata*	The activity was found to be elevated	Abercrombie et al. (2008), Srivastava and D'Souza (2009)
Serine acetyltransferase (SAT) activity	*H. verticillata*, *C. demersum*, *B. juncea*, *O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)
Cysteine synthase (CS) activity	*H. verticillata*, *C. demersum*, *B. juncea*, *O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)
Glutathione-S-transferase	*H. verticillata*, *C. demersum*, *B. juncea*, *O. sativa*	Increased activity suggested role of GSH in As detoxification	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)
γ-Glutamyl cysteine synthase (γ-ECS) activity	*H. verticillata*, *C. demersum*, *B. juncea*, *A. thaliana*, *O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava and D'Souza (2009), Srivastava et al. (2009), Sung et al. (2009), and Tripathi et al. (2012a, b, 2013a)
Phytochelatin synthase (PCS) activity	*C. demersum*, *B. juncea*, *A. thaliana*, tobacco, *O. sativa*	As exposure increased the activity for more synthesis of PCs	Li et al. (2004), Gasic and Korban (2007), Mishra et al. (2008), Sung et al. (2009), and Tripathi et al. (2012a, b, 2013a)
γ-Glutamyl transpeptidase (γ-GT)	*C. demersum*, *B. juncea*, *O. sativa*	As exposure increased the activity, while in contrasting lines of *B. juncea*, activity was more pronounced in tolerant variety	Mishra et al. (2008), Srivastava et al. (2009), and Tripathi et al. (2012a, b, 2013a)

accompanied by increased levels of both Cys and GSH, indicating that increased sulfur metabolism may be a viable mechanism for increasing As tolerance in plants. *Arabidopsis* may behave similarly, with transcripts for a γ-ECS, GSH synthetase, and PC synthase being induced during As exposure (Sung et al. 2009).

5.2 Antioxidant Enzymes

Arsenic intraconversion inside the plant may produce oxygen imbalance which causes oxidative damage after production of ROS (Tripathi et al. 2012a, b). To combat oxidative stress, various antioxidant enzymes have been increased their activities in the plants (Mishra et al. 2008, Tripathi et al. 2012a, b). The hyperaccumulator plants can accumulate a significantly higher concentration of As in their bodies, but they have evolved a variety of As tolerance mechanisms against higher levels of free radicals (Khan et al. 2009).

Upon exposure to As, the enzymes involved in the balancing of these free radicals include superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) (Souri et al. 2018). The antioxidative enzymes are generally electron donors and react with ROS to form a neutral and nontoxic end product. Under biotic or abiotic stress, the activation or the suppression of antioxidant enzymes to scavenge ROS takes place in conjugation with each other. Among these antioxidant enzymes, SOD is a key player that constitutes the first line of defense by dismutation of superoxide free radicals (O_2^-) into O_2 and H_2O_2 in plants (Tripathi et al. 2017). Arsenic-induced increase in the activity of SOD can be due to an enhanced level of O_2^- or the direct action of As on SOD gene overproduction.

Souri et al. (2018) observed a significantly higher increase (116%) in SOD activity in *Isatis cappadocica*, which was exposed to 800 μM As under hydroponic conditions. As-induced SOD activities have been observed in many other studies as well (Singh et al. 2006, Srivastava et al. 2007, Mishra et al. 2008, Tiwari and Sarangi 2017, Tripathi et al. 2017).

Catalase scavenges H_2O_2 level in peroxisomes in many stress conditions including As stress in an energy-efficient manner (Srivastava et al. 2017). Arsenic stress may modulate CAT activity depending upon the strength, extent, and kind of stress in various plants (Tripathi et al. 2012a, b, 2017). Accordingly, Souri et al. (2018) also observed that increased activity of CAT was responsible for H_2O_2 detoxification during lower level of SOD in the shoots of *I. cappadocica*.

Ascorbate peroxidase is a key enzyme of ascorbate–glutathione cycle and plays an important role for H_2O_2 detoxification (Singh et al. 2006, Tripathi et al. 2013a, b, 2017). The deviation of APX activity during As stress has been well-documented in ameliorating the harmful effects of H_2O_2 in various plants (Srivastava et al. 2007, Mishra et al. 2008, Souri et al. 2018, Tripathi et al. 2012a, b, 2017).

Glutathione reductase assists plants in the NADPH-dependent reduction of oxidized glutathione to maintain the ratio of GSH/GSSG for proper functioning of the cells (Srivastava et al. 2009, Begum et al. 2016). The As-induced oxidative damage was partly mitigated by induced activity of GR, in order to sustain the balance of GSH (Souri et al. 2018). Similarly, GR overproduction has been noticed in many other plants under As stress (Mishra et al. 2008, Srivastava et al. 2007, 2009, Begum et al. 2016). It is established fact that As significantly affects the GSH level in plants by converting it into PCs. The higher demand for GSH is managed by higher GSH turnover through enhanced GR activities in As-stressed plants (Mishra et al. 2008).

In general, plants exhibit remarkable difference in their antioxidant potential during As stress (Singh et al. 2006, Srivastava et al. 2007, Mishra et al. 2008, Tiwari and Sarangi 2017, Tripathi et al. 2017). As-hyperaccumulators tolerate As stress by activation of antioxidative defense system in order to maintain lower ROS level, further leading to As detoxification and significant accumulation in tissue (Khan et al. 2009). Arsenic exposure also activates the antioxidant machinery in various plant species, such as *H. verticillata*, *C. demersum*, *B. juncea*, *O. Sativa*, and *Glycine max* L. (Srivastava et al. 2007, Mishra et al. 2008, Bustingorri et al. 2017, Hasanuzzaman et al. 2017).

5.3 Nonenzymatic Antioxidants (Ascorbate, Polyamines, and Phenols)

Ascorbate and dehydroascorbate (low-molecular antioxidants) act as nonenzymatic antioxidants in glutathione–ascorbate cycle for free radical scavenging. They are measured in various As-stressed plants, and increased ratio of AsA/DAsA was noticed in *P. vittata*, *P. ensiformis*, *H. verticillata*, and *O. sativa* (Singh et al. 2006, Srivastava et al. 2011, Tripathi et al. 2012a, b). These insights suggest the prominent role of ascorbate for removal of As stress.

The polyamines are also known to involve in ROS scavenging (Drolet et al. 1986) and the diminution of lipid peroxidation (Borrell et al. 1997) to protect the plant under As stress. Mascher et al. (2002) confirmed that levels of polyamines, viz., spermidine and spermine, are raised merely at lower As exposure; however, diamine is induced at higher doses during As(V) exposure in red clover plants (*Trifolium pratense*). The phenolic content was also showed their variation in rice during As stress (Chauhan et al. 2017). Out of eight, seven phenolic compounds have been identified from roots and shoots of the rice during As–Se interaction. Out of eight, all polyphenols were found to decrease during As stress in shoots and roots except rutin and ferulic acid in roots, which were increased about 25-fold and 87%, respectively. However, quercetin was detected only during As stress in both roots and shoots, while caffeic acid was not detected in shoots during As stress imparting its tissue-specific modulation (Chauhan et al. 2017). Consequently, differential metabolite profiling in plant species denote that plants may modulate their metabolome up to variable degree for As tolerance and toxicity mechanisms.

5.4 Amino Acids

Amino acids, the building block of protein, are known to chelate metals ion for metal tolerance inside the plants. The role of various essential and nonessential amino acids was explored for As tolerance. Dwivedi et al. (2010) analyzed the amino acid profile in grain of various rice genotypes during As stress. They concluded that most of the essential amino acid (EAA) metabolites such as valine, methionine, leucine, and alanine, and nonessential amino acids (NEAAs), viz., histidine, alanine, proline, glutamic acid, and cysteine, increased in most of the rice genotypes during As(V) exposure. Some other amino acids, viz., proline, glutamic acid, aspartic acid, and alanine, were also increased during As(V) stress in *Spinacia oleracea* (Pavlik et al. 2010). Among stress-responsive amino acid, proline is the most studied molecule and can function as an osmolyte and free radical scavenger and also can protect cell membrane damage. The level of proline was also elevated in *O. sativa* during As (III) stress (Mishra and Dubey 2006). The S-containing amino acid, cysteine, plays a central role in As detoxification, used as a primary metabolite for synthesis of GSH and PCs for effective complexation of As. The cysteine content increased in some aquatic plants such as *H. verticillata*, rootless plant *C. demersum*, and crop plants *B. juncea* and *O. sativa* (Srivastava et al. 2007, 2009, Mishra et al. 2008, Tripathi et al. 2012a, b) during As stress.

5.5 Carbohydrates

The carbohydrate metabolism is negatively affected during As stress in plants, and soluble sugars can be accumulated for As tolerance (Gramss 2012). In rice shoots, the relative amount of nonreducing sugars was decreased than reducing sugars during As stress (Jha and Dubey 2004) which imitates suppression of sucrose formation than accessible hexose monophosphate. The possible cause behind this variation is the reduced activities of starch-degrading enzymes, i.e., α- and β-amylase, and starch phosphorylase leading to As-induced plant toxicity (Jha and Dubey 2004). On the contrary, an increase of about 77–120% in starch phosphorylase activity was reported in *O. sativa* and *Phaseolus aureus* seedlings during As exposure, ensuing in a raised level of soluble sugars (Jha and Dubey 2004).

In addition, stimulation of sucrose-hydrolyzing enzymes, viz., acid invertase and sucrose synthase, was studied along with the inhibition of sucrose phosphate synthase activity during As toxicity (Jha and Dubey 2004). Acid invertases are responsible for the sucrose degradation for the formation of glucose and fructose (hexoses), which activity was directly correlated with hexose level (Kaur et al. 2012). The acid invertase and sucrose synthase are the key enzymes for the synthesis of hexoses, which might be shortly oxidized through the glycolytic pathway (Baud and Lepiniec 2009). The metabolic impairments as discussed above will lead to altered plant growth and development under As stress.

5.6 Nitric Oxide

Nitric oxide (NO) is a free radical signaling agent in plants which regulates several cellular functions under normal and stressed situations (Sahay and Gupta 2017). NO is considered as a reactive nitrogen species, and its useful and injurious effects are based upon its level and localization inside plant cells (Sahay and Gupta 2017).

The defensive role of NO against heavy metal stress occurs through unambiguous mechanisms: (i) prevention of Fenton reaction through reaction of NO with OH radicals to form HNO_2, thus defending cells against hazardous OH radical; (ii) avoidance of radical-mediated lipid peroxidation through reaction with lipid radicals; (iii) reaction with superoxide radical to form peroxynitrite ($ONOO^-$), which has lesser toxicity and lifespan than highly toxic H_2O_2; (iv) activation of antioxidants like SOD, CAT, APX, and POX; and (v) working as a signaling molecule for biological reactions leading to variation in gene expression (Saxena and Shekhawat 2013).

As a signaling molecule, NO mediates activation of antioxidants and the biosynthetic pathway of PCs in roots, which facilitate the induced uptake of sulfate by enhanced synthesis of amino acids and nonprotein thiols (Farnese et al. 2013). NO supply improves As tolerance in *Luffa acutangula* through dawdling As absorption and accumulation by increased cell wall thickness of the root epidermis (Singh et al. 2013). Hasanuzzaman and Fujita (2013) demonstrated that the exogenous NO promoted relative water content, chlorophyll pigment, antioxidant enzymes, and glyoxalase activities in As-treated wheat seedling. In *Vicia faba* seedlings, NO supplementation resulted in enhanced seed yield, photosynthetic content, plant hormones, and mineral nutrients to combat As toxicity (Mohamed et al. 2016). The internal concentration of NO was also increased during As(V) stress in *H. verticillata* and *O. sativa* (Srivastava et al. 2011, Tripathi et al. 2012a, b, 2015). Similarly, NO addition considerably lowered root As uptake and its transport to shoots in rice plants during As(III) treatment (Singh et al. 2017) and improved chlorophyll through increased Fe uptake.

Nitric oxide was effectively known to lessen adverse impact on growth and ROS-imposed injuries during As stress in *O. sativa* (Singh et al. 2015), *Vigna radiate* (Ismail 2012), and *Phaseolus vulgaris* L. (Talukdar 2013). NO pretreatment in plant scavenges ROS entirely for improvement of As tolerance (Farmer and Mueller 2013, Sahay and Gupta 2017).

5.7 Salicylic Acid

Salicylic acid (SA) is categorized in the group of phenolics and acts as endogenous phytohormone, which contributes in growth, photosynthesis, flowering, nitrate metabolism, and ethylene production (Vicente and Plasencia 2011). SA imparts its crucial job for signaling of protective act next to the pathogen attack and also responds to other undesirable surroundings to plants (Pandey et al. 2013).

SA also plays signaling role for abiotic as well as toxic metal stress like As (Wani et al. 2017). In hydroponics, SA application was capable for amelioration of As stress through increased internal concentrations of both SA and NO in rice plants during As exposure suggesting SA-mediated modulation of NO, correlated with increased activity of nitrate reductase (Singh et al. 2017). Furthermore, reduced expression of *OsLsi*2 gene during SA and NO interaction was accountable for lesser As uptake.

SA supplementation was reported to induce the level of proline, an antioxidant in plants, thus helpful against As and other metals toxicity (Yang et al. 2009). Similarly, exogenous SA imparts its role in pigment synthesis exhibiting better photosynthesis and dynamic growth in plants (Singh et al. 2017). The SA addition was supportive in extenuating chlorosis by enhancing Fe uptake. In another study, 1 mM SA addition showed induction in chlorophyll and protein content along with reduced lipid peroxidation to combat As toxicity in wheat seedlings (Zengin 2015).

SA also may indirectly involve in antioxidant stimulation through NO induction. Application of SA with As was more effective than SA pretreatment in lowering As uptake in rice. Enhanced level of enzymatic and nonenzymatic antioxidants and thiols plays an important role for As tolerance in plants (Singh et al. 2015). Similarly, Saidi et al. (2017) also reported that SA reduced the As-induced oxidative stress through increased synthesis of APX, CAT, and GPX in seedling of *Helianthus annuus*.

5.8 Growth Hormones and Brassinosteroids

Plant growth hormones are the bioactive molecules which are responsible for plant growth and development. Various studies reported that plant growth hormones have protective role against heavy metals toxicity such as Cu, Cd, Pb, As, etc. Initial research into plant hormones identified five major classes: auxin, cytokinins, gibberellins, ethylene, and abscisic acid (Thomas and Thomas 1979). This list was later expanded and brassinosteroids, jasmonates, and strigolactones are now considered as major plant hormones. An inadequate knowledge is available on the role of growth hormones in As stress amelioration, which needs to be elucidated through detailed analysis in plant.

The role of auxin hormone was identified during As(III) stress in *A. thaliana* (Krishnamurthy and Rathinasabapathi 2013). Auxin transporter mutants aux1, pin1, and pin2 showed its sensitive behavior to As(III) stress compared to the wild type (WT). Inhibitors of auxin transport considerably reduced As(III) tolerance in the WT, whereas addition of indole-3-acetic acid enhanced As(III) tolerance of aux1 but lesser than WT. Uptake assays of labeled H3-IAA exhibited As(III)-modulated auxin transport in WT roots. As(III) enhanced the H_2O_2 levels in WT although not in aux1, signifying a positive role for AUX1 for auxin transport through ROS-mediated signaling (Krishnamurthy and Rathinasabapathi 2013).

The role of cytokinins (CKs) was also investigated in *A. thaliana* for As tolerance (Mohan et al. 2016). CK signaling mutants and transgenic plants with reduced endogenous CK levels demonstrated tolerance to As(V) stress through induced level of thiol compounds and lowered uptake of As indicating regulatory role of CK for As tolerance (Mohan et al. 2016). The protective role of brassinosteroids (Brs) against As toxicity was also investigated in rice plants (Xu et al. 2018). The pooled effect of 24-epibrassinolide (Br24) or 28-homobrassinolide (Br28) on As uptake was studied in rice seedlings. The finding concluded that Br24 and Br28 may possibly hamper As accumulation and may ameliorate the As toxicity (Xu et al. 2018).

Methyl jasmonate (MJ) is also a significant plant growth regulator, concerned with plant protection against abiotic stresses; nevertheless, its potential function against metal stress is not very implicit. The impact of MJ was analyzed on physiological and biochemical parameters of As-treated (200 μM) *Brassica napus* (Farooq et al. 2016) showing considerable raise in leaf chlorophyll fluorescence and biomass than As-stressed plants. The findings revealed that MJ scavenge ROS through improved antioxidant defense system, secondary metabolite, and decreased As accumulation.

6 Conclusion

It is apparent from the above critical discussion that As exposure adversely affects plants at biochemical and molecular levels and influences a majority of physiological responses, such as inhibition in overall growth processes, photosynthetic efficiency, and biomass accumulation. Arsenic can induce oxidative stress via the enhanced production and/or inefficient elimination of ROS and consequently damage biomolecules and interferes with various metabolic pathways, either directly or indirectly, by intervening in activities of certain key enzymes.

Plants adapt several strategies to overcome As stress, such as complexation of As with S-peptides and compartmentalization and induction of stress-responsive amino acids and various enzymatic and nonenzymatic antioxidants (phenolics and ascorbate). Moreover, the signaling metabolites such as NO and SA are typically endowed with As tolerance involving different mechanisms. The fate and recognition of various unidentified sulfur metabolites must be studied during As stress in plants. Our knowledge on the role of growth hormones during As stress is scanty, which must be enriched through in-depth research. Besides, potential research is required to unravel the toxic effects of As on seed germination and plant development through metabolome modulation.

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Arsenic Transport, Metabolism, and Possible Mitigation Strategies in Plants

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1 Introduction

Arsenic (As), an ubiquitous metalloid and potential carcinogen, is introduced into the environment by processes such as weathering of rocks, volcanic eruptions, burning of fossil fuels, mining, smelting of ores, arsenical pesticide and herbicide usage, and usage in wood preservatives (Zheng et al. 2013). Arsenic can combine with other elements mainly oxygen, sulfur, and chlorine to produce its inorganic and organic forms. Its inorganic form is mainly used as a wood preservative and organic form as pesticides. It exists in four different oxidation states: −3, 0, +3, and +5 (Asere et al. 2017). In the environment, As is present in inorganic forms, viz., arsenious acids (H_3AsO_3), arsenic acids (H_3AsO_4), arsenites (As (III)), and arsenates (As (V)), and in organic forms, viz., monomethylarsonic acid (MMA), dimethylarsinic acid (DMO), trimethylarsine oxide (TMAO), and also in gaseous forms (Shukla and Srivastava 2017). In water, As is generally present in As (V) and As (III) forms (Mines 2014). Arsenate has a similar electronegativity and ionization energies to phosphorous, so compounds of As also bear a resemblance to

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phosphorous compounds. Thus, As can inhibit glycolysis by replacing inorganic phosphatase (Vasudevan et al. 2013) and Krebs cycle by inhibiting pyruvate conversion to acetyl-CoA which can cause severe diseases like cancer, multiorgan failure, etc. (Tharmalingam et al. 2017). On the other hand, As (III) binds to protein's sulfhydryl groups and disturbs their structures or catalytic functions (Tripathi et al. 2007; Zhao et al. 2010).

Arsenic poisoning symptoms include severe stomach pain, nausea, and headaches and can lead to death (Jomova et al. 2011). The WHO has currently recommended limit of 10 μg/L of As in drinking water (George et al. 2014), which means over 50 billion people are drinking As-contaminated water. Akinbile and Haque (2012) have reported cases of As poisoning in China, Mexico, Taiwan, India, Argentina, Chile, and Bangladesh. Recently, it has been reported that 80% infant foods contain As in rice products like baby cereals, puffs, etc. The use of As-contaminated water for irrigation also leads to an increase in As concentration in soil. By using deep tube wells as alternative water sources, drinking water crisis can be solved, but for irrigation, the use of deeper pumping may induce migration of As from upper to downward aquifer that will permanently destroy the deepwater source (Roy et al. 2015).

Several methods for treatment of As-contaminated soil and water involve either excavation or on-site management and later dump to a landfill site that causes migration of contaminant to the nearby environment. Soil washing is also an alternative, but it is very costly and requires further treatment as it produces metal-rich residues (Jena and Dey 2017). Plants have unique mechanism for uptake, accumulation, and detoxification of contaminants. Direct use of plants for treatment of contaminated soil, sludge, sediments, ground, and surface water is known as phytoremediation, an environment-friendly and cost-effective technique (Mosa et al. 2016). Plant root system is very specific for uptake of nutrients from soil and removes unwanted substances by translocation, bioaccumulation, and finally degradation. Uptake of As in plants as As (V) occurs through phosphate transporters, whereas As (III) enters into the plants through the nodulin 26-like intrinsic protein (NIP) and plasma membrane intrinsic protein (PIP) aquaporin channels (Zhao et al. 2010; Mosa et al. 2012). Plant species exposed to As release excessive reactive oxygen species (ROS) in plant tissues, which leads to oxidative stresses, for instance, lipid peroxidation (Ahsan et al. 2008; Tripathi et al. 2012a, b). Arsenate (As (V)) and arsenite (As (III)) exposure also boost antioxidant responses (Requejo and Tena 2005; Rai et al. 2011). After exposure to As (V), glutathione (GSH) synthesis and metabolism are augmented for As sequestration in rice seedlings exposed to As (V) (Ahsan et al. 2008; Norton et al. 2008) that reflect a higher consumption of GSH under As stress. Arsenate is readily reduced to As (III) in plant. Further, As (III) form compound with phytochelatin (PC) or glutathione and undergo vacuolar sequestration.

Phytoremediation technique, which is an environment-friendly and low-cost method, has been widely accepted for As removal (Wani et al. 2017). The revelation of *Pteris vittata* as As hyperaccumulator plant by Ma et al. (2001) stimulated the possible discovery of other As hyperaccumulator/accumulator plants to improve the use of phytoremediation technique. This chapter focuses on the mechanisms of As transport, metabolism, and phytoremediation strategy to mitigate As toxicity. Greater understanding of these mechanisms will help in the development of As-tolerant plants that can sequester As in their tissues and avoid risks caused by As toxicity.

2 Arsenic Transport in Plants

Intake of As-contaminated water and food leads to As toxicity which affects millions of people around the world mainly South and Southeast Asia. People use shallow tube water for irrigating staple food crops such as rice, which subsequently gets highly contaminated with As (Verbruggen et al. 2009). Plants uptake As from contaminated water and store it in their grains, which is then consumed by humans and leads to serious health issues (Martinez et al. 2011; Bundschuh et al. 2012). Hence, it is essential to understand the As uptake and its metabolism by plants to avoid the risks caused by As toxicity. Arsenic is nonessential for plants and affects growth and yield of crop by limiting other nutrients essential for plant growth (Duan et al. 2013; Brackhage et al. 2014). Thus, increased attention is being paid to reduce its level and develop As-tolerant plants especially the important cash crops.

In nature, As is available in both organic forms (methylated As) and inorganic forms, mainly As (V) and As (III). Among the various forms of As, As (V) and As (III) are the most abundant and toxic for plants. The predominant form of As (V) occurs in soil aerobic conditions. As (V) may be higher in the rice rhizosphere due to oxygen release by rice roots (Seyfferth et al. 2010). Hence, it's important to understand how As (V) is taken up by rice plants. As (V) is a structural analogue of inorganic phosphate and taken up by phosphate transporters in plants. It affects nucleotide synthesis and energy homeostasis of the cell (Meharg and Hartley-Whitaker 2002). Plant aquaporins that transport water and neutral molecule in and out of the plant cell also efficiently transport As (III). Soil also contains several methylated As species due to As-containing herbicides and pesticides. Uptake and transport mechanism of methylated As in plants are not fully known. Interestingly, rice grains are known to accumulate double the amount of methylated As than inorganic As (Zheng et al. 2013). Table 1 summarizes the various arsenic transporters identified in different plant species that are reported to transport As (V), As (III), and methylated As species.

3 Transport of As (III)

In anaerobic conditions, As (III) is the dominant form due to microbial reduction activity. As (V) is rapidly reduced to As (III) with the help of arsenate reductase enzyme, the first step in As metabolism (Duan et al. 2005). Several transporters

Table 1 Arsenic transporters in different plant species

As species	Gene name	Gene family	Plant source	Subcellular localization	References
As (III)	AtNIP1;1	Aquaporins	*Arabidopsis thaliana*	Plasma membrane	Kamiya et al. (2009)
	AtNIP1;1	Aquaporins	*Arabidopsis thaliana*	Plasma membrane	Kamiya et al. (2009)
	AtNIP3;1	Aquaporins	*Arabidopsis thaliana*	Plasma membrane	Xu et al. (2015)
	AtNIP5;1	Aquaporins	*Arabidopsis thaliana*	Plasma membrane	Bienert et al. (2008b), Kamiye et al. (2009)
	AtNIP6;1	Aquaporins	*Arabidopsis thaliana*	Plasma membrane	Bienert et al. (2008b)
	AtNIP7;1	Aquaporins	*Arabidopsis thaliana*	Plasma membrane	Isayenkov et al. (2008)
	OsNIP1;1	Aquaporins	*Oryza sativa*	Plasma membrane	Ma et al. (2008)
	OsNIP3;1	Aquaporins	*Oryza sativa*	Plasma membrane	Ma et al. (2008)
	OsNIP3;2	Aquaporins	*Oryza sativa*	Plasma membrane	Bienert et al. (2008b)
	OsNIP3;3	Aquaporins	*Oryza sativa*	Plasma membrane	Katuhara et al. (2014)
	OsLsi	Aquaporins	*Oryza sativa*	Plasma membrane	Ma et al. (2008), Bienert et al. (2008a)
	OsLsi2	Aquaporins	*Oryza sativa*	Plasma membrane	Ma et al. (2008)
	OsLsi6	Aquaporins	*Oryza sativa*	Plasma membrane	Ma et al. (2008)
	OsPIP2;4	Aquaporins	*Oryza sativa*	Plasma membrane	Mosa et al. (2012)
	OsPIP2;6	Aquaporins	*Oryza sativa*	Plasma membrane	Mosa et al. (2012)
	OsPIP2;7	Aquaporins	*Oryza sativa*	Plasma membrane	Mosa et al. (2012)
	LjNIP5;1	Aquaporins	*Lotus japonicus*	Plasma membrane	Bienert et al. (2008b)
	LjNIP6;1	Aquaporins	*Lotus japonicus*	Plasma membrane	Bienert et al. (2008b)
	HvNIP1;2	Aquaporins	*Hordeum vulgare*	Plasma membrane	Katsuhara et al. (2014), Besse et al. (2011)
	PvACR3	Aquaporins	*Pteris vittata*	Tonoplast	Indriodo et al. (2010)

(continued)

Table 1 (continued)

As species	Gene name	Gene family	Plant source	Subcellular localization	References
As (V)	AtPHT1;1	Phosphate transporter	*Arabidopsis thaliana*	Plasma membrane	Catearecha et al. (2007), LeBlanc et al. (2013)
	AtPHT1;4	Phosphate transporter	*Arabidopsis thaliana*	Plasma membrane	Catearecha et al. (2007), LeBlanc et al. (2013)
	AtPHT1;5	Phosphate transporter	*Arabidopsis thaliana*	Plasma membrane	Nagarajan et al. (2011)
	AtPHT1;7	Phosphate transporter	*Arabidopsis thaliana*	Plasma membrane	LeBlanc et al. (2013)
	AtPHT1;8	Phosphate transporter	*Arabidopsis thaliana*	Plasma membrane	Remt et al. (2012)
	AtPHT1;9	Phosphate transporter	*Arabidopsis thaliana*	Plasma membrane	Remt et al. (2012)
	OsPHT1;8	Phosphate transporter	*Oryza sativa*	Plasma membrane	Wu et al. (2011), Jia et al. (2011)
	OsOHT1;1	Phosphate transporter	*Oryza sativa*	Plasma membrane	Kamiya et al. (2013)
Methylated As	OsLsi	Aquaporins	*Oryza sativa*	Plasma membrane	Ma et al. (2011)

belonging to the superfamily of aquaporins have been identified in plants which uptake As (III). Aquaporins are membrane channels that transport water and small neutral molecules including As (III) (Li et al. 2014; Mukhopadhyay et al. 2014; Kumar et al. 2018). Plant aquaporins have been classified into five major categories based on their localization and sequence similarities: nodulin 26-like intrinsic proteins (NIPs), plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), small basic intrinsic proteins (SIPs), and the uncategorized (X) intrinsic proteins (XIPs) (Maurel et al. 2015). NIP and PIP subfamilies of aquaporins are known to transport essential nutrients such as silicon and boron (Ma et al. 2006; Takano et al. 2006; Bienert et al. 2008a; Mosa et al. 2012; Kumar et al. 2018). Additionally, these two subfamilies of proteins have been reported to be involved in As (III) transport (Bienert et al. 2008a, b; Isayenkov and Maathuis 2008; Ma et al. 2008; Mosa et al. 2012; Xu et al. 2015) (Table 1). Transgenic *Arabidopsis* lines having T-DNA insertion in *NIP1;1* gene showed induced tolerance to As (III) (Kamiya et al. 2009). *Arabidopsis* mutant *nip3;1* plants exhibited significantly less As (III) compared to wild-type plants when exposed to As (III) indicating the NIP3;1 role in root to shoot transport of As (III) (Xu et al. 2015). Likewise, several other NIP mutants (*nip1;1*, *nip1;2*, *nip3;1*, *nip5;1,* and *nip7;1*) also showed reduced plant As levels and among these *nip1;1*, *nip3;1* and *nip7;1* showed increased tolerance to As (III) (Isayenkov and Maathuis 2008; Kamiya et al. 2009; Xu et al. 2015). *Arabidopsis NIP7;1,* expressed in both vegetative and inflorescence phases of the tissues, has a major role in transporting As (III) into xylem and phloem. Loss-of-function mutants of *nip7;1* showed increased tolerance to As (V) and spatiotemporal distribution of As (Lindsay and Maathuis 2016). *Arabidopsis thaliana*, NIP5;1 and NIP6;1, rice (*Oryza sativa*) NIP2;1 and NIP3;3, and *Lotus japonicus* NIP5;1 and NIP6;1 exhibited As (III) bidirectional transport when expressed in a yeast strain lacking As (III) transport systems (Bienert et al. 2008b) (Table 1). In rice (*O. sativa*) NIP2;1 which is known as silicon influx transporter (called as OsLsi1) also mediates As (III) uptake in rice roots (Ma et al. 2006, 2008). OsNIP3;3 and HvNIP1;2 also showed As (III) transport activity in yeast cells (Katsuhara et al. 2014) (Table 1).

Along with several NIPs, members of plasma membrane intrinsic protein (PIP) family are also involved in As (III) transport. OsPIP2;4, OsPIP2;6, and OsPIP2;7 are reported to transport As (III) across membranes (Mosa et al. 2012). *Arabidopsis* roots expressing OsPIP2;6, when exposed to As (III), showed increased efflux and bidirectional transport of As (III) by PIPs (Mosa et al. 2012). Heterologous expression of OsPIP2;4, OsPIP2;7, and OsPIP2;7 in *Xenopus laevis* oocytes showed increased As (III) influx. Overexpression of these genes in *Arabidopsis* showed increased tolerance to As (III), exhibited influx and efflux activity when As was exposed for short duration, and resulted in no accumulation of As when exposed for long period (Mosa et al. 2012) (Table 1).

Tonoplast intrinsic protein (TIP) is a subfamily of aquaporins. Among several TIP proteins, TIP4;1 showed uptake of As (III) (He et al. 2016). Heterologous expression of *PvTIP4;1* in yeast showed As (III) diffusion, and the *Arabidopsis* plants overexpressing *PvTIP4;1* exhibited induced As (III) levels and induced sensitivity to As (III) (He et al. 2016).

4 Transport of As (V)

Arsenate [As (V)] is the dominant form of As, available in aerobic conditions (Meharg and Hartley-Whitaker 2002). Arsenate has similar chemical structure as inorganic phosphate and can be taken up by plant phosphate transporters (Table 1). In *Arabidopsis thaliana*, AtPht1;1 and AtPht1;4 are high- and medium-affinity phosphate transporters and are known to uptake As (V) (Shin et al. 2004; González et al. 2005) (Table 1). AtPht1;5 transports both Pi and As (V) from source to sink organs of phosphorous. Loss-of-function mutant of *AtPht1;5* plants showed As (V) resistance (Nagarajan et al. 2011). In the *atpht1;1–3* mutant Pi activation genes were downregulated, and As (V)-related genes were upregulated (Catarecha et al. 2007). The overexpression of AtPht1;7, which specifically expresses only in reproductive tissues of *Arabidopsis*, showed higher As (V) accumulation (LeBlanc et al. 2013). *AtPht1;9* which expresses in roots and is involved in P_i uptake during phosphate starvation conditions also showed uptake of As (V) (Remy et al. 2012). Additionally, the involvement of *AtPht1;8* in As (V) uptake along with P_i has been demonstrated (Remy et al. 2012) (Table 1). High arsenic content 1 (HAC1), arsenate reductase, an enzyme which reduces As (V) to As (III), controls As accumulation in both roots and shoots of *Arabidopsis* (Chao et al. 2014).

T-DNA insertion line in which expression of *OsPT1* was knockdown showed decreased As (V) accumulation in the shoots compared to wild type, suggesting its involvement in root to shoot transport of As (V) in rice (Kamiya et al. 2013). *OsPHF1* mutant, defective in phosphate transporter traffic facilitator 1 (which regulates the exit of P_i transporter form ER to plasma membrane), loses its ability to take up P_i and As (V) (Wu et al. 2011). On the other hand, the overexpression of *OsPT8* increases the uptake of As (V) (Wu et al. 2011). Recent study showed that a mutation in this gene (*OsPT8*) reduced the uptake of As (V) (Wang et al. 2016). *OsHAC1;1* and *OsHAC1;2* are induced upon As (V) exposure. Loss of function of these two genes leads to decreased reduction of As (V) to As (III) and reduced efflux of As (III) to the medium, whereas overexpression of either gene leads to reduced As accumulation in rice grains (Shi et al. 2016). Similarly, rice (*Oryza sativa*), *OsPht1;8,* showed the uptake of As (V). Overexpression of *OsPht1;8* showed higher uptake and translocation of As (V) (Jia et al. 2011; Wu et al. 2011), which is regulated by *OsPHR2*, a transcription factor. OsPht1;1, a high-affinity phosphate transporter in rice, is also involved in As (V) uptake (Sun et al. 2012; Kamiya et al. 2013). Phosphate deficiency and As (V) induce *P. vittata Pht1;3* and lead to accumulation of high amount of As (V). Yeast cells expressing *PvPht1;3* show high sensitivity to As(V) and accumulate high As (V) compared to *Arabidopsis thaliana Pht1;5* (DiTusa et al. 2016).

Several WRKY transcription factors were reported to be involved in As (V) uptake. WRKY6 and WRKY45 regulate As (V) uptake by inducing the expression of *AtPht1;1* which further accumulates As (V). WRKY6 transcription factors downregulate *Pht1;1* expression to prevent transposon expression in response to As (V), and once As (V) is removed, it relocates AtPht1;1 and induces its expression (Castrillo et al. 2013). WRKY45 positively regulates As (V) uptake by inducing *AtPht1;1* expression (Wang et al. 2014).

5 Transportation of Methylated Arsenic

Due to arsenal pesticide, herbicide usage, and microorganisms in soil, trace amounts of methylated As are synthesized, mostly monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Ye et al. 2012; Huang 2014). Earlier studies showed that methylated As is taken up by plants in lesser amount than inorganic As(V) or As(III) (Marin et al. 1992; Carbonell-Barrachina et al. 1998; Burlo et al. 1999; Carbonell-Barrachina et al. 1999). But, recent studies showed two-fold higher amount of methylated As in rice grains compared to inorganic As (Zheng et al. 2013). Abedin et al. (2002) hypothesized that the uptake of MMA follows Michaelis-Menten kinetics in rice. However, the uptake of DMA is unclear. Abbas and Meharg (2008) demonstrated the uptake mechanism of DMA in maize (*Zea mays*) by Michaelis-Menten with linear function.

In rice, low silicon rice1 (Lsi1) is responsible for the uptake of both MMA and DMA in roots. Mutants of *OsLsi1* showed reduced uptake of both MMA and DMA by approximately 80% and 50%, respectively, compared with the wild type (Li et al. 2009). Both MMA and DMA follow the same entry route as glycerol, which is aquaporins in rice roots (Rahman et al. 2011). Hirano et al. (2004) found that DMA is less toxic than As (III) (100-fold), which could reduce As toxicity in rice grains.

6 Arsenic Metabolism in Plants

Various steps are known to be involved in the As metabolism in plants. Reduction of As (V) to As (III) is considered an early step in As metabolism, and arsenate reductase (AR) can efficiently catalyze this reduction step (Mukhopadhyay and Rosen 2002). In addition, other enzymes or pathways are also suggested to exist for As (V) reduction in plants (Rathinasabapathi et al. 2006). As (III) production enables As detoxification either through efflux of As (III) or complexation with thiol compounds. A clear correlation between As uptake and enhancement in As-chelating capacity has been observed. In nonhyperaccumulator plants, As (III)-thiol complexes are sequestered into vacuoles of root cells by transporters present on vacuolar membrane (Dhankher et al. 2002; Wang et al. 2002). In hyperaccumulator plants, most of the As (V) taken up from soil is transported to aboveground parts and stored as free As (III). In numerous plant species, methylated As species have been reported. However, whether plants methylate As themselves or take up methylated As generated by microorganisms is unclear. A deeper understanding of mechanisms of As metabolism in As-sensitive and -tolerant plants will enable scientists to develop As-tolerant plants to combat the danger posed by As. This section will focus on different steps of As metabolism in plants.

7 Reduction of Arsenate to Arsenite

Arsenate reduction to As (III) is regarded as the first step in the detoxification of As in plants (Fig. 1). Arsenic speciation studies revealed that As is mainly found in the form of As (III) in plants, even when treated with As (V) suggesting that following uptake, As (V) is reduced efficiently to As (III) in plants (Pickering et al. 2000; Dhankher et al. 2002; Xu et al. 2007; Zhao et al. 2009; Finnegan and Chen 2012; Mishra et al. 2017). Methylarsonate (MA (V)) is also reduced to MA (III) efficiently, but trivalent form of dimethylarsinate (DMA (V)) has not been detected yet (Mishra et al. 2017). Magnetic resonance study established the role of reduced glutathione (GSH) in the reduction of As (V) to As (III) in vitro (Delnomdedieu et al. 1994). However, this reaction is considered too slow to be responsible for efficient reduction. Arsenate reductase (AR) enzyme catalyzes As (V) reduction to As (III) efficiently. AR enzyme was first identified in yeast and bacteria (Mukhopadhyay et al. 2000). It utilizes GSH as electron donor, and the mixed disulfide formed between GSH and AR is known to be resolved by glutathione reductase (GRX)

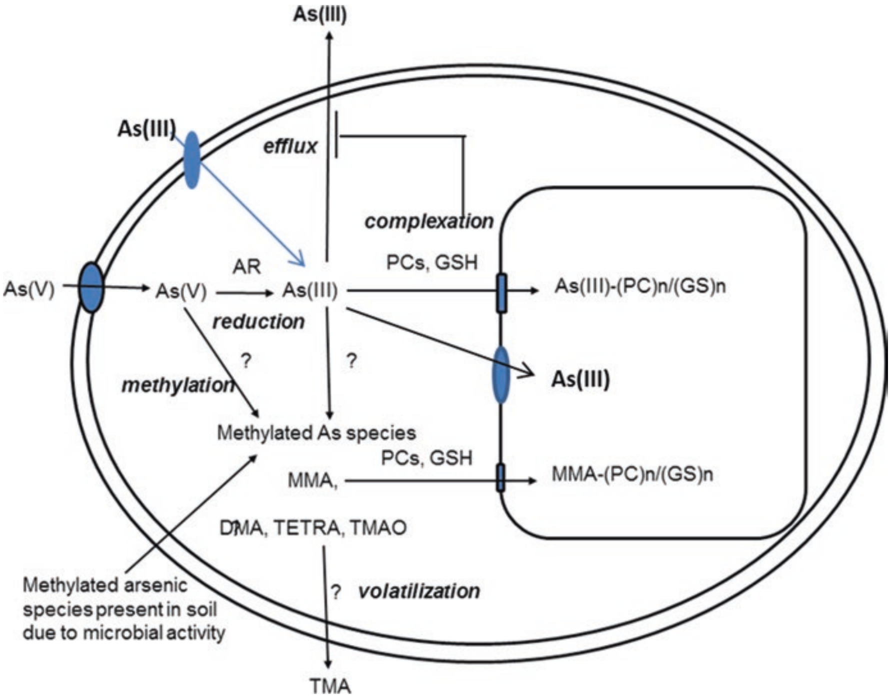

Fig. 1 Arsenic metabolism in plant root. Arsenate reductase (AR) catalyzes the reduction of As (V) to As (III). As (III) can either be effluxed or make complexes with the thiol group containing phytochelatins (PCs) or glutathione (GSH) and get sequestered into root vacuoles. Complexation of As (III) with thiols reduces As (III) efflux. As(III) can also be transported to the vacuole by arsenical compound resistance3 (ACR3) effluxer. Methylated As species monomethyl arsenic acid (MMA), dimethylarsenic acid (DMA), tetramethylarsonium ions (TETRA), and trimethylarsonium oxide (TMAO) present in plant are either due to capability of plants to methylate inorganic As themselves or due to uptake of methylated As generated by microorganisms. It is not clear whether nonhyperaccumulator plants can volatilize As as trimethylarsine (TMA). Question marks (?) denote uncertain/unclear steps or mechanisms

(Mukhopadhyay et al. 2000). In plants, AR genes showing AR activity have been cloned and characterized in *P. vittata* (*PvACR2*), *A. thaliana* (*AtACR2*), *O. sativa* (*OsACR2*), and *Holcus lanatus* (*HlAsr*) (Dhankher et al. 2006; Ellis et al. 2006; Duan et al. 2007). Sánchez-Bermejo et al. (2014) identified *AtARQ1* member of a new group of As (V) reductases which contributes to *A. thaliana* natural variation for arsenate tolerance. This role coincides with the enhanced As (V) reductase activity observed in naturally selected hyperaccumulator plants with increased capacity to detoxify As (III). As (V) treatment led to enhanced *OsACR2* and *HlACR2* expression (Bleeker et al. 2006; Duan et al. 2007). In As hyperaccumulator *P. vittata* roots exposed to 2 mM As (V) for 9 days, sevenfold higher AR-specific activity was observed compared to those of roots and shoots of As (V) non-tolerant plants (Duan et al. 2005).

As (V) reductases of eukaryotes possess rhodanase domain (Bordo and Bork 2002). A new qtl ATQ1 (AR) identified in *Arabidopsis* was found to code for rhodanase-like protein. *ATQ1* encodes a protein which participates in detoxification of As. When grown on As-contaminated media, plants with T-DNA insertion mutant of *ATQ1* were found to be more sensitive to AsV compared to the wild type. Chao et al. (2014) revealed that in *Arabidopsis*, *ATQ1* plays an important role not only in the reduction of As (V) to As (III) but also in efflux of As (III) into soil. As (III) is effluxed considerably from root tissues in various plant species, and this may be important in reducing As load and enhancing As tolerance in plants. In *P. vittata,* higher As efflux compared to nonhyperaccumulator plants has been associated with plant growth maintenance during As accumulation (Chen et al. 2016). Recently, Shi et al. (2016) identified two genes *OsHAC1*;*1* and *OsHAC1*;*2* in rice as AR. Both genes were found to be abundant in roots and induced by As (V) exposure. They suggested that the product of these genes limits As accumulation by mediating As (III) efflux to the outside medium. *OsHAC1*;*1* and *OsHAC1*;*2*, when expressed in *E. coli* mutants that lack the endogenous AR activity, showed AR activity. *OsHAC1*;*1* and *OsHAC1*;*2* gene knockout resulted in reduced As (III) efflux and enhanced As accumulation in shoots and grain, whereas overexpression of either *OsHAC1*;*1* or *OsHAC1*;*2* enhanced As (V) tolerance, As (III) efflux, and reduced accumulation of As. Similarly, *OsHAC4* was also found to play critical role in As (V) tolerance and regulation of As accumulation in rice (Xu et al. 2017). In *Arabidopsis* plants, knockdown of *ACR2* led to the development of plant lines with more sensitivity to high concentrations of As (V), but not As (III) than wild type. These lines showed less As concentration in roots and 10- to 16-times higher As concentrations in shoots than wild type (Dhankher et al. 2006). In contrast, Liu et al. (2012) reported no significant changes in As redox status and As (III) efflux between wild-type and *AtACR2* overexpression or knockout lines and suggested the role of other enzymes/pathways in reduction of As (V) in plants. The glycolytic enzyme cytosolic triosephosphate isomerase (TPI) from *P. vittata* has been demonstrated to play direct or indirect role in As (V) reduction (Rathinasabapathi et al. 2006). *PvTPI* gene, when expressed in the *E. coli* mutant strain that lacks *ArsC*, increased the As (III) content and As (V) resistance indicating that nonspecific enzymes may also contribute partly to As (V) reduction (Rathinasabapathi et al. 2006). In mammalian systems, glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GDPH) and purine nucleoside phosphorylase (PNP) are known to catalyze As (V) reduction to As (III) (Gregus and Németi 2005; Radabaugh et al. 2002). However, it remains unclear whether these two enzymes participate in As reduction in plant also.

8 Methylation

Plants treated with inorganic As exhibited a small concentration usually <1% of the total As concentration of MMA, DMA, and trimethylarsine oxide (TMAO) in tissues and xylem sap, indicating that plants themselves methylate As (Marin et al. 1992;

Meharg and Hartley-Whitaker 2002; Francesconi and Kuehnelt 2002; Quaghebeur and Rengel 2003; Xu et al. 2007; Raab et al. 2007; Mishra et al. 2017). Methylation of As (V) is reported in macrophages, phytoplankton, and some terrestrial plants (Phillips 1990; Koch et al. 2000). Methylated As is further metabolized to arsenosugars and organophospholipids (Phillips 1990). However, it remains unclear whether methylated As forms are actually metabolized by the plant or they are merely taken up by plants from soil solution (Fig. 1).

Tomato (*Solanum lycopersicum*) plants grown in perlite in phosphorus (P) and nitrogen (N)-starved condition and exposed to ^{74}As-labelled As (V) showed significant level of methylated forms of As including MMA (III), MMA (V), and DMA (V) in roots indicating that plants can methylate As under severely nitrogen- and phosphorus-deficient conditions (Nissen and Benson 1982). Considerable amounts of methylated forms of As were also present in *Helianthus annuus* and *Holcus lanatus* grown hydroponically and treated with inorganic As (Quaghebeur and Rengel 2003; Raab et al. 2007). As these investigations were performed under nonsterile conditions, there is a possibility that microorganisms produced these methylated species (Zhao et al. 2010). Also, no evidence of external methylation in the soil or water around the plants was observed, and yet many As-methylated species such as MMA, DMA, tetramethylarsonium ions (TETRA) and TMAO, arsenocholine, arsenobetaine, arseno-sugar, and glycerol-ribose were present in many plant species (Koch et al. 2000). Although there was no evidence of TMAO found in the surrounding environments, TMAO was present in *Mimulus* sp. indicating the possibility of methylation in plant. In vitro As methylation activity has been demonstrated in *Agrostis tenuis* leaf extract using S-adenosyl-L-methionine (SAM) as methyl donor. As (V) pre-exposure led to induced As methylation activity in plants, and MMA was formed initially and DMA accumulated later. As (III) is the preferred substrate for methylation, and it is suggested that roots uptake As (V) and reduce it to As (III) before methylation through induced As methyltransferase activities (Wu et al. 2002).

Arsenic methylation in plants has been suggested to follow the Challengers pathway, which is already known in fungi and bacteria (Bentley and Chasteen 2002). In microbes S-adenosyl methyl transferase methylates As (III) to MMA (V) using SAM as the methyl-group donor. MMA (V) is converted to MMA (III) by a reductase using thiols. Further, methylation and reduction reactions lead to production of DMA (V), DMA (III), TMAO, and trimethylarsine (TMA; a volatile gas). Rice microarray gene expression study revealed enhanced expression of a gene methyl transferase (Os02g51030) in response to As (V) treatment (Norton et al. 2008). Similar to arsM genes of microbes, rice methyl transferase possess UbiE/Coq5 family protein motif (Qin et al. 2006). An alternative pathway to that of the Challenger pathway involving AS3MT enzyme, which utilizes As (III) and the GSH complex (ATG) as the substrates and catalyzes the transfer of methyl group to the final products MMA and DMA, has been suggested (Hayakawa et al. 2005). Methylation of As (V) is not considered as an effective detoxification mechanism. As-methylating activities were found to be suppressed in As accumulators. The As-tolerant *Boehmeria nivea* and As hyperaccumulators, *P. vittata* and *P. cretica*, accumulated significantly less DMA in comparison to As (V) (Huang et al. 2008). The concentration of DMA was higher in rhizoids compared to fronds in As hyperaccumulators, *P. vittata* and *P. cretica*. In contrast, *B. nivea* showed higher concentration of DMA in fronds in comparison to rhizoids (Huang et al. 2008). Although As (V) reduction to As (III) in plants has been convincingly demonstrated, methylation remains controversial (Van den Broeck and Vandecasteele 1998; Pickering et al. 2000). Methylated species are present in the soil due to activity of microorganisms. Therefore, methylated As species present in plants could be due to uptake of methylated species existing in soil solution (Cullen and Reimer 1989; Koch et al. 1999). Lomax et al. (2012) strongly suggested that plants cannot methylate inorganic As rather they take up methylated As generated by microbial activity. Enzyme arsM catalyzes the production of various methylated intermediate species from As (III), with volatile trimethylarsine (TMA) as the final product (Qin et al. 2006). Sakakibara et al. (2010) collected vapor samples to determine volatilization of As compounds from fronds of *P. vittata* grown in As-polluted soil. Around 90% of As taken up by *P. vittata* was removed suggesting that *P. vittata* can volatilize As effectively. However, when sunflower plant was exposed to As (V) concentration for 1 day, concentration of As (V) remained unchanged over the following 32 days without As exposure to roots indicating that even if volatilization happened, it should probably be very small (Raab et al. 2007).

9 Complexation and Sequestration of Arsenic

Due to high affinity toward thiol (-SH) groups, As (III) can form complexes with thiol containing glutathione and/or phytochelatins (PCs) and get stored in vacuole of plants root (Fig. 1). As (III) forms complexes with PCs inhibiting As (III) efflux and root to shoot As (III) translocation in *Arabidopsis* (Liu et al. 2010). In As hyperaccumulator plants, thiols have restricted role and As is mainly stored as As (III) (Pickering et al. 2006; Zhao et al. 2009). X-ray absorption spectroscopy (XAS) studies revealed that in roots and shoots of *A. thaliana* and *B. juncea* nearly all (96–100%) As are complexed with the thiol group (Pickering et al. 2000 and Dhankher et al. 2002). However, using high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS)/molecule-specific electrospray-ionization mass spectrometry (ES-MS), it was revealed that <60% of the As complexed with the thiol group in roots and leaves of sunflower (Raab et al. 2005). In *Thunbergia alata*, the HPLC-ICP-MS/ES-MS method revealed 55–64% of As present as As (III) complexed with thiol compounds, whereas XAS showed 53% of As (III) complexed with thiols.

In vitro, As (III) forms $(GS)_3$-As (III) complex with GSH which remains stable over pH range 1.5–7.5, but gets dissociated at higher pH (Delnomdedieu et al. 1994). As (V) forms $(GS)_3$-As (V) complex only when molar ratio of As:GSH exceeds 1:2. MMA and DMA form CH_3∗As(SG)z and (CH~)Z∗A~.SG complex, respectively. In vivo, As (V) in DMA has been shown to form dimethylarsinothioyl glutathione complex in *Brassica oleracea.* Undissociated complexes of As (III) and PCs have been purified from plant (Sneller et al. 1999; Schmöger et al. 2000). Both PCs-As (III) and GSH-As (III) complexes are transported and sequestered into vacuole. The PCs-As (III) complex is stable in acidic environment; thus, its sequestration into vacuole is important for complete detoxification of As (Guo et al. 2012). In *Arabidopsis*, the PC-deficient mutant was considerably more sensitive to As (V) compared to wild type. When *Arabidopsis thaliana* wild type and its mutants (glutathione-deficient Cad 2–1 and PC-deficient Cad 1–3) were treated with different concentrations of As (V), mutants were found to be about 20 times more sensitive to As (V) compared to wild type, suggesting that the GSH and PCs are involved in As (V) detoxification (Aborode et al. 2016). Overexpression of genes involved in synthesis of PCs and GSH led to enhanced As (V)/As (III) tolerance in plants. In *A. thaliana* leaves, overexpression of two genes *ArsC* (gene involved in As (V) reduction) and γ-glutamylcysteine synthetase (γ-ECS) (gene involved in GSH synthesis) enhanced As (V) tolerance considerably (Dhankher et al. 2002). Also, As concentration in shoot was enhanced by two- to threefold. Constitutive overexpression of *E. coli ArsC* gene was found in both root and shoot tissues, whereas overexpression of *E. coli γ-ECS* was observed only in leaves. *AtPCS1* overexpression in *A. thaliana* led to enhanced production of thiol peptides, including many unidentified compounds in roots upon As (V) exposure compared to wild type (Li et al. 2004). However, overexpression of *AtPCS1 did not* increase As concentration in shoots as As (III) thiol complex transport from roots to shoots is unfavorable. Similarly, overexpression of *AtPCS1* gene in *B. juncea* caused a moderate increase in tolerance to As (V) but not As accumulation in shoots (Gasic and Korban 2007). Overexpression of *AtPCS1* in the *A. thaliana* cytoplasm led to As tolerance, whereas targeting of *AtPCS1* to chloroplasts causes significant As (V) sensitivity (Picault et al. 2006).

Enhanced formation of nonprotein thiols plays a key role in detoxification of MA (V) in plants. Both MA (III) and MA (V) form complexes with PCs (Mishra et al. 2013; Raab et al. 2007). Up to 14 different species of As complexes such as As (III)-(PC2)2, As (III)-PC3, GS-As (III)-PC2, and MMA-PC2 have been identified in As (V)- or As (III)-treated sunflower plants using HPLC-ICP-MS and ES-MS. In sunflower, GS-As (III)-PC2 complex was found to be the most abundant complex initially but later As (III)-PC3 was the main As-PC complex (Raab et al. 2005). In the As (V)-tolerant *H. lanatus*, As (III)-PC3 was the dominant complex (Raab et al. 2004). In *Rauwolfia serpentina* cell culture, *Silene vulgaris* and tolerant populations of *H. lanatus* PC-SH to As molar ratio were found to be ≥3:1 (Schmöger et al. 2000; Sneller et al. 1999; Schat et al. 2002; Hartley-Whitaker et al. 2002). Arsenic and MA form various types of complexes with cysteine, desGly-phytochelatin, hydroxymethyl-phytochelatin, and hydroxymethyl-GSH. Rice plants treated with As (V) showed that up to 48% of As in roots were complexed with thiol forming up to 20 different As species, whereas up to 83% of root As in MA (V)-treated plants formed complexes with various thiols leading to formation of up to 16 As different species. In spite of high complex formation in roots, more As was translocated to shoot in MA (V)-treated plants compared to As (V)-treated plants and As transfer factor was in order DMA(V) > MA(V) > As (V). Around 78% of MA (III) and 71% of As (III) in shoot are known to be weakly bound and thus considered more cytotoxic compared to As (III) (Mishra et al. 2017). In general, plants with higher ability to form As-thiolate showed lower ability for As to translocate (Huang et al. 2008). In plants, all synthesized PCs do not complex with As, and reduction of As (V) to As (III) may be considered a controlling step in thiol complex formation.

From the cytoplasm, As (III)-thiol complexes are transported and sequestered in the vacuoles, where the acidic pH (~5.5) is favorable to the stability of the complexes. The *Arabidopsis* ATP-binding cassette protein ABCC1 confers As resistance by sequestering As-PC complexes in the vacuole. Bleeker et al. (2006) observed efficient transport of As (III)-$(GS)_3$ into the tonoplast membrane vesicles prepared from *H. lanatus* roots. ABC transporter was suggested to be involved in this process as the transport was MgATP-dependent and charge-independent (Bleeker et al. 2006). Transport rate was similar in As (V)-tolerant and non-tolerant *H. lanatus* plants. Free As (III) was taken up at about one-fifth of the rate of that of As (III)-$(GS)_3$ into the vesicles. Zhang et al. (2017) provided the first evidence that AtABCC1 activity is mediated by phosphorylation. Phosphomimetic mutant study demonstrated that phosphorylation of Ser846 is essential for the As transporting function of AtABCC1. *PvACR3*, a well-characterized *ACR3* As (III) efflux protein located in *P. vittata* tonoplast, is involved in sequestration of As into vacuoles. *P. vittata* with nonfunctional *PvACR3* exhibited an As (III)-sensitive phenotype (Indriolo et al. 2010). Duplication of *ACR3* in *P. vittata* and the loss of *ACR3* in angiosperms have been suggested to be responsible for the difference between the As tolerance of *P. vittata* and angiosperm (Indriolo et al. 2010).

10 As Phytoremediation

Phytoremediation is an emerging technique to remediate the contaminated soil and water by using plants without harming the environment (Jiang et al. 2015). Phytoremediation of toxic metals uses plants with high metal tolerance and hyperaccumulating properties (Ghori et al. 2016; Mosa et al. 2016). Researchers have explored a number of plant mechanisms and optimized the factors for enhanced uptake of heavy metals (Tangahu et al. 2011). Plants act as “excluders” and “accumulators” for pollutants. Some plants have the properties to restrict the uptake of heavy metals, and they are known as excluders (Rudin et al. 2017). On the other hand, some plants have the abilities to accumulate heavy metals and biodegrade and biotransform them to reduce their toxicity. Some plants are able to attain high plant biomass to soil metal concentration called as bioaccumulation factor (BF) and a shoot-to-root metal-concentration ratio called as translocation factor (TF). Plants with TF greater than 1 are defined as hyperaccumulators (Tangahu et al. 2011 and Ma et al. 2001). Also, hyperaccumulators should have total accumulation

>1000 mg kg^{-1} As in plant biomass grown on soil containing 100 mg kg^{-1} of As (Gonzaga et al. 2006; Meharg et al. 2002). The first reported As hyperaccumulator was *P. vittata* (Ma et al. 2001) followed by *Pityrogramma calomelanos* (Francesconi et al. 2002). Other related fern species which are studied as hyperaccumulators are *P. longifolia*, *P. umbrosa*, *P. argyraea*, *P. cretica* (Zhao et al. 2002), *P. biaurita*., *P. ryiunkensis* (Srivastava et al. 2005). Several studies on the mechanism of As uptake, accumulation, and tolerance by *P. vittata* have revealed valuable aspects to improve phytoremediation techniques (Danh et al. 2014). The effectiveness of various phytoremediation methods used by various researchers is explained in Table 2.

Table 2 Effectiveness of phytoremediation for arsenic treatment in plant species

Plant	Technique	References
Pteris vittata	Phytoaccumulation/phytoextraction	Ye et al. (2011)
Brassica juncea		Pickering et al. (2000)
Polypodium calomelanos and *Pteris vittata*		Niazi et al. (2012)
Ceratophyllum demersum		Weis and Weis (2004)
Hydrilla verticillata		Nigam et al. (2013)
Urtica dioica		Viktorova et al. (2016)
Eichhornia crassipes		Jasrotia et al. (2017)
Bambusa vulgaris		Srivastava and Dwivedi (2016)
Aloe vera		Tripathi et al. (2016)
Arundo donax	Phytovolatilization, phytoextraction	Mirza et al. (2011)
Helianthus annuus	Phytoextraction and rhizofiltration	McCutcheon and Schnoor (2003)
Pteris vittata	Phytoextraction	Zhao et al. (2002)
Pteris cretica		
Pteris longifolia		
Pteris umbrosa		
Pteris vittata		Srivastava et al. (2005)
Pteris ensiformis		
Nephrolepis exaltata		
Pistia stratiotes	Rhizofiltration	Roy et al. (2015)
Hydrilla verticillata		
Nasturtium officinale		
Nasturtium microphyllum		
Lemna gibba		
Lemna minor		
Spirodela polyrhiza		
Azolla caroliniana		
Helianthus annuus	Phytoextraction and rhizofiltration	McCutcheon and Schnoor (2003)

11 Various Phytoremediation Strategies Used for As

11.1 Rhizofiltration

This method involves removal of contaminants from flowing water by plant roots. Arsenic is precipitated or adsorbed around the roots, and after saturation with adsorption, roots are harvested. Wetlands with facultative microbial system are maintained, and wastewater containing contaminants is passed through it. Root system and wetlands will facilitate and precipitate toxic elements. Plant roots secrete compounds such as amino acids, sugars, organic acids, vitamins, and secondary metabolites near rhizosphere. Roots regulate the soil microbial flora by exudation of various compounds. Hydroponically grown moonlight and brake fern have shown successful rhizofiltration (Baldwin and Butcher 2007) due to their ability to produce root exudates that precipitate As by changing rhizosphere pH (Roy et al. 2015). A number of aquatic plants have been reported for their rhizofiltration property, but among them duckweeds, water hyacinth, water spinach, water ferns, water cabbage, hydrilla, and watercress (Roy et al. 2015) are suggested as ideal plants for removal of As. However, due to their small and slow-growing roots, terrestrial plants are presently under research for their rhizofiltration property as they have long and strong roots to cover larger As-contaminated area (Rofkar and Dwyer 2013).

11.2 Phytostabilization

Phytostabilization refers to the formation of a plant cover on contaminated area, thereby minimizing contaminants to transport in water and air. Phytostabilization contains the contaminants within the vadose zone of lithosphere by accumulation or precipitation of metals by root surfaces. Phytostabilization can be enhanced by utilization of soil amendments, which are effective in immobilization of contaminants and plant species tolerating increased levels of contaminants (Bolan et al. 2011). *Eucalyptus cladocalyx* in plants showed tolerance to increased levels of As and accumulated less As in aboveground tissues and potential plant species for phytostabilization of As-contaminated area (King et al. 2008). In pot experiments with different arsenic treatments, a mangrove (*Aegiceras corniculatum)* seedling showed normal growth and development without showing As toxicity, which demonstrate that *A. corniculatum* is an As excluder species and tolerates As stress. Arsenic was mainly accumulated in roots with relatively high bioconcentration factor (BCF) in roots and low BCF in stem and leaf suggesting *A. corniculatum* as a potential mangrove species for As phytostabilization (Wu et al. 2015). The *Atriplex* genus is distributed worldwide, and many plants of this family are halophytes and xerophytes that grow in deserts and arid regions. Fernández et al. (2016) evaluated the accumulation of As and the growth parameters of *Atriplex atacamensis*, *A. halimus*, and *A. nummularia* established in an arid area and shown to have a phytostabilization capacity of As.

Phytostabilization studies of tree species *Conocarpus erectus* and *Populus deltoides* revealed that these tree species are nonhyperaccumulators of As, but due to their large biomass production and relatively high quantity of extracted metal per plant, large quantities of As could be extracted, during their long-term life, and can be used for phytostabilization of As-contaminated sites (Hussain et al. 2017). *Prosopis juliflora*, a halophytic tree, has the property for long-term phytostabilization of arsenic in pyritic mine tailings in semiarid climate (Hammond et al. 2018).

11.3 Phytoaccumulation/Phytoextration

It is a common property of hyperaccumulator plants that involves accumulation of contaminants in aboveground portion of plants through roots. After plantation of these plants, they are allowed to grow for some time, and after that they are harvested. Further, they are either reduced to ashes or composed to revive metals. Zhao et al. (2002) in their study reported accumulation up to 6200–7600 mg/kg in the 500 mg/kg As treatment within *P. vittata, P. cretica, P. longifolia,* and *P. umbrosa* through phytoextraction of arsenic. *Arundo donax* has also shown its ability to treat contaminated waters containing As concentrations up to 600 mg/L using phytoextration technique (Mirza et al. 2011). Srivastava et al. (2005) reported that concentration of As in *P. biaurita*, *P. quadriaurita*, and *P. ryukyuensis* (using phytoextraction technique) ranged from 1770 to 3650 mg/kg in fronds and 182–507 mg/kg in the roots when treated with 100 mg/kg As in soil. Recently, Ye et al. (2011) have observed the role of *P. vittata* to reduce arsenic concentration in rice from arsenic-contaminated environment. The authors also observed reduced dimethyl arsenic acid (DMA) content in treated sample. *Brassica* is also reported as a good metal hyperaccumulator by Pickering et al. (2000). Niazi et al. (2012) studied that two hyperaccumulator ferns (*P. calomelanos* and *P. vittata*) were able to reduce As level in soil up to 20 mg/kg within 6–15 years. Generally, wetland plants are not included as hyperaccumulators, but *Ceratophyllum demersum* has shown 20,000-fold accumulation of As (Weis and Weis 2004). *Hydrilla verticillata* an aquatic plant has also reported 96.4% arsenic adsorption at 100 ppb with 0.005 g of biomass per ml at pH 6 for 5 hours (Nigam et al. 2013). Recently, a group of scientists in China has evolved new in situ phytoremediation technique by using chelating agents, ethylenediamine-N-N′-disuccinic acid (EDDDS) and citric acid (CA), to support the hyperaccumulator for enhanced remediation. It was reported that plant biomass increased by 3.5-fold as compared to control with significant increase in As extraction. Addition of chelating agents, EDDDS and CA, helped to enhance the solubility of heavy metals in aboveground plant parts and translocation from roots to shoots. Further in their study, they also revealed that EDDDS reduced the risk of leaching of heavy metals to groundwater (Francis et al. 2017). PvACR3 is a key antiporter of As (III) in hyperaccumulator fern *P. vittata*. Chen et al. (2013) overexpressed PvACR3 in *Arabidopsis* with CaMV 35S promoter and reported increased tolerance of As in *Arabidopsis*. After As(III) treatment, transgenic seeds also reported 7.5-fold higher accumulation of As than control seeds. PvACR3 increased the As (III) efflux in *Arabidopsis* to external environment, and transgenic seeds were able to survive in highly concentrated As treatments. Thus, their study revealed the behavior of PvACR3 and their role in As accumulation. Recently, *Urtica dioica* has been studied to remediate the heavy metal-contaminated soil. Viktorova et al. (2016) reported the role of *Urtica dioica* for the treatment of heavy metal (HM) or polychlorinated biphenyl (PCB)-contaminated soil with endophytic bacteria *Bacillus shackletonii* and *Streptomyces badius*. In their study, they revealed no accumulation of As by *Urtica dioic*a from As-contaminated (10 mg/kg) soil. Boshoff et al. (2014) reported 11 mg/kg As accumulation in *Urtica dioic*a as their soil was 14 times more contaminated than soil used by Viktorova et al. (2016). Effectiveness of water hyacinth (*Eichhornia crassipes)* and two algae (*Chlorodesmis* sp. and *Cladophora* sp.) has been investigated to determine their As tolerance and As remediation capacity (Jasrotia et al. 2017). The study found algae species more tolerant to As (III and V) than water hyacinth. *Cladophora* sp. has shown survival under extreme As stress condition (6 mg/ml) and remediates the As-contaminated soil from 6 mg/L to less than 0.1 mg/L within 10 days. On the other hand, water hyacinth has shown survival not more than 2 mg/L As stress condition with only 20% remediation of As-contaminated soil. Jasrotia et al. (2017) reported that *Cladophora* sp. is appropriate to treat As-contaminated wastewater that can be further used for irrigation. Brima and Haris (2014) studied the efficiency of water hyacinth (WH), banana pseudostem (BN), sugarcane bagasse (SC), and jute for As removal. The study revealed proficiency of WH > BN > SC > jute in increasing order for As treatment. Further they also prepared a prototype filter from WH (20 g) for the treatment for As from 250 and 1000 μg/l As-contaminated drinking water that was reported 80% and 84%, respectively. Srivastava and Dwivedi (2016) reported the role of *Bambusa vulgaris* (bamboo) leaf biomass for the treatment of As-contaminated water sample. They showed 55% reduction of As concentration by using 8 g of bamboo leaf biomass for 4 hours. Pearson correlation analysis showed positive significance for the use of *Bambusa vulgaris* for remediation of As. Tripathi et al. (2016) studied that 30 g leaves of *Aloe vera* can completely eliminate As from 1 liter As-contaminated water (up to 43 ppb). A greenhouse pot experiment was performed by Lampis et al. (2015) to assess the proficiency of As phytoextraction by the fern *P. vittata* that have grown in As-contaminated soil, in the presence and absence of selected rhizobacteria isolated from the contaminated site. They used four bacterial strains of two varieties (i) siderophore-producing and arsenate-reducing (*Pseudomonas* sp. P1III2 and *Delftia* sp. P2III5 (A)) and (ii) siderophore and indoleacetic acid-producing (MPV12, *Variovorax* sp. P4III4, and *Pseudoxanthomonas* sp. P4V6 (B)). These bacterial strains have the ability to reduce As(V) to As(III) and help in plant growth. Further, they have reported the role of bacterial strain that have increased plant biomass up to 45% and increased As removal efficiency by *P. vittata* from 13% to 35%. Although a lot of new strategies have been introduced along with the time to remediate As from polluted area, still more research is needed for the management of As-polluted sites.

11.4 Phytovolatilization

Plants interact with various organic and inorganic compounds, which affect the nature and transportation of contaminants. Volatile compounds gets volatilized from aerial parts of plants, which are called as direct phytovolatilization, or from soil mainly due to plant root activities called as indirect phytovolatilization (Limmer and Burken 2016). This process involves uptake of As by plant along with water and nutrients. After passing through root to leaves, transpiration process takes place causing its evaporation into the environment. Mirza et al. (2011) studied phytovolatilization as the principal mechanism of As removal by bioenergy crop *Arundo donax* and reported 7.2–22% As removal in 300–1000 μg L^{-1} As concentration. Sakakibara et al. (2010) also reported removal of 90% of As by *P. vittata* from As-contaminated site. It was reported that intermediate methylated As compounds (mono- and dimethylarsenious acid) are more harmful than inorganic As species (Petrick et al. 2000) and 100 times more genotoxic than their nonvolatile form (Andrewes et al. 2003). Meng et al. (2011) expressed *arsM* gene in Japonica rice (*Oryza sativa* L.) from the soil bacterium *Rhodopseudomonas palustris* and observed increased methylation and volatilization in transgenic rice after 12-day exposure to As (III). Jia et al. (2012) showed that the rice plant reduces trimethylarsine oxide (TMAs(V)O) to volatile trimethylarsine (TMAs), which can further be volatilized from rice shoots. When rice plants were treated with TMAs(V)O, volatilization of TMAs from rice plants was observed, but plants treated with As(V), DMAs(V), and MMAs(V) showed no volatilization. Arsenic volatilization from plants will decrease As accumulation in rice.

12 Conclusion and Future Perspective

Arsenic as metal contaminant is an emerging issue. FDA has reported arsenic as group I carcinogenic agent as it is entering into the food chain. Consumption of As-contaminated food leads to severe diseases like cancer. Arsenic is predominantly present in two inorganic forms: As (V) as phosphate analogue and As (III) as silicon and boron analogue. Arsenate enters through the food chain in human and disrupts the Krebs cycle and ATP production. For treatment of As pollutant, several techniques like microbial, chemical, physical, and phytoremediation are available. Chemical and physical techniques are very costly and also have some drawback like partial removal, high-energy requirements, and generation of toxic sludge. Introduction of *P. vittata* (fern) as hyperaccumulator has paved new ways to remediate the contaminants, i.e., economic and environment friendly. *P. vittata* has shown a higher rate of uptake of As through the aquaporins as a transporter. Several studies performing knockdown and overexpression of genes encoding aquaporin families (NIP, PIP) have revealed their role in As transportation through the root. Further studies of the As metabolism of plants revealed how these plants are able to survive in the high As-concentrated area. To survive in highly contaminated area, plants involve reduction of arsenate, chelation, sequestration and volatilization. But the mechanism of volatilization is not yet clear (Fig. 2). Although phytoremediation is widely accepted over other physical and chemical methods, some improvement is required.

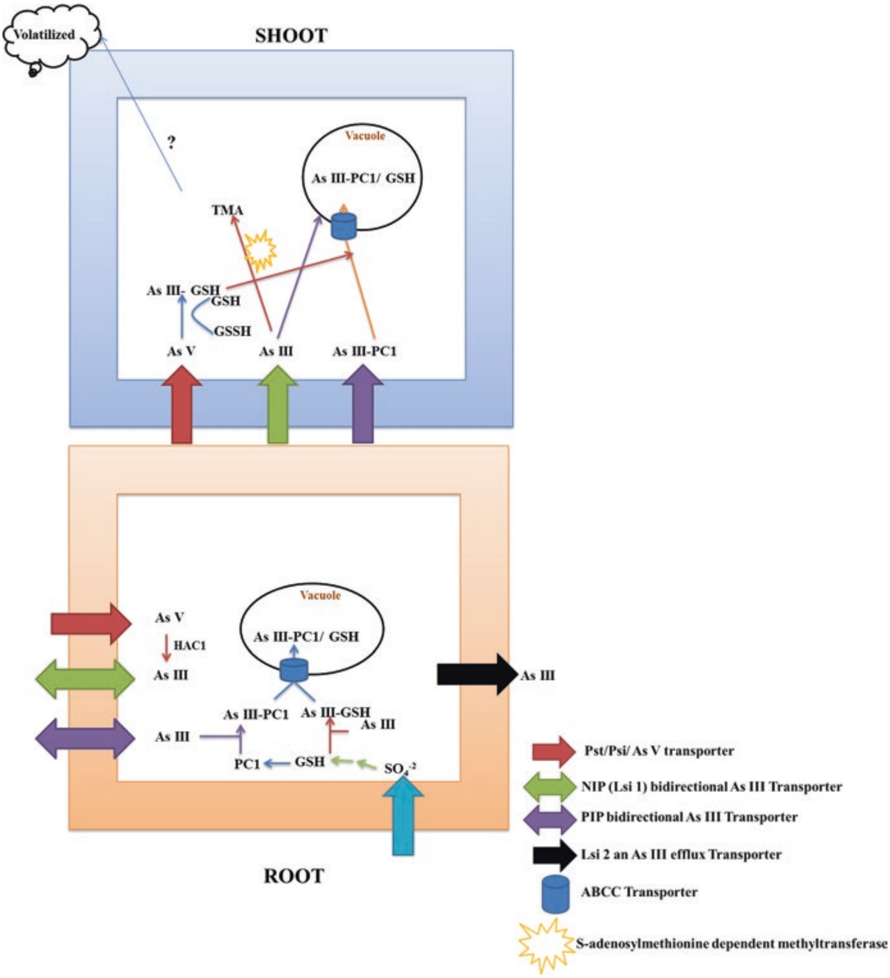

Fig. 2 Accumulation and detoxification by hyperaccumulator. In this figure, mechanism of arsenic accumulation and detoxification has been explained. Usually arsenic is present in inorganic form (mainly As (V) and As (III)) in nature. Uptake of arsenic occurs in the form of arsenate through As (V) or phosphate transporters, and As (III) transport occurs through NIP (silicon transporter) and PIP transporters. Arsenate reductase enzyme or high arsenate content 1(HAC 1) converts As (V) to As (III) which does not mimic phosphate. Further, As (III) forms compound with phytochelatin (PC 1) or glutathione and enters in vacuole through ABCC1 tonoplast arsenic transporter. Through roots arsenic enters into shoots where sequestration process occurs in the same manner as in roots except the step of volatilization. Arsenite is converted to trimethylarsine (TMA) in the presence of S-adenosylmethionine-dependent methyltransferase that acts as a catalyst. TMA further volatilizes into atmosphere with an unknown mechanism. Thus, hyperaccumulators can detoxify and accumulate high concentrations of arsenic leading to reduced contamination of soil and water

Although considerable progress has been made in understanding As transport and metabolism in plants, knowledge gaps exist which needs to be filled through further research. Direct determination of novel forms of complexed and uncomplexed As present in different plant parts in the presence and absence of different forms of As in growth media will be helpful in giving further insights into As transport and metabolism in plants. Several genes and pathways involved in As transport and metabolism have been identified. However, there is a possibility that other/alternate enzymes or pathways are involved. Further research should be focused on identification of novel genes and pathways involved in As transport and metabolism in different plant species. A deeper understanding of the differences in As transport and metabolism between tolerant and sensitive varieties will reveal potential genes that could be useful in developing plants with increased tolerance and hyperaccumulating ability and safer crops with reduced As levels.

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Plant-Chromium Interactions: From Toxicity to Remediation

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Abbreviation

Cr	chromium
Kg	kilogram
mg	milligram
ppb	parts per billion
ppm	parts per million

1 Introduction

In the last 120 years, the human race has witnessed the most dynamic period of industrial and technological developments. Extensive use of natural resources, disposal of wastes into water bodies and intensive use of heavy metals, fossil fuels, xenobiotics, petroleum products, dyes, hydrocarbons, poly-aromatic hydrocarbons, pesticides and various other pollutants have resulted in serious environmental problems (Mohamed et al. 2016; Rodgers-Vieira et al. 2015; Wasilkowski et al. 2014; Wojcieszyńska et al. 2014). Out of these pollutants, heavy metal contamination has posed a major threat, as these heavy metals and metalloids persist in the ecosystem due to their non-degrading nature, slowly intoxicating the entire ecosystem. In recent years, the contamination level of heavy metals in the environment has increased beyond recommended levels, posing detrimental effects on almost all kinds of life forms (Tak et al. 2013). Heavy metal contamination [such as chromium (Cr), arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg)] poses a threat not just to those who are in direct contact with contaminated sites, but their capability to get into the food chain is a major cause of concern. Out of all the heavy metals, Cr is being utilised in a wide range of industrial processes right from stainless steel

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manufacturing to electroplating and from leather tanning to wood preservation. Identified by the US Environmental Protection Agency (USEPA) as one of the 17 chemicals posing threat to humankind, hexavalent Cr [Cr(VI)] is highly toxic, reactive, mobile and hazardous to both flora and fauna. Hence, several research groups from all around the world are involved in the development of technologies to remove the Cr(VI) from contaminated sites or convert Cr(VI) to the less mobile and less toxic form, i.e. Cr(III).

This chapter links chemistry and speciation of Cr, its bioavailability and uptake by plants, its toxicity and detoxification mechanism and finally how the various mechanisms used by plants to survive Cr stress can be used for phytoremediation.

2 Occurrence and Chemistry of Chromium

Chromium (Cr) is the 17th most abundant element in the Earth's mantle. It is a hard, steel-grey-coloured metal occurring naturally as chromite ($FeCr_2O_4$). Cr is a transition metal and is the first element of the group VI of the periodic table. Cr can be found in the air, water, soil and almost all the phases of the environment, and depending on the paternal source, its abundance in soil may range from 10 to 50 mg kg^{-1}. It has the capability to exist in various oxidation states, ranging from C(II) to Cr(VI); however, among these, Cr(0), Cr(III) and Cr(VI) are the most common states due to their high stability (Dhal et al. 2013; Jobby et al. 2018).

One of the most remarkable facts about Cr is its simultaneous and spontaneous interconversion between Cr(III) and Cr(VI) oxidation states. In this redox process, the manganese oxide (MnO2) present in the soil and sediments spontaneously oxidises Cr(III) into Cr(VI)[a], while various reduced carbon compounds present in the soil donate electrons to Cr(VI) reducing it to Cr(III)[b].–

$$Cr3^{+} + 1.5MnO_2 + H_2O \rightarrow HCrO_4^{+} + 1.5Mn_2^{+} + H^{+} \qquad (a)$$

$$C_6H_6O_2 + CrO_2^{-4} + 2H_2O \rightarrow 0.5Cr_2O_3 + 1.5C_6H_4O_2 + 2.5H_2O + 2OH^{-} \qquad (b)$$

The physical and chemical characteristics of Cr depend on its present oxidation state. Cr(0) is the solid, metallic form with high fusion point. Cr(III) and Cr(VI) exist mostly in ionic state. Cr(VI) is the most toxic form of Cr due to its high oxidation potential, solubility and mobility; and it exists as chromate (CrO_4^{2-}), dichromate ($Cr_2O_7^{2-}$) and CrO_3. On the other hand, Cr(III) is less mobile, insoluble in water, mainly bound to organic matter in soil existing in the form of oxides, sulphates or hydroxides and hence is almost non-toxic. However, depending on factors such as pH, concentration of MnO_2, etc., the oxidation states of Cr may vary. The next section describes the pH-dependent speciation of Cr (Dhal et al. 2013; Jobby et al. 2018). The natural Cr cycle in the environment is depicted in Fig. 1.

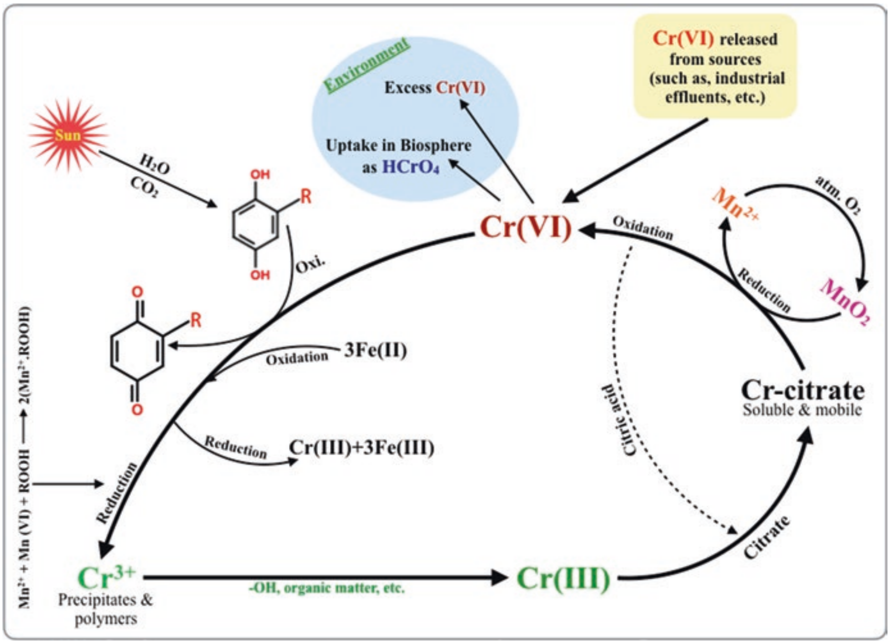

Fig. 1 The chromium cycle. (Modified from Jobby et al. (2018))

3 Chromium Speciation

Under oxidising conditions, Cr(VI) is the predominant state, while Cr(III) dominates when conditions are reducing. In aqueous solution, depending on various factors such as pH, the presence and concentration of oxidising and reducing compounds, the kinetics of redox reactions and total redox potential, Cr(VI) can exist in several forms, namely, $Cr_2O_7^{2-}$, CrO_4^{2-}, H_2CrO_4 and $HCrO_4^-$. Independent of its concentration, Cr(VI) exists only as CrO_4^{2-} ions at pH above 7, whereas in the pH range of 1–6, $HCrO_4^-$ is the predominant species.

$$H_2CrO_4 \rightarrow H^+ + HCrO_{4^-} \qquad \left(pH \sim 1 \quad 6\right) \tag{c}$$

$$HCrO_{4^-} \rightarrow H^+ + CrO_{4^-} \qquad \left(pH > 7\right) \tag{d}$$

$$2HCrO_{4^-} \rightarrow Cr_2O_7^{2^-} + H_2O \tag{e}$$

4 Sources of Chromium in the Environment

Cr might enter into the environment via various natural and anthropogenic sources. The global average emission of Cr from natural sources was estimated to be more than 43,000 tons/year (Nriagu 1988).

The corrosion-resistant quality and hardness is the major reason for the tremendous industrial application of Cr in tanning, electroplating, metallurgical, wood preservation, stainless steel manufacturing, paint production and pulp and paper industries (Jobby et al. 2018). Cr-rich effluents from these industries are the major source of chromium contamination in the environment. As per the Environmental Protection Agency (EPA), the maximum contamination level (MCL) for Cr (VI) in drinking water is 100 μg L^{-1} (ppb)(Atsdr.cdc.gov. 2019). But in many countries, the total Cr concentration in drinking water is way above the permissible limit due to its untreated release from industries (Shiller and Boyle 1987; Xingzhen and Xiuxia 1987). Most of the industrial wastes are dumped to reclaim marshlands and for backfills at sites. The Cr from these contaminated sites leaches and seeps into the underground water reserves contaminating them in the process and posing a considerable health hazard (Barrera-Díaz et al. 2012). In 1987, underground water in various regions of the USA was reported to contain Cr up to 50 mg L^{-1}(US Environmental Protection Agency 1987).

The natural composition of rocks and sediments plays a major role in describing the concentrations of Cr in soil. In natural soil, Cr concentration ranges from 5 to 1000 mg kg^{-1} depending on the soil type; however, it can go up to 125 g kg^{-1} in serpentine soil (ATSDR (Agency for Toxic Substances and Disease Registry) 1998). Anthropogenic depositions, such as dumping of Cr-contaminated solid and/or liquid wastes, effluents, etc., result in increased levels of Cr in the soil (Kimbrough et al. 1999). Similar to that in the aquatic environment, Cr in soil undergoes sorption, precipitation, dissolution and oxidation-reduction and can exist in both forms of Cr(III) and Cr(VI). MnO_2 is the major oxidising agent present in soil that oxidises Cr(III) to Cr(VI); however, oxidation by dissolved O_2 is negligible when compared to that by MnO_2 (Fendorf and Zasoski 1992). The reduction of Cr(VI) to Cr(III) can be brought about by iron, sulphides, vanadium and organic materials (Cary 1982). However, if the soil loses its reducing capacity over time, Cr(VI) becomes persistent for years.

5 Interaction of Chromium with Plant

5.1 Uptake

The uptake of Cr by plants is the first step towards plant-chromium interaction. The pathway involved in this process is not very clear; however, Cr is a non-essential element; hence, plants do not possess any specific mechanism for its uptake.

Cr uptake is also speciation dependent. It has been speculated that the uptake of Cr(III) by the plant is an energy-independent, passive process (Zayed and Terry 2003; Skeffington et al. 1976). Cr(VI) is taken up through carriers that are meant for the uptake of essential element for plant metabolism, such as sulphates (Kim et al. 2006; Cervantes et al. 2001a). Cr is also known to compete with Fe, S and P for carrier binding sites (Shanker et al. 2005). Factors such as pH of soil, concentration of Cr, its oxidative state, salinity of soil and dissolved salts affect the Cr uptake by the plant (Babula et al. 2008). Out of the two stable oxidation states, Cr(VI) is more toxic at lower concentrations, owing to its higher solubility and hence bioavailability. Moreover, studies have also demonstrated that Cr(VI) forms stable complex with organic acids and mycorrhizal fungi present in the soil and aid in Cr uptake by the plants (Davies et al. 2001). Even to date, authors and researchers are unclear if Cr(VI) is reduced to Cr(III) at the plant's root surface or it is taken up without reduction. Over the years, certain studies have shown that maximum accumulation of Cr occurs in plants of the family Brassicaceae (also known as sulphur-loving plants) suggesting that sulphate carriers of the sulphur uptake mechanism are involved in Cr uptake and translocation (Barceló and Poschenrieder 1997). Since S and Cr compete for the same binding site, the presence of S in the soil reduces the extent of uptake of Cr by the plant; in contrast, the presence of Ca enhances the uptake of Cr by the plant (Shewry and Peterson 1974; Skeffington et al. 1976).

5.2 Accumulation and Translocation

In various studies, it has been found that Cr accumulation mostly occurs in roots and shoots of the plant (Paiva et al. 2009). However, it is seen that the major part of absorbed Cr accumulates in the root (~98%), while a small portion is transported to the shoots, and only about 0.1% of it accumulates in the seeds (Paiva et al. 2009; Sundaramoorthy et al. 2010; Huffman Jr and Allaway 1973). This higher root accumulation could probably be due to immobilisation of Cr in vacuoles of the root cells, thus making it less toxic and may act as a natural response by the plant towards heavy metal toxicity (Shanker et al. 2004). Cr(VI) and Cr(III) both can cross the endodermis via symplast, but Cr(VI) might readily reduce to Cr(III), which is then retained in the root cortex cells, explaining the lower toxicity of Cr(III) in comparison to Cr(VI). However, such reducing enzymes have not been found in higher vascular plants, while they have been widely reported in various bacteria and fungi (Cervantes et al. 2001b). Inside the vacuoles, Cr(III) can remain soluble by formation of stable complexes such as Cr-EDTA; it can then be translocated to the aerial parts of the plant (Barceló and Poschenrieder 1997). Such ability of Cr to form complexes with chelators and organic ligands increases its uptake by the plants, which then moves in the xylem of the plants.

5.3 Growth and Development

The accumulation or concentration of Cr in plants affects the overall growth and development of plants. Cr can enhance the growth of certain species at lower concentrations, while it is highly toxic at higher concentrations, especially Cr(VI), which can inhibit various enzymatic activities in plant metabolism severely damaging the plant. Inhibition of seed germination, seedling growth, root growth, induction of leaf chlorosis and necrosis, along with wilting, are some of the symptoms of Cr toxicity in plants (Chandra and Kulshreshtha 2004). Studies have reported the toxic effects of Cr in terrestrial plants, such as pulses, cereals, vegetables (Sanita di Toppi et al. 2004; Haas and Brusca 1961; Shanker and Pathmanabhan 2004; Jun et al. 2009), as well as in some aquatic plants (Chandra and Kulshreshtha 2004).

5.4 Effects on Seed Germination

Being the first physiological process to be affected by Cr toxicity, the ability of a plant's seed to germinate in a Cr-contaminated environment is an indication of its capacity to tolerate this metal (Peralta et al. 2001). Depending on the plant species as well as the source of Cr, a variety of response towards Cr contamination and toxicity is exhibited by the plants (Lopez-Luna et al. 2009). A reduction of 75% was observed in germination of seeds of the weed *Echinochloa colona* treated with 200 μM Cr (Rout et al. 2000). Studies have reported reduction of seed germination in *Triticum aestivum* (Datta et al. 2011), *Brassica oleracea* var. *acephala* (Ozdener et al. 2011), *Daucus carota*, *Lycopersicon esculentum* (Sundaramoorthy et al. 2010) and many more.

The reduction in germination of seeds under Cr stress has been correlated to the decrease in activity (or inhibition) of α-amylase and β-amylase (carbohydrates hydrolysing enzymes), which subsequently reduces the supply of sugar to the embryo axes (Zeid 2001). On other hand, Cr treatment increases the protease activity in seeds, contributing to the reduction in germination (Zeid 2001). Studies have also reported interference of Cr in structure and function of male gametophyte; inhibition in pollen germination and pollen tube growth along with induction of alterations in pollen tube shapes have been reported (Speranza et al. 2007, 2009). Speranza et al. (2009) found that arabinogalactan protein distribution and callose deposition in pollen were altered by Cr, and Cr(III) was more toxic in this case than Cr(VI) (Speranza et al. 2009).

5.5 Effects on Root Growth

Being the first organ to come in contact, the root of a plant is affected the most by the presence of Cr. The adverse effects of Cr toxicity on the root growth have been demonstrated in a number of studies. Under Cr(III) stress, decrease in root length of

Allium cepa was reported (Liu et al. 1992). A stress of 100 μM of Cr(III) resulted in reduction in overall development of plant and its biomass in *Helianthus annuus* (Davies et al. 2002). Chen et al. (2001) reported decrease in total root weight and root length of wheat under Cr stress (20 mg Cr(VI) kg^{-1}) as $K_2Cr_2O_7$ in soil (Chen et al. 2001).

The decrease in root growth as a result of Cr toxicity could be due to arrest of cell division or inhibition of root cell elongation or both in the root tip (Liu et al. 1992). Reduction in mitotic index in growing root tips under Cr(III) and Cr(VI) stress has been reported (Liu et al. 1992). It was observed that Cr(VI) caused cell cycle extension, leading to inhibition of cell division and thus root growth (Sundaramoorthy et al. 2010). The decrease in root growth can further inhibit shoot growth and development since this can cause a corresponding decreased absorption of water and nutrients by roots and their subsequent transport to aerial parts of the plants. This can be explained by the fact that decreased root surface area under Cr(VI) stress will result in reduced capacity of plant to search for water and nutrients in the soil, leading to water stress and affecting overall development of the plant.

5.6 Effects on Stem Growth

Cr stress has also shown adverse effects on plant height and shoot growth. Back in 1953, *A. sativa* plants under Cr stress were reported to have poor development along with necrotic lesions (Hunter and Vergnano 1953). The height of *Curcumas sativus*, *Lactuca sativa* and *Panicum miliaceum* was also reported to be reduced under Cr(VI) stress (Joseph et al. 1995). Similarly, various other research groups have reported decrease in plant height and total biomass. As discussed in the previous section, this decrease in plant height may be due to decreased root growth, which can lead to a consequent reduction in uptake and transport of water and nutrients by the plant. Moreover, the cellular metabolism of shoots can also be affected as a consequence of Cr transport to aerial parts of the plant, contributing to reduction in plant height.

5.7 Effects on Leaf Growth and Number

Cr toxicity is known to cause reduction in number of leaves and leaf area, along with induction of structural abnormalities, chlorosis and tissue necrosis. A 50% reduction in number of leaves per plant was observed when wheat was subjected to 0.5 mM Cr that was added in nutrient media (Sharma and Sharma 1993). Reduction of total leaf area and biomass was reported in *Albizia lebbeck* at 200 ppm Cr(VI) stress (Tripathi et al. 1999). Cr toxicity causes reduction of total leaf area due to reduction in the number of cells in the leaves or reduction in cell size (Nieman

1965). Some authors have suggested that these leaf growth traits can be used as bioindicators of heavy metal pollution as well as for the selection of resistant species.

5.8 *Effects on Grain Yield*

Adverse effects on grain yield in crop plants have been reported if the soil is contaminated with Cr. In *P. sativum*, reduction in number of flowers and pods per plant was reported (Bishnoi et al. 1993a). Moreover, drastic reduction in number of seeds per pod was also observed (Bishnoi et al. 1993a). A Cr(VI) stress of 1 mM induced severe effects in *T. aestivum* and no grain formation occurred (Sharma et al. 1995). Similarly, various studies have reported reduction in total yield under Cr stress.

6 Physiological Changes Seen Under Chromium Stress

Various physiological processes such as photosynthesis, enzyme-catalysed reactions, mineral nutrients, water relations, etc. are affected by Cr toxicity (Fig. 2 and Fig. 3).

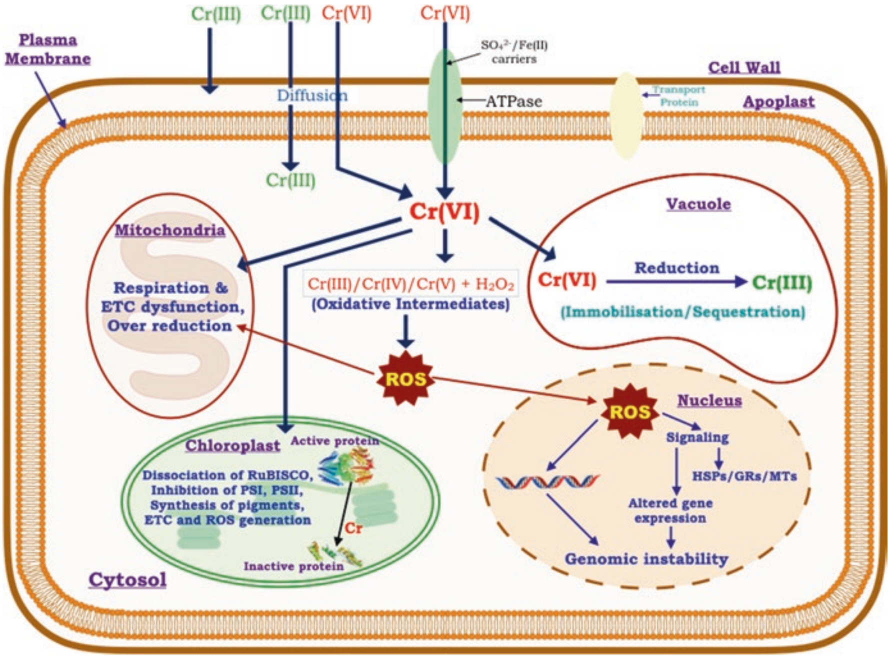

Fig. 2 Effects of Cr at cellular level in plants (a hypothetical overview) (Modified from Singh et al. (2013))

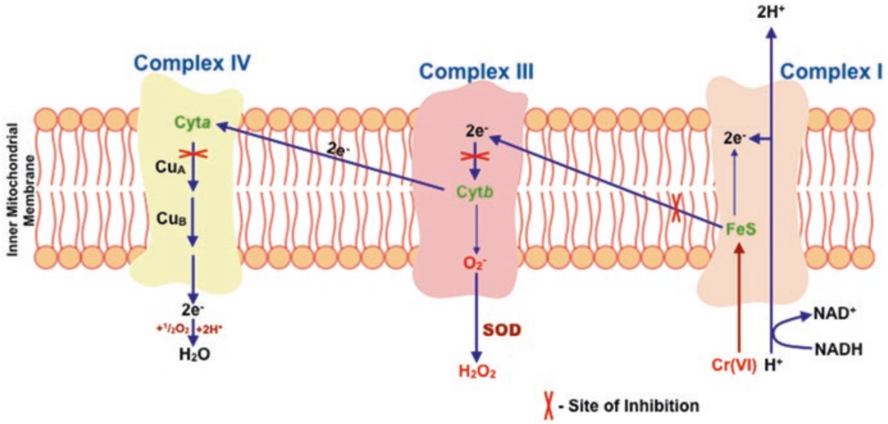

Fig. 3 Inhibition of transport of electrons at various sites of the electron transport chain (ETC) in mitochondria. [SOD, superoxide dismutase; NAD, nicotinamide adenine diphosphate] (Modified from Singh et al. (2013))

6.1 Effects on Photosynthesis

Various heavy metals, including Cr, affect the photosynthesis in plants, mainly in terms of carbon fixation, electron transport across the electron transport chain (ETC) [Fig. 3], photophosphorylation and various enzymatic activities leading to decrease in productivity and in some cases, ultimately, to death (Clijsters and Van Assche 1985). Although various researchers have reported the effects of Cr on photosynthesis in higher plants and trees, the exact mechanism for induction of inhibition of photosynthesis is not well understood. However, it is thought to be due to disorganisation of chloroplast structure, electron transport inhibition or the enzymes involved in the Calvin cycle. Reduction in chloroplast autofluorescence and volume in pea plant after exposure to Cr(VI) was reported (Rodriguez et al. 2012). Changes in ultrastructure of chloroplast as a result of Cr toxicity have also been reported (Panda and Choudhury 2005). A decrease in chlorophyll a and chlorophyll b contents in leaves was observed in mungbean cultivars on exposure to Cr(VI)(Tiwari et al. 2009). The decrease in ratio of chlorophyll a:b is indicative of reduction in size of the peripheral part of the antenna complex as a result of Cr toxicity (Bishnoi et al. 1993b). Moreover, it has been observed that Cr(VI) has a more pronounced effect on photosystem (PS) I than on PS II in peas (Bishnoi et al. 1993b). The Cr-dependent reduction in photosynthetic rate has also been linked to diversion of electrons from the electron-donating side of PS I to Cr(VI); moreover, it is possible that the electron produced might not be used for carbon fixation in Cr-stressed plants. Moreover, Cr(VI) has a tendency to induce abnormalities in the chloroplast ultrastructure such as poor development of lamellar system with increased space in thylakoid and fewer grana. Such abnormalities affect the photosynthesis and transfer of excitation energy (Krupa and Baszynski 1995; Van Assche and Clijsters 1983). Many studies have also shown decrease in chlorophyll content with increase in Cr concentration (Paiva et al. 2009; Zeid 2001), suggesting that chlorophyll synthesis and/or chlorophyllase activity is being affected.

6.2 Effects on Mineral Nutrition

Cr is likely to affect the mineral nutrition of plants owing to its structural similarity to that of certain essential elements. Such interaction of Cr with the uptake and accumulation mechanism of essential elements has gained much attention, and as already discussed, Cr(VI) and Cr(III) exploit different mechanisms of uptake by plants (Zaccheo et al. 1985). Studies have suggested that both the species are likely to interfere with uptake of several ionically similar species (such as Fe and S); Sundaramoorthy et al. (2010) observed a decrease in the uptake of both macro-(N, P, K) and micronutrients when concentration of Cr in growth media increases (Sundaramoorthy et al. 2010). Interference of Cr with uptake of Fe, Mo, P and N has been reported (Adriano 1986). Cr is also known to inhibit the translocation of P, K, Zn, Cu and Fe within the plant parts (Barcelo et al. 1985). Such inhibition is likely to be due to displacement of nutrients from the physiological binding sites by Cr, resulting in decreased uptake and translocation of essential elements.

6.3 Effects on Water Balance

The exact effect of Cr on water relations in higher plants is not clear; however, it was observed that the effect of Cr on water balance is concentration dependent (Barceló et al. 1986); also wilting of various species due to Cr toxicity has been reported (Turner and Rust 1971). In cauliflower, decrease in the water potential and transpiration rates was observed when subjected to Cr, along with increase in diffusion resistance and relative water content in leaves (Barceló et al. 1986). Cr toxicity was found to cause reduction in longitudinal water movement due to decrease in treachery vessel in beans (Vazques et al. 1987). An indirect effect of Cr toxicity on water balance is due to reduction in root surface in Cr-stressed plants, reducing the plant's capability to explore surface for water.

7 Biochemical Changes During Chromium Stress

7.1 Enzymatic Alteration During Stress

Several enzymes are inhibited as well as induced. These enzymes are inhibited either due to (1) binding of the metal to sulphydryl groups changing structural integrity of enzymes and (2) analogue substitution of toxic metals with essential metal in metalloproteins or metal-protein complexes.

In *A. lebbeck*, the activity of nitrate reductase increased over control values in leaves, and a negative correlation with leaf area, root and shoot length, and biomass was observed, indicating Cr(VI) stress (Tripathi et al. 1999), whereas inhibition of nitrate reductase activity at higher concentrations of Cr was observed in *Nelumbo nucifera* (Vajpayee et al. 2000) and *Nymphaea alba* (Panda and Patra 2000).

It is observed that Cr toxicity in plants results in chlorosis due to low Fe content in plants; this suggests and correlates with the effects of Cr stress on Fe mobilisation and uptake. Roots are known to absorb Fe(II) only; hence, under Fe-deficient conditions, the activity of reductases in roots of various plants is enhanced, thus increasing the capacity to reduce Fe(III) to Fe(II)(Alcantara et al. 1994). H + ATPase is another enzyme altered during chromium stress (Schmfger 2001).

7.2 Cr-Induced Oxidative Stress and Antioxidant Response

Generation of ROS is a common response to a wide range of biotic and abiotic stress. These ROS are usually normally produced in cells during reduction of O_2 to H_2O. Cr is also known to initiate production of ROS like H_2O_2, O_2^-, OH^- etc., accumulation of which causes oxidative damage to plants (Panda and Patra 2000; Dixit et al. 2002). The redox and nonredox character of a particular metal governs the nature and extent of ROS produced in its response. Metals like Cu and Fe have high redox potential and can undergo Fenton reactions generating toxic ROS. Although the mechanism of ROS production by Cr is not clearly understood, some studies have demonstrated that just like Cu and Fe, Cr also participates in Fenton and redox reactions (Strile et al. 2003). The oxidative stress as a result of Cr toxicity leads to disruption of cellular functions and structure of plants. Lipid peroxidation is a major indication of oxidative damage in plants, resulting in loss of membrane integrity and functionality (Shanker et al. 2003).

Major heavy metal detoxification mechanism in plants involves induction of antioxidant enzymes, namely, superoxide dismutase (SOD), peroxidase, catalase (CAT) and glucose-6-phosphate dehydrogenase. An increased antioxidant activity was observed when plants were exposed to low heavy metal stress, while at higher concentrations, no further increase in SOD activity was observed, and CAT activity decreased (Gwozdz et al. 1997). A 29% increase in activity of SOD was observed when exposed to 20 µM Cr(VI), whereas 200 µM Cr(VI) produced significant inhibition (Dixit et al. 2002). Studies have also shown decline in specific activity of catalase with increase in Cr concentration (Jain et al. 2000) and complete restriction of activity in the presence of excess of Cr (Chatterjee and Chatterjee 2000). The increased enzyme activity is in response to the generation of ROS, or superoxide radicals were observed, which probably is due to Cr-induced blockage of the electron transport chain (ETC) in the mitochondria. On other hand, the decrease in enzyme activity at higher Cr concentration is likely to be due to inhibitory effect of Cr ions on the antioxidant enzyme itself (ref) (Boonyapookana et al. 2002). Moreover, it was observed that Cr(VI) induces greater ROS generation than Cr(III).

7.3 Alterations at Molecular and Protein Levels Under Cr Stress

Although the molecular events as a result of Cr toxicity are not well known, several studies have suggested that Cr accumulation in plants induces the expression of certain genes involved in defence-related signal transduction and represses the activity of other genes. This induction and repression of genes depend on the level of Cr stress and defence mechanism active in the plants.

Several studies have reported changes in protein profile analysis of plants under Cr stress using 2D gel electrophoresis followed by mass spectroscopy (Labra et al. 2006). In *Z. mays*, several qualitative and quantitative changes in the protein expression were reported, and 22 proteins were identified in Cr-treated plants using mass spectroscopy; 4 of which were antioxidants (SOD, RAB24 protein/1-Cys proxidation), 3 were stress-related, 6 sugar metabolism linked and 9 others(Labra et al. 2006). Although the cellular function of antioxidant protein 1-Cys peroxiredoxin/Rab is not well understood, it seems to play an important role in the protection of DNA in stressed conditions (Stacy et al. 1996). However, it was found that in transgenic tobacco, this protein was involved in the elevation of resistance against oxidative stress, contrary to its role in dormancy in test plants (with no Cr stress); this suggests that the primary and major function of 1-Cys peroxiredoxin/Rab is defence against oxidative stress (Lee et al. 2000).

A study of altered protein expression in four *Salix* species under Cr(III) stress suggests that the gene expressions in response to Cr(III) stress are similar to the ones observed in pathogen attack and senescence-mediated apoptosis. This suggests a cross talk of signalling pathways in response to stress of both biotic and abiotic origins (Quaggiotti et al. 2007). Another study on hydroponically grown tomato plants under Cr(VI) stress showed up-regulation of HSP (heat shock proteins), MTs and GR isoforms, and Hsp90-1 was found to be more sensitive to stress conditions as well as it accumulated to a greater extent than other indicators (MT-2 and GR-1-like transcripts), suggesting that Hsp90-1 is an important biomarker of stress (Goupil et al. 2009). Also, the role of these molecular chaperones in protein protection and repair mechanisms under stress conditions has been reported (del Razo et al. 2001).

On analysis of protein profile in leaves of *Typha angustifolia* under Cr(VI) stress showed that stress induces the expression of ATP synthase, RuBisCO small subunit and coproporphyrinogen III oxidase (CPO). Overexpression of ATP synthase suggests that plant requires greater energy to cope up with such stress, and so the up-regulation of RuBisCO and CPO can also be correlated with their protective role in photosynthesis (Bah et al. 2010). The microarray analysis of genome in *O. sativa* roots exposed to Cr stress showed that 1138 genes out of 51,279 genes were up-regulated, while expression of 1610 genes was downregulated. Further analysis showed that these genes were involved in regulating transport, defence, stress response, growth and development and secondary metabolism (Dubey et al. 2010). Of the up-regulated genes, 35 were related to cytochrome P450 and 3 were HSPs, while downregulated ones included genes belonging to peroxidase family, 2 cysteine synthase, and 1 glutathione synthase genes. This study thus suggested that response to Cr stress is a complex network of up- and downregulation of the regulatory pathways in *O. sativa* (Dubey et al. 2010).

Studies also showed up-regulation of genes encoding for γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS) (enzymes involved in glutathione synthesis) under Cr stress, suggesting the defence role of glutathione under oxidative stress (Zulfiqar et al. 2011). Also, up-regulation of genes involved in glutathione-S-transferases (GSTs) synthesis (i.e. GSTF6 and GSTU4) was observed (Zulfiqar et al. 2011), which was similar to the findings in As-related stress (Abercrombie et al. 2008). A study on the molecular effects of Cr(III,VI) on kiwi pollen by comparative proteomics and DNA laddering analysis showed reduction of two proteins involved in mitochondrial oxidative phosphorylation. This leads to decline on intracellular ATP content and hence decrease in pollen germination. Furthermore, pollen showed up-regulation of proteins involved in cell redox homeostasis, scavenging response and lipid synthesis along with alteration in the ubiquitin proteolytic pathway. Cr(III) and Cr(VI) employed different mechanisms in alteration as Cr(III) showed 20S proteasome dysfunction (in terms of activity and abundance), while Cr(VI) does not affect 20S proteasome. Instead, a reduction in Rpn11 proteasome subunit is observed under Cr(VI) stress, affecting 26S-mediated proteolysis, which in turn results in impairment of protein turnover in affected pollens (Vannini et al. 2011).

In *Zea mays*, Cr(VI) has also been reported to cause increase in *Zm*MPK5 kinase activity (a 45-kDa myelin basic protein) with MAPK-like characteristics (MAPK, mitogen-activated protein kinase). The activation of this protein kinase is H_2O_2 dependent; as in the absence of H_2O_2 or the presence of H_2O_2 scavenger, the activity of this kinase is inhibited. Moreover, it was found that nitric oxide (NO) and calcium-dependent protein kinase (CDPK) are also involved in Cr(VI)-induced *Zm*MPK5 pathway (Ding et al. 2009).

8 Chromium Detoxification and Phytoremediation in Plants

Various heavy metals including Cr, As, Cd, Pb and Hg are highly toxic to living cells. However, some heavy metals, like Zn, Fe and Cu, are essential micronutrients, but they become toxic above certain threshold concentrations. Hence, to maintain a required physiological balance, plants have developed a complex system of mechanisms by which they regulate the uptake and accumulation of heavy metals (Cobbett and Goldsbrough 2001). Metal-binding ligands play a role in detoxification by metal binding. In plants, phytochelatins (PCs) and metallothioneins (MTs) are the two classes of metal binding that are involved in chelation and sequestration of metal ions for uptake and transport in plant (Cobbett and Goldsbrough 2001). MTs are low molecular weight cysteine-rich metal-binding proteins. MTs have been reported to play an important role in Cr detoxification in plants. Reports suggest the expression of MT-like proteins in sorghum under Cr stress (Shanker and Pathmanabhan 2004). Due to lack of thorough studies, the role of MTs and PCs in Cr detoxification in plants has not been well understood. Studies have also suggested that production of ROS as a result of Cr stress might trigger MT mRNA transcription (Shanker and Pathmanabhan 2004). Organic acids like citric and malic acids act as essential chelators that bind to insoluble forms of metals in soil, making them available for uptake by the plants. Also, the roots have been known to exudate important agents that form complexes and affect the redox behaviour of trace metals (Hale and Griffi 1974). Study showed that the presence of carboxylic acid and amino acids enhances the Cr uptake in *Lycopersicon esculentum* (Srivastava et al. 1999). However, citric acid, oxalic acid and aspartic acid are more effective in mobilising Cr than carboxylic and amino acids and that too for longer period of time (Dixit et al. 2015).

Certain plants have tendency to resist high metal concentrations and can grow well in the soil contaminated with metals such as Pb, Cr, Hg, As, Zn, Fe, etc. This can be attributed to the tendency of such plants to uptake metals from surroundings and accumulate them in vapour tissues. The plants that can accumulate more than 1000 mg of Cr /kg biomass (0.1% of dry weight) have been categorised as Cr hyperaccumulators (Reeves and Baker 2000). As discussed above, the major portions of absorbed Cr accumulate in the roots and are hardly translocated up to the shoot and aerial parts, making Cr removal from contaminated sites problematic. However, studies and trials have shown that phytoremediation has great potential for implications as cost-effective, in situ Cr remediation technology at contaminated sites. Most of such plants produce reductase enzymes, such as ones produced by bacteria, and mediate the conversion of Cr(VI) to Cr(III). The less mobile and less reactive Cr(III) thus formed is accumulated in plant tissues, mostly in vesicles of root cells. Various species of hyperaccumulators have been identified.

Since long, a wide range of plants have been tried and tested for phytoremediation of chromium. Many plants have been labelled as hyperaccumulators due their ability to grow at very high concentration of metals and also to absorb these metals in them. Most of these plants have been found growing in close vicinity of chromium-contaminated sites (Wild 1974). The high hyperaccumulators can have capacity ranging from 600,000 to 30,000 mg kg^{-1}, for example, *Laguncularia racemosa* (560,000 mg kg^{-1}, in roots) (Rocha et al. 2009) and *Dicoma niccolifera* (30,000 mg kg^{-1}, in leaves) (Peterson and Girling 1981). Many moderate (Cr = 2000–3000 mg kg^{-1}) and low (Cr = 1000–2000 mg kg^{-1}) hyperaccumulators have also been identified, but such plants are not ideal for in situ applications due to their low accumulation capacity. Table 1 lists some plant species which have been studied for their accumulation of Cr.

Various aquatic species have also been identified as Cr hyperaccumulators. *Nymphaea spontanea* was found to accumulate up to 2.2 mg of Cr(VI) per gram of plant biomass (Choo et al. 2006). *Typha angustifolia* was found to be a hyperaccumulator with a capacity to bioaccumulate up to 20,120 mg of Cr per kg biomass (Firdaus-e-Bareen 2008). Other species, such as *Salvinia natans* (Dhir et al. 2009),

Table 1 Various studies employing plants over a range of different species for phytoremediation of Cr

Plant species	Cr accumulation	Plant part	Reference
Dicoma niccolifera	2400 mg kg^{-1}	Dry leaves	Wild (1974)
Sutera fodina	1500 mg kg^{-1}	Dry leaves	Wild (1974)
Convolvulus arvensis	2800 mg kg^{-1}	Dry leaves	Torresdey et al. (2004)
Chinese brake fern	5717 mg kg^{-1}	Roots	Su et al. (2005)
Chinese brake fern	1145 mg kg^{-1}	Shoots	Su et al. (2005)
Leptospermum scoparium	20,000 mg kg^{-1}	Foliage ash	Lyon et al. (1968)
Nopalea cochenillifera	25,263 mg kg^{-1}	Roots	Adki et al. (2013)
Spartina argentinensis	15,100 mg kg^{-1}	Whole plant	Redondo-Gómez et al. (2011)
Laguncularia racemosa	560,000 mg kg^{-1}	Roots	Rocha et al. (2009)
Leersia hexandra	5608 mg kg^{-1}	Leaves	Zhang et al. (2007)
Leersia hexandra	18,656 mg kg^{-1}	Roots	Zhang et al. (2007)
Aerobryopsis longissima	7500 mg kg^{-1}	Moss ash	Lee et al. (1977)
Dicoma niccolifera	30,000 mg kg^{-1}	Leaves	Peterson and Girling (1981)
Pearsonia metallifera	20,000 mg kg^{-1}	Foliage ash	Wild (1974)
Eichhornia crassipes	3951 mg kg^{-1}	Roots	Zhu et al. (1999)
Thlaspi caerulescens	3400–3500 mg kg^{-1}	Roots	Shahandeh and Hossner (2000)
Brassica juncea	4100 mg kg^{-1}	Shoots	Diwan et al. (2010)
G. americana	3841 mg kg^{-1}	Roots	Barbosa et al. (2007)
Phragmites australis	4825 mg kg^{-1}	Rhizome	Calheiros et al. (2008)
Nymphaea spontanea	2200 mg kg^{-1}	Whole plant	Choo et al. (2006)
Typha angustifolia	20,120 mg kg^{-1}	Whole plant	Dhir et al. (2009)
Azolla sp. (A. microphylla, A. pinnata and A. filiculoides)	5000–15,000 mg kg^{-1}	Whole plant	Arora et al. (2006)
Salvinia natans	5700–9000 mg kg^{-1}	Whole plant	Dhir et al. (2009)
Callitriche cophocarpa	1000 mg kg^{-1}	Whole plant	Augustynowicz et al.(2010)

Callitriche cophocarpa (Augustynowicz et al. 2010) and *Ipomoea aquatica* (Weerasinghe et al. 2008), are known to hyperaccumulate Cr.

In 2011, Revathi et al., for the first time, attempted phytoremediation of Cr-polluted soil of Ranipet Tanneries in Tamil Nadu using *Sorghum* (Revathi et al. 2011). The impact of Cr(VI) on chlorophyll content and plant biomass was also investigated. Moreover, an increase in plant biomass was obtained upon addition of vermicompost to the contaminated site; this in turn creates more room for bioaccumulation of Cr and increases the efficiency of the process.

9 Conclusion

Chromium does not have any metabolic role in plants. But its presence in soil in high concentration can bring about adverse changes in plant growth and metabolism. Plants have the ability to tolerate Cr toxicity through various mechanisms like induction of enzymes for detoxification and production of antioxidants and metal-binding ligands like phytochelatins (PCs) and metallothioneins (MTs). Though there is a varied degree of difference in the tolerance and accumulation levels of various plant species, some plants have proven to have a great potential for hyperaccumulation of Cr. If this technology of phytoremediation could be expedited by helping plant growth, then this technology can be commercialized throughout the globe to solve this menace.

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Metals, Crops and Agricultural Productivity: Impact of Metals on Crop Loss

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1 Introduction

Agriculture plays a strategic role in the world's economy, both in developed and developing countries. In developing countries like India, where more than 50% of the population is dependent on agriculture, the agricultural sector acts as the backbone of the economy providing basic ingredients to the population and raw materials for industrialization. As per reports, 54.6% of the Indian population is engaged in agriculture and allied activities and contributes nearly 17% to the country's gross value added (Annual Report 2016–17, Department of Agriculture, Cooperation and Farmers Welfare). But due to population explosion in recent decades, there has been a tremendous pressure on this sector for production of surplus food grains in order to feed such a large population. The current world population of around 7.5 billion includes more than a billion malnourished individuals (UN 2014). Today's agricultural practices fall short in providing the required food for the increasing world population under the impact of climate change and several types of biotic and abiotic stresses (Meena et al. 2015). Agricultural crops are exposed to innumerable adverse environmental conditions including biotic and abiotic stresses, of which heavy metal stress is of major concern as it has notable consequences on crop

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growth and yield (Gill 2014). During the last few decades, agricultural lands have become vulnerable to heavy metal contamination due to increased anthropogenic activities, modern agricultural practices, rapid industrialization and urbanization (Eapen and D'souza 2005; Kavamura and Esposito 2010; Miransari 2011; Singh et al. 2016). Nowadays, urban areas have become a prominent hub of various stationary and mobile sources which release huge amount of heavy metals into the environment, exceeding the natural emission rate (Nriagu 1989; Bilos et al. 2001). In addition to heavy metal release from smelters and metalliferous mines, indiscriminate use of fertilizers and pesticides has also contributed to contamination of large areas of land (Yang et al. 2005). Plants have adapted and evolved against adverse environments by cross-wired metabolic processes to maintain the course of development. Plants' resistance against biotic stresses mostly relies on inheritable traits, whereas the genetically composite response against abiotic stresses is multigenic and difficult to trace (Munns 2005). The crucial part for depiction of the nature of phenotypic variations leading to successful stress tolerance responses is dependent on plant–environment interaction. This kind of interaction acts as a key determinant of phenology, plant anatomy and development (Meena and Yadav 2013).

Metal stress plays a crucial role in crop quality and productivity along with the economical parameters and human food security. This adverse condition is mostly manifested at phenological, biochemical, and molecular levels such as inhibition of growth, accretion of compatible organic solutes, and changes in phytohormones and other endogenous contents (Meena and Yadav 2013). Under natural conditions, generally the percentage of metal in soil is below the phytotoxic level, but metals occur more frequently from industrial and agricultural activities. Mining operations, waste disposal sites, non-ferrous metal industries, fertilizers, pesticides or metal-containing sludge are major sources of dispersion of metals in the aquatic and terrestrial ecosystem (Lepp 1981).

The term "heavy metals" represents the section of metals and metalloids with atomic density greater than 4 g/cm^3 or 5 times or more (greater than water) (Hawkes 1997). However, the most important factor in heavy metals is their chemical properties compared to their density. Heavy metals include Lead (Pb), Mercury (Hg), Iron (Fe), Cadmium (Cd), Antimony (Sb), Nickel (Ni), Copper (Cu), Molybdenum (Mo), Manganese (Mn), Strontium (Sr), Arsenic (As), Zinc (Zn), Silver (Ag), Cobalt (Co), Selenium (Se), Chromium (Cr) and elements of the Platinum group. Of these metals, Cd, Hg, Sb, As and Pb have potential to reduce crop productivity to a great extent if their concentration rises critically above threshold levels (Rascio and Navari-Izzo 2011; Park et al. 2011; Foucault et al. 2013; Shahid et al. 2013; Xiong et al. 2014; Pierart et al. 2015). Bioavailability of heavy metals in various media differs and its uptake in excess amount to plants requirements causes phytotoxic effects in plants (Blaylock and Huang 2000; Monni et al. 2000). Some of them are known as trace elements due to their presence in trace (10 mg kg^{-1} or mg L^{-1}) or in ultra-trace (1 µg kg^{-1} or µg L^{-1}) quantities in the environmental matrices.

Cd, Zn and Cu are widely spread pollutants and can reach high levels in soil. They are readily taken up by plant roots, whereas Pb, Co and Ni are phytotoxic only under specific soil conditions (Foy et al. 1978; Lepp 1981). The bivalent cations of the above metals belong to the "borderline" class according to their ligand affinity. They react with O-containing ligands (characteristic for "class A" elements) and with S- or N-ligands (typical for "class B" elements) as well. They can be arranged in the following series of increasing "class B" character (their "class A" character increases in the opposite direction) (Nieboer and Richardson 1980; Woolhouse 1983). Both types of metals can be phytotoxic for plants when they are assimilated in excess amount (Vallee and Ulmer 1972).

$$\mathrm{Zn} < \mathrm{Ni} < \mathrm{Co} < \mathrm{Cd} < \mathrm{Cu} < \mathrm{Pb}$$

The series mentioned above contain essential trace elements, and the functions of each metal at the enzyme level have been reviewed by Sandmann and Boger (1983). Heavy metal exposure results in reduced crop growth and biomass, and in extreme cases it causes plant death. It may lead to disorganization of grana structures, decreased chlorophyll synthesis, reduced growth of root and shoot, interfering with processes like photosynthesis and respiration (Bah et al. 2010; Cenkci et al. 2010; Ali et al. 2011; Pourrut et al. 2011, 2013; Austruy et al. 2014). Stunted growth, leaf epinasty, chlorosis, necrosis and red-brownish discoloration are visible symptoms of severe metal phytotoxicity (Lepp 1981; Woolhouse 1983). Changes like capacity of enzyme inhibition have also been observed at low levels of phytotoxicity before visible symptoms become evident. The metal enters the plant from soil mostly via the root (Uzu et al. 2009; Pourrut et al. 2011). Metals' entry from soil to root is indirect; rather they are first adsorbed on plant roots followed by binding to carboxyl groups of uronic acid around the roots or directly to the mucilage polysaccharides of the rhizoderm cell surface (Pourrut et al. 2011). Several steps are involved in the uptake of metals by plants, including desorption of metal from soil particles, transport of metals towards plant roots, uptake of metals by roots and translocation of metals towards shoot (Meers et al. 2009). Transportation of heavy metal towards aerial plant parts is via the xylem and is most probably activated by transpiration (Liao et al. 2006; Verbruggen et al. 2009). Factors like pH, temperature, organic matter, moisture and availability of nutrients play a major role in absorption and accumulation of heavy metals in plants. Heavy metal contamination of agricultural soil may deteriorate soil health and reduce the productivity of crops (Cai et al. 2012). Heavy metal uptake in excess amount by plants and consequent accumulation at the trophic level along with the food chain is a potential threat to animal and human health (Sprynskyy et al. 2007).

Excessive uptake of heavy metals by plants from contaminated soil impairs several biochemical, physiological and morphological functions in plants. Heavy metals mainly accumulate in the plant root cells and their entry is blocked either by the Casparian Strips or they get trapped by the root cell wall (Shahid et al. 2015). When heavy metal toxicity increases in plants, oxidant species like ROS are produced which cause oxidative stress, disturbing the redox balance of the system; but in counter-action, plants develop such defence mechanisms which not only help them to reduce toxicity but also maintain productivity. Such mechanisms include heavy metal binding by phytochelatins, sequestration into vacuoles and production of various antioxidants (Shahid et al. 2015). At higher concentration of heavy metals, plants show distinct toxicity symptoms and their uptake, utilization and assimilation are modulated by the plant cells (Janicka-Russak et al. 2008; Saito et al. 2010; Singh et al. 2012; Srivastava et al. 2012; DalCorso et al. 2013; Farias et al. 2013; Fidalgo et al. 2013; Singh et al. 2016). Excessive heavy metal uptake by plants leads to reduced crop yield as the process of photosynthesis, nitrogen metabolism and intake of water and mineral is influenced due to heavy metal uptake by plants (Cambrollé et al. 2011; Buendía-González et al. 2010; Dirilgen 2011; Shahid et al. 2012; Austruy et al. 2014).

2 Different Sources of Heavy Metals

The various sources of heavy metals in the environment are: (1) natural sources, (2) agricultural sources, (3) industrial sources, (4) domestic effluent, (5) atmospheric sources and (6) other sources. Heavy metal pollution originates from both anthropogenic and natural sources. Activities like smelting, mining and modern agricultural practices have contaminated large areas by heavy metals like Cd, Zn and Cu in countries including China, Japan and Indonesia (Herawati et al. 2000); Cu, Cd and Pb in Albania and North Greece (Shallari et al. 1998; Zanthopolous et al. 1999); and Cd, Cr, Ni, Pb, Zn and Cu in Australia (Smith 1996). Heavy metal occurs within the Earth's crust and its formation can be explained simply by the process of weathering.

2.1 Natural Sources of Heavy Metals

The most significant natural source of heavy metals is rock outcropping or geologic parent matter. Type of rock, environmental conditions and weathering process determines the concentration and composition of heavy metals. Generally, geologic plant samples contain high concentrations of Pb, Ni, Cr, Sn, Mn, Co, Cu, Hg, Cd and Zn. Pedogenesis (soil formation) initiates generally from sedimentary rock. Within sedimentary rocks category, shale contains highest concentrations of Mn, Ni, Cr, Co, Cu, Zn, Cd, Pb, Hg and Sn followed by limestone and sandstone. Igneous rocks such as olivine, hornblende and augite contribute significant amounts of Zn, Mn, Ni, Co and Cu to soil. High concentrations of Pb, Al, Zn, Ni, Mn, Cu and Hg along with harmful gases are emitted by volcanoes (Seaward and Richardson 1989). Marine aerosols and forest fires act as carriers for some heavy metals in several environments. Transportation of dust arising in Asia, the Pacific, the Antarctic and the Arctic has also been investigated (Davidson et al. 1985). Mn and Cu from marine sources have been detected in precipitation as an input to terrestrial environments (Vermette and Bingham 1986). A "bubble bursting" phenomenon is a natural process which also brings airborne Zn, Pb, Cu, Cd and Ni through sea salt particles (Pacyna 1986). Prairie and forest fires initiate emission of airborne heavy metals (Ross 1994). Carbonaceous matter produced during fire contains volatile heavy metals (like Hg and Se). Heavy metals are emitted into soil and the atmosphere through leaching process by stems and leaves via decomposition and volatilization. Several heavy metals have been detected in coastal areas due to sea sprays and subsequent aerosol production in oceanic activities.

2.2 Agricultural Sources of Heavy Metals

Inorganic and organic fertilizers are the most common sources of heavy metals in agricultural soil. Apart from that liming, pesticides, sewage sludge and irrigation water carry high amounts of heavy metals in agricultural soils (Nagajyoti et al. 2010). Cadmium is of specific concern in the plant community since it accumulates at very high levels in leaves which may be consumed by animals or humans. Application of manure, limes and sewage sludge leads to enrichment of Cd into soil (Nriagu 1988; Yanqun et al. 2005). Generally, the amount of heavy metals in agricultural soil is very small but repetitive application of phosphate fertilizer and its persistent nature results in the accumulation of various metals in topsoil (Verkleji 1993). Manure from animals enriches soil and also adds Co, Cu, Mn and Zn, whereas sewage sludge adds Cu, Cd, Pb, Ni, Cr and Zn (Verkleji 1993). Increase in heavy metal concentrations in agricultural soil depends on the rate of application, individual elemental concentration and characteristics of the soil to which it is applied. Sewage sludge in soil is considered as one of the most significant sources of heavy metal contamination (Ross 1994). Several pesticides used to manage and control diseases of vegetables, fruit crops and grain are heavy metal based (Verkleji 1993; Ross 1994). Frequent usage of such compounds leads to contamination of soil with very high rate of Fe, Cu, Hg, Pb, Mn, As and Zn (Ross 1994). Pesticide, labelled as lead arsenate was used in Canadian orchards for duration of more than 60 years and currently is having greater consequences for food contamination due to enriched level of As, Pb and Zn. Irrigation water sources like deep wells, rivers, lakes or irrigation canals can accumulate heavy metals such as Pb and Cd upon continued use of such chemicals (Ross 1994).

2.3 Industrial Sources of Heavy Metals

Mining activities and refineries are examples of heavy metal sources from industrial backgrounds. Heavy metal contamination through emission from mining operations depends on the type of mining (Table 1), for example, As, Fe and Cd are discharged by coalmines, which causes direct or indirect contamination of soil around coalfields. Utilization and mobilization of Hg in significant amounts in gold

Table 1 Industrial sources of metals

Industry	Metals
Mining operations and ore processing	Al, As, Cd, Hg, Mn, Mo, Pb, U, V
Metallurgy and electroplating	Ag, As, Be, Bi, Cd, Cr, Cu, Hg, In, Pb, Ni, W, Zn
Chemical industries	Al, As, Ba, Cd, Cr, Cu, Fe, Ga, Hg, Os, Pb, Sn, Ta, Ti, U, Zn
Dyes and pigments	Al, As, Cd, Cu, Fe, Pb, Sb, Ti, Tl
Ink manufacturing	Co, Cu, Fe, Hg, Ni
Pottery and porcelain	As, Cr, Sb, U
Alloys	Be, Ga, In, Os, Pd, Ta
Print	Ba, Cr, Os, Pb, Ti, Zn
Photography	Ag, Au, Cd, Cr, Mo, Pd, U
Glass	As, Ba, Co, Ni, Ti, W
Paper mills	Al, Cr, Cu, Hg, Pd, Sb, Ta, Ti, W
Leather tanning	Al, As, Ba, Cd, Cu, Fe, Hg, Zn
Pharmaceuticals	Al, Cu, Fe, Ga, Hg, Os, Ta
Textiles	Al, As, Ba, Cd, Cu, Fe, Hg, Os, Ni, Sb
Nuclear technology	Ba, Cd, In, U
Fertilizers	Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, Ni, Zn
Chlor-alkali production	Al, As, Cd, Cr, Fe, Hg, Mn, Pb, Sn, Zn
Petroleum refining	Al, As, Cd, Cr, Fe, Ga, Hg, Os, Ni, Zn

Adapted and modified from Nagajyoti et al. (2010)

mining industries have become the main sources of this pollutant to the environment (Lacerda 1997). These practices of mining and refineries have rapidly spread in the recent past throughout the tropics which had been nearly forgotten since 1960, particularly in Asia and Latin America concerning over ten million individuals (Lacerda 1997). High-temperature-demanding processes such as smelting and casting emit metals in particulate and vapour forms. Water plus vapour of heavy metals such as Zn, Cu, Pb, Cd, As and Sn in atmosphere accumulate to form aerosols. These are either dispersed by wind (dry deposition) or precipitated by rainfall (wet deposition), causing soil or water contamination. Erosive runoff of mines, dusts from transportation of crude ores, corrosion and leaching of heavy metals to soil and groundwater have the potential to contaminate soil and water bodies. Different types of processing in refineries also cause heavy metal pollution in soil systems. Power stations involved in energy supplying such as nuclear power stations, coal burning power plants, petroleum combustion plants and high-tension lines contribute many heavy metals such as B, Ni, Cd, Se, Cs, Zn and Cu to the environment (Verkleji 1993). Microelectronics, plastics processing, wood preservation, paper processing and textile manufacturing are the other sources of industry-based heavy metal pollution. Plantations growing beneath power supply lines with high Cu concentration have been reported to be toxic to the grazing community (Kraal and Ernst 1976).

2.4 Heavy Metals in Domestic Effluents

Domestic effluents in the form of waste water constitute the biggest source of metal pollution in lakes and rivers. Domestic effluents may consists of (1) untreated or mechanically treated waste water, (2) substances which have passed through the filters of biological treatment, and (3) waste water substances passed over sewage outfalls and discharged to receiving water bodies which often end up in the sea from coastal residential areas (Table 1). Possible pollution hazard arises with the use of detergents, since the common household detergent has a massive impact on water quality. Most detergents carry trace amounts of Fe, Sr, Mn, B, Co, Zn and Cr. Pollution with respect to urbanized areas clearly indicates the problem of metal contamination. A summary based on statistical analysis showed that urban storm water runoff has long been identified as a key source of pollutants to surface waters (Bradford 1997). Studies by Bolter et al. (Bolter 1974) indicate that leaching of Pb increased its availability to runoff rather than percolation into the topsoil.

2.5 Atmospheric Sources of Heavy Metals

Metal containing airborne particulates have been shown to originate from natural and anthropogenic processes. Depending on existing climate conditions, these particulates may become windblown over great distances; nonetheless, they are ultimately returned to the lithosphere as precipitations by rain or snowfall. Additional atmospheric sources include high-temperature anthropogenic sources of special importance on a global scale and geothermal sources such as volcanic eruptions, which have caused significant atmospheric pollution (Eshleman et al. 1971).

2.6 Other Sources of Heavy Metals

Trash incineration, landfill sites and transportation (diesel-powered vehicles, automobiles, and aircraft) are other sources of heavy metals. Anthropogenic sources which contaminate the soil are fly ash production due to coal combustion and corrosion of commercial heavy metal waste products which add Pb, Cr, Cu and galvanized metals (primarily Zn) (AL-Hiyaly et al. 1988) to the environment. Coal burning adds some heavy metals into the soil such as Al, Cd, Mn, Ni, Ti, Fe and Hg (Verkleji 1993). V, Fe, Ni and Pb are added into the environment due to oil combustion. Emission of metal during vehicular transportation includes Zn and Ni from tyres, Cu and Cd from diesel engines, Al by catalyst, and Zn and Ni from aerosol emissions. Lubricants are antiwear protectants of vehicles, which emit Zn, Cd, Cr, Ni, Pb and Hg particularly in case of incompetent engines performance. Leaded gasoline is a primary source of Pb in the environment. Municipal waste management by incineration generates considerable concentrations of Fe, Cu, Zn, Sn, Pb and Al.

3 Effects of Heavy Metals on Plants

Since plants are sessile, they are unable to withstand the frequent changes occurring in their environment (Singh et al. 2016), and the primary contact site for heavy metal ions is the root of a plant (Gill 2014). Heavy metals are bioaccumulative in nature (Gill 2014); they do not undergo biodegradation and are not easily metabolized. Since these metals cannot be degraded, they start to accumulate in plants, causing various diseases and disorders (Pehlivan et al. 2009). A reliable transport system is required for the entry of the heavy metal into the plant system (Singh et al. 2016).The entry of the metals into the plant system takes place mainly via the roots (Uzu et al. 2009; Pourrut et al. 2011) and is followed through by a number of pathways. Heavy metals firstly absorb on the roots of the plant, bind to carboxyl groups of uronic acid near the roots or simply bind to the mucilage polysaccharides of the rhizoderm cell surface (Pourrut et al. 2011). A plant regulates several other processes in order to make heavy metals bioavailable, some of which include desorption of metal from soil particles, transport of metals towards plant roots, uptake of metals by roots and translocation of metals towards shoot (Saifullah Meers et al. 2009; Shahid et al. 2015).

A broad range of physiological and metabolic alterations occur due to toxicity of heavy metals in plants (Dubey 2010; Villiers et al. 2011). However, due to different heavy metals, the site of action and overall visual toxic responses differ. Plant growth reduction is a common effect due to heavy metal toxicity including leaf necrosis, chlorosis, turgor loss, decreased seed germination rate and a crippled photosynthetic apparatus, often correlated with the process of senescence in plants or with plant death (Carrier et al. 2003; Sharma and Dubey 2007; DalCorso et al. 2010). The plants are able to absorb only those heavy metals which are present in soil as soluble components or which are readily solubilized by root exudates. There are some other heavy metals required in trace amounts for the proper growth and functioning of the plant, but when present in excess can cause phytotoxicity. When heavy metal accumulation exceeds the desirable limits, it directly or indirectly hampers the plant functioning morphologically, biochemically and physiologically, which ultimately reduces the crop productivity. All these toxic phenomena occurring within a plant depend on duration of exposure, concentration of heavy metal exposed to plant, stage of plant growth and development, intensity of stress and the plant species (Shahid et al. 2015). The most common evidence of heavy metal toxicity is reduced plant growth (Sharma and Dubey 2007) including leaf chlorosis, necrosis, turgor loss, decrease in the rate of seed germination and crippled photosynthetic apparatus, often correlated with the process of senescence or with plant death (DalCorso et al. 2010; Carrier et al. 2003; Gill 2014). The germination of the seed is one of the most crucial stages in plant growth and development and it can be variably affected by heavy metal toxicity. Different species of plants show different impacts on the morphological and anatomical structure of seed coats (Munzuroglu and Geckil 2002) depending on the penetrating ability of metals to reach the seed coat and embryonic tissues (Ko et al. 2012; Marquez-Garcia et al. 2013). Due to oxidative stress caused by metals, germination gets hampered due to interruption in the supply of metabolites for respiration by the seed reserves (Ko et al. 2012). The metal ions make their passage to plant tissues via the soil solution along with nutrients. Once they reach the tissues they start to induce physiological changes in the leaf structure, which in turn alters the photosynthesis and respiration rates resulting in decreased energy production. Thus, as a result of heavy metal toxicity, plant metabolic activities are badly affected. The heavy metal mainly targets the enzymes and reduces the soil enzyme activity (Tyler et al. 1989) and has potential to stimulate the formation of free radicals and ROS (Dietz et al. 1999; Gallego et al. 1996; Groppa et al. 2001; Sandalio et al. 2001; Fornazier et al. 2002). Metals generally attack the ligands present in enzymes and mask the catalytically active groups, which ultimately turns the whole process to produce toxic effects. They even have the potential to bind with sulphhydryl groups present in proteins, causing inhibition of an activity or disruption of specific structures (Van Assche and Clijsters 1990). Other processes carried out by plants such as flowering, seed germination and embryo formation also get affected (Guala et al. 2010). Chlorosis, necrosis, reduced nutrient uptake, decline in nitrogen-fixing capacity and reduced plant growth and yield are some other effects of concern occurring in plants. All these impacts are due to biochemical, molecular and ultrastructural modification in plant cells and tissues induced by heavy metal toxicity (Gamalero et al. 2009). Contamination of agricultural soils by heavy metals has become a main concern due to their possible impact on ecological parameters (Fig. 1). Here, we discuss the impact of some heavy metals on plants.

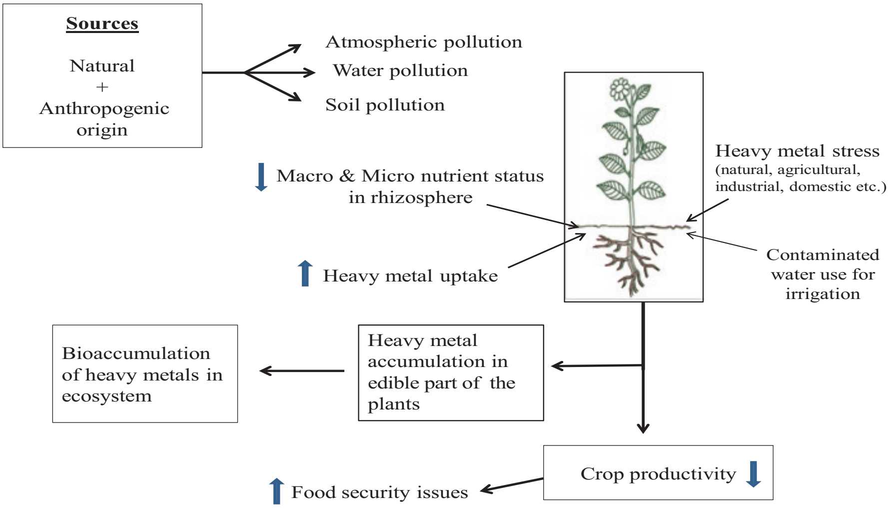

Fig. 1 Heavy metal contamination of agricultural soil and their possible impact on ecological parameters

3.1 Copper

Copper is a key micronutrient for plants. Both deficiency and excess of Cu beyond permissible limits impede the plant growth and influence biochemical processes (Gang et al. 2013; Adrees et al. 2015). It plays a vital role in the assimilation of CO_2 and ATP synthesis. Copper is a fundamental element of proteins involved in photosynthetic system like plastocyanin and cytochrome oxidase involved in mitochondrial respiratory electron transport chain respectively (Demirevska-Kepova et al. 2004). The disproportionate application of Cu-containing pesticides and fungicides against plant diseases has resulted in Cu accumulation on the agricultural topsoil (Scheck and Pscheidt 1998; Zheng et al. 2004; Michaud et al. 2007; Mackie et al. 2012). Brun et al. (1998) estimated 3–5 kg Cu ha^{-1} vineyards soils in the vicinity of Champagne (France) by a single application of Bordeaux mixture ($Ca(OH)_2 + CuSO_4$). The factors governing the phytoavailability of Cu are the physiochemical properties of soil and various biological processes (soil pH, dissolved organic matter) that occur in rhizospheric zone of the plants (Hinsinger et al. 2009). Excess Cu in soil leads to cytoxicity by inducing stress, causing damage to plants. This results in plant growth retardation and chlorotic leaves (Lewis et al. 2001). Copper in excess not only reduces the biomass with the advent of chlorosis in leaves (Quartacci et al. 2000) but also interferes with the ETS of photosynthesis (Patsikka et al. 2002). Ouzounidou (1994) observed growth inhibition of *Alyssum montana* due to Cu toxicity. Antagonistic impacts on germination of seed, quality and quantity of lateral root of *Solanum melongena* were observed in presence of both Cu and Cd (Neelima and Reddy 2002). Singh et al. (2007) observed 40% reduction in wheat seed germination with respect to control when treated with 100 ppm Cu. Reduction in seed germination of *Vicia sativa* L. (common vetch) was observed on exposure to Cu concentration by 5 mM Cu (Muccifora and Bellani 2013). Leaf area and diameter of maize plant reduced at higher Cu levels (100 g ha^{-1}) due to Cu toxicity (Barbosa et al. 2013). The reduction in root and shoot length of wheat at 50 ppm Cu concentration upto 72% and 31%, respectively, was observed compared to control in nutrient medium for 6 days (Gang et al. 2013). Biochemically, Cu toxicity leads to oxidative stress, which ultimately results in ROS formation (Stadtman and Oliver 1991). Oxidative stress disturbs metabolic pathways and often results in loss of macromolecules (Hegedüs et al. 2001). Activity of defence enzyme SOD increases in maize (Mocquot et al. 1996; Meng et al. 2007; Liu et al. 2014).

3.2 Zinc

Zinc disturbs various biochemical and physiological processes of plants due to its long biological half-life (Rout and Dass 2003). Phytotoxicity of Zn has been observed in plants like *Phaseolus vulgaris* (Cakmak and Marschner 1993), *Ipomoea batatas* (Kim et al. 2010), *Fontinalis antipyretica* (Dazy et al. 2009), *Brassica* *juncea* (Prasad and Hagmeyer 1999) and *Nicotiana tabacum* (Tkalec et al. 2014). Zinc altered the enzymatic efficiency of pea plants (Romero-Puertas et al. 2004) and *Phaseolus vulgaris* (Van Assche et al. 1988; Somashekaraiah et al. 1992). Zn induced lipid peroxidation in plant species like *Solanum lycopersicum* (Cherif et al. 2011) and *Vetiveria zizanioides* (Weihong et al. 2009). Zn concentration in contaminated soil frequently exceeds to required concentration as nutrients and leads to phytotoxicity. Zn concentrations of polluted soil have been measured to be around 150–300 mg/kg (Davies Jr et al. 2002; Warne et al. 2008). Growth of shoot and root is adversely affected by zinc phytotoxicity (Malik et al. 2011). On chronic exposure of Zn in soil, chlorosis in young leaves tends to extend to the older leaves as well (Ebbs and Kochian 1997). The phenomenon of chlorosis may increase as hydrated Zn^{2+} and Fe^{2+} ions exhibit similarity in their radii allowing Zn to induce Fe deficiency (Marschner 1986). Excessive Zn uptake by plant leads to Cu and Mn deficiencies. Micronutrients transportation from root to shoot is hampered by such deficiencies. Ebbs and Kochian (1997) observed comparatively higher Mn and Fe concentration in roots than shoots when plants were grown in Zn-rich media. Change in leaf colour to purplish-red, recognized as phosphorus deficiency, is however another distinctive effect of Zn cytotoxicity (Lee et al. 1996).

3.3 Cadmium

Cadmium contamination, mainly in agricultural topsoil, has become one of the major environmental concerns over the years (Du et al. 2013). Cadmium is a widely known and exceptionally substantial pollutant due to its relatively high toxicity, large water solubility and relative mobility in soil and it does not bind to form strong complexes with organic matter (Pinto et al. 2004; Nelson and Campbell 1991). The photosynthetic apparatus is impaired with the sequestration of Cd in plant tissues (Dias et al. 2013). The Cd^{2+} ions inhibit RuBisCo activation, decreasing its activity and altering its structure by substituting Mg^{2+} ions which act as co-factor in carboxylation reactions and may also shift RuBisCo activity towards oxygenation reactions (Pietrini et al. 2003). Cadmium is very toxic to photosynthesis especially in higher plants. High Cd concentration in leaves can deteriorate structure, composition and functioning of PSI and PSII (Janik et al. 2010; Molins et al. 2013). Chlorophyll content decreases with increasing concentration of Cd ions in cells; Chl-a is affected more than Chl-b. Additionally, Cd inhibits the stomatal openings (Hasan et al. 2011), water uptake and its transportation (Vassilev et al. 1997). Chlorophyll reduction on exposure to Cd has been primarily observed in barley (Vassilev et al. 2002), tomato (Ammar et al. 2008; López-Millán et al. 2009), maize (Ekmekçi et al. 2008), mustard (Mobin and Khan 2007) and garden cress (Gill et al. 2012). Perfus Barbeoch et al. (2002) suggested that Cd^{2+} mimics Ca^{2+} and it enters the guard cell through energy-dependent Ca^{2+} channels. Intercellular Ca^{2+} dynamics study can explain the toxicity of Cd^{2+}. Cadmium ions have potential to substitute for Ca^{2+} in calmodulin (a ubiquitous Ca^{2+} binding protein) regulation, resulting in interference of Ca^{2+} signalling (Suzuki et al. 1985; Cheung 1988). Intercellular combat against Cd^{2+} toxicity results in modifications of membrane permeability and ROS generation (Mahmood et al. 2009). Cadmium in the plants drain down photosynthesis by altering enzymes of Kelvin cycle (Nazar et al. 2012) and thus reduces the carbohydrate metabolism (Khan et al. 2009). Cadmium restricts the activity of several enzymes forming a strong affinity with the thiol group (Mendoza-Cózatl et al. 2005). Cadmium is absorbed by the rhizospheric region and is translocated to aerial parts of the plant, resulting in degradation of the quality as well as yield of crops by constraining not only the absorbance but also the regulation of macro/micronutrients in crops (Sandalio et al. 2001; Hassan et al. 2005), thus impeding several physiological processes (Gussarson et al. 1996; Nazar et al. 2012). Translocation of Cd to the vegetative parts of rice has also been reported (Wang et al. 2014; Song et al. 2015). Such situations pose a threat to agricultural sustainability in terms of world rice production and thus food safety can be attained by minimizing the bioavailability of Cd (Rizwan et al. 2016).

3.4 Arsenic

Arsenic is ubiquitous and is extremely toxic for the environment (Tu and Ma 2002; American Agency for Toxic substances and Disease Registry (ATSDR), 2007). Countries like Argentina, Bangladesh, Mongolia, Chile, Poland, China, Mexico, Nepal, India, Taiwan, and various parts of the USA have reported groundwater As with lethal concentrations (Chowdhury et al. 2000; Smith et al. 2000; Mitra et al. 2002; Anawar et al. 2002; Pandey et al. 2002; Das et al. 2004). In India (especially in some of parts of West Bengal), irrigational practices carried out with As-polluted groundwater result in higher concentration of As in plants as well as topsoil (Brammer and Ravenscroft 2009). Arsenic exists primarily in two inorganic forms; As(V) and As(III) of which As(III) is highly toxic (Tripathi et al. 2007). Since, As(V) is a structural analogue of phosphate, its transport across membrane takes place through phosphate transporters (Stoeva and Bineva 2003). Cytoplasmic As toxicity impedes metabolic processes related to phosphate metabolism (Garg and Singla 2011). It contests with phosphate in ATP replacing it to form unstable adenosine diphosphate-As(V), consequently interruption in energy flow in cells is observed (Hartley-Whitaker et al. 2001; Meharg and Hartley-Whitaker 2002; Cozzolino et al. 2010). Stoeva et al. (2003) suggested that in the vegetative phases of maize, As induces increase in lipid peroxidation. Arsenic reduced transpiration rates in Oat cultivars, compared with control plants. This may be due to the stressed water translocation (Stoeva and Bineva 2003). Ahmed et al. (2006) observed decrease in leaf number, root length, plant height and biomass per pot with increase in As concentration. Similar observations have been reported regarding reduction in plant biomass, decrease in crop yield and fruit production and morphological changes when plants are exposed to As-treated soil (Carbonell-Barrachina et al.

1998; Srivastava et al. 2009; Mokgalaka-Matlala et al. 2008; Shaibur et al. 2008). Shaibur et al. (2008) reported reduction in plant biomass and yield in sorghum (*Sorghum bicolor*) due to toxic As levels in soil.

3.5 Chromium

Chromium is extremely lethal to plants and shows detrimental effects to growth and development of plants (Davies Jr et al. 2002). Primarily, Cr inhibits seed germination. Threshold for Cr tolerance can be determined initially by the germination potential of a seed in Cr medium (Peralta et al. 2001). Reduced seed germination in *Echinochloa colona*, a weed, was observed by 25% with 20 ppm Cr concentration (Rout et al. 2000). Parr and Taylor (1982) reported up to 48% decline in germination of bush bean (*Phaseolus vulgaris*) in soil on exposure to 500 ppm of hexavalent Cr. Similar results of reduction in 23% seed germination of Lucerne (*Medicago sativa* cv. Malone) in presence of 40 ppm of Cr (VI) (Peralta et al. 2001) were reported. Reduced germination rate was also observed in *Lolium perenne* (Chigbo and Batty 2013) and *Allium* (Nematshahi et al. 2012). Reduced bud germination in sugarcane by 32% and 57% was also reported on exposure to 20 and 80 ppm Cr, respectively (Jain et al. 2000). Zeid (2001) suggested adverse effects on amylases activity due to Cr, inhibiting the sugar regulation in embryo axis, which could be the probable reason for such decline in seed germination. Decreased root development in presence of heavy metals in crops and trees is a common and well-documented effect (Tang et al. 2001). Prasad et al. (2001) observed comparative variation in metal toxicity with respect to new root primordia of *Salix viminalis*, where magnitude of toxicity ranged Cd < Cr < Pb. However, Cr affected the root length relatively more than any other heavy metal. Chromium stress plays a central role in the process of photosynthesis involving electron transport, photophosphorylation, CO_2 fixation and enzyme activities (Clijsters and Van Assche 1985). The effect of Cr in higher plants in terms of photosynthesis is well documented (Van Assche and Clijsters 1983). Chromium-induced inhibition in the process of photosynthesis is probably due to disassembly of chloroplast's ultra-structure; however, the exact mechanism is not clear as yet (Vazques et al. 1987). Desmet et al. (1975) used chromate as Hill reagent in isolated chloroplast. However, Bishnoi et al. (1993) reported that Cr (VI) showed more prominent effects on PS I than on PS II in isolated chloroplast of pea. Probable metabolic plant modifications under Cr stress are: (i) alterations in production of chlorophyll, anthocyanin and other pigments (Boonyapookana et al. 2002); (ii) enhanced defence mechanism in the cells against Cr stress (Shanker et al. 2003); (iii) progression in production of new metabolites (e.g., phytochelatins, histidine) which may possess resistance or exhibit tolerance to Cr stress. An increased antioxidant enzymatic activity at lower concentration of heavy metal was observed, whereas SOD activity did not increase. However, Gwozdz et al. (1997) stated that with increasing metal concentration catalase activity decreased.

3.6 Lead

Lead is a hazardous environmental pollutant ubiquitously distributed in soil. It exists in soil as: (i) dissolved (soil solution), (ii) interchangeable (organic and inorganic forms), (iii) precipitated insolubly with supplementary soil components and (iv) structural components of soil lattices (Cecchi et al. 2008; Tabelin and Igarashi 2009; Sammut et al. 2010; Vega et al. 2010; Shahid et al. 2012). The phytoavailable forms—(i) and (ii) as stated above—are major forms which cause Pb persistency in the ecosystem. Dissolved Pb^{2+} ions enters the root passively in rice by adhering to root surface; it disturbs translocation of other mineral nutrition as Pb tends to restrict mineral translocation (Zhang et al. 2006; Pourrut et al. 2011). It enters in plants through pathways like ionic channels, proton pumps, co- and anti-transporters (Ashraf et al. 2015). High adsorption of Pb is assisted by cells with thin cell wall (young cells) at root apices (excluding root cap cells) (Xin and Yan-yu 1997). According to Verma and Dubey (2003), absorbed Pb in rice is mainly confined to root cells and only a small fraction is translocated to aerial plant parts. Lead exposure in *Brassica pekinensis* significantly diminished nitrate content in shoots, nitrate reductase and free amino acid (Xiong et al. 2006). Elevated Pb accumulation within the cells interferes with cellular enzymatic activities by reacting with sulphydryl groups, causes modifications in membrane permeability and interrupts mineral nutrition (Sharma and Dubey 2005; Gill 2014). Chatterjee et al. (2004) suggested with reference to plant–nutrient relationship that Pb^{2+} limits the influx of divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}) and anions like NO^{3-} in several plants. Decreased mineral regulation by plants from soil on exposure of Pb might be due to the antagonistic nature of similar sized metal ions (similar atomic radii) like K^+ ions (Sharma and Dubey 2005). The imbalance in plant nutrients results in down-regulation of several physiological processes. Moreover, on exposure of 20 days old rice sapling to 0.5–1 mM of Pb^{2+} concentration, a considerable effect on the root length of rice by about 40% was observed; ceasing of root cell viability followed by cell death through up-regulation of ROS production was also evident (Verma and Dubey 2003). Gu et al. (1989) observed reduction in rice biomass and yield up to 12% on exposure to Pb contamination in soil.

3.7 Nickel

Nickel occurs naturally in soil under the category of trace elements as a transition metal. Anthropogenic events such as exploitation of fossil fuels, inappropriate sewage disposal, mining works, inadequate usage of pesticides and fertilizers have increased Ni^{2+} concentration in soil of certain areas (Gimeno-García et al. 1996). Ni^{2+} concentration has increased in contaminated soil 20- to 30-fold (200–26,000 mg/kg) from the overall range (10–1000 mg/kg) found in natural soil (Izosimova 2005). Increase in Ni^{2+} concentrations in soil induced alterations to physiological condition and may have varied toxicity symptoms like chlorosis and necrosis in rice plants (Das et al. 1997; Pandey and Sharma 2002; Rahman et al. 2005). Chen et al. (2009) stated that the acidic soil or solution with exposure to Ni^{2+} is one of the vital factors besides organic matter composition which influences Ni^{2+} uptake in plants. Increase in Ni^{2+} uptake in *Lathyrus sativus* was observed ranging pH up to 5.0; contrary results were observed when pH was increased up to 8.0 (Panda et al. 2007). Moreover, plants exposed to Ni^{2+}-rich soil exhibited imbalance of nutrients and impairment of cell membrane functions. Lipid composition of plasma membrane and its H-ATPase activity affected by Ni^{2+} is well reported in rice shoots (Ros et al. 1992). Relatively increased MDA concentration is observed in Ni^{2+}-sensitive rather than Ni^{2+}-tolerant plants (Gonnelli et al. 2001). Such alterations interrupt membrane functions and lead to an imbalance of ion exchange in cytoplasm, especially K^+, the most transportable ion across cell membrane. Decreased water content in plants is induced by increased uptake of Ni^{2+}. The reduced water uptake is an indicator of advancement of Ni^{2+} phytotoxicity (Pandey and Sharma 2002; Gajewska et al. 2006).

3.8 Mercury

Mercury is a distinct metal in itself as it predominately exists in different forms, e.g. HgS, Hg^{2+}, Hg and methyl-Hg. However, Hg^{2+} abundantly exists in agriculturally ploughed soil (Han et al. 2006). Plant and metal interaction is of particular importance due to the wide application of Hg-containing fertilizers, seed disinfectants and herbicides (Cavallini et al. 1999). Some Hg compounds used on tree foliage as fungicides can be translocated and redistributed in plants (Ross and Stewart 1962). The Hg^{2+}ions get freely sequestered in aquatic and higher plants (Kamal et al. 2004; Wang and Greger 2004; Israr et al. 2006). Phytotoxic concentration of Hg^{2+} in plant cell has potential to induce evident physiological injuries in plants (Zhou et al. 2007). Elevated levels of Hg are observed in edible plant parts when they are grown in industrialized zones. Probable mechanism involved in phytoxicity may be due to modification in cell membrane permeability, high affinity for sulphydryl and phosphate groups, potential to replace essential ions and thus, disruption in nonprotected proteins (Patra et al. 2004; Patra and Sharma 2000). The Hg^{2+} ions induce changes in physiology of leaf stomatal opening and creates physical barrier for the flow of water within plant by binding with the aqua channel proteins (Zhang and Tyerman 1999). Concentration of Hg^{2+} within cell not only interferes with mitochondrial activity but also initiates oxidative stress, resulting in interruption of biomembrane lipids (Messer et al. 2005; Cargnelutti et al. 2006). Mercury is reported to distress the antioxidant defence system by disrupting the non-enzymatic antioxidant modulation in glutathione as well as the enzymatic antioxidant machinery glutathione reductase and SOD (Sparks 2005; Ortega-Villasante et al. 2005; Israr et al. 2006). Carrasco-Gil et al. (2012) reported lipid peroxidation in *Medicago sativa* due to elevated levels of Hg.

4 Future Outlook

Heavy metals are naturally occurring elements that have multiple industrial, domestic, agricultural, medical and technological applications leading to their widespread distribution in the environment, raising concerns over their potential effects on plants and the environment. Their toxicity depends on several factors including concentration, exposure, and chemical species. Background knowledge of the sources, chemistry, and potential risks of toxic heavy metals in contaminated soils is necessary for the selection of appropriate remedial options. Remediation of heavy-metal-contaminated soils is necessary to reduce the associated risks, make the land resource available for agricultural production, enhance food security and scale down land tenure problems arising from changes in land use patterns. Alongside, elucidation of the mechanistic basis of heavy metal interactions is also essential for risk assessment and productivity loss. Hence, research is needed to further elucidate the molecular mechanisms and plant health impact associated with exposure to toxic metals either alone or in combination.

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Agroecotoxicological Aspect of Arsenic (As) and Cadmium (Cd) on Field Crops and its Mitigation: Current Status and Future Prospect

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1 Introduction

Arsenic (As) is the 53rd most abundant element in the earth's crust with a background concentration below 3.0 ng m^{-3}, 100.0 mg Kg^{-1}, and 10.0 µg L^{-1} in the atmosphere, lithosphere (in soil), and in freshwater, respectively. In nature, As mainly coexists with other elements such as iron (Fe), nickel (Ni), copper (Cu), and sulfur (S). Among the common minerals of As, realgar (coexists with S) and arsenopyrite (coexists with Fe and S) are generally commercially exploited (Matschullat 2000). According to the observations made by the British Geological Survey and the United States Geological Survey (USGS), China (25,000 MT) holds the highest position in As production (with 69% share) followed by Morocco and Russia in the year 2015. At present, 200+ minerals of As exist in nature. Among these minerals, the majority, i.e., ≈ 60% are arsenate followed by ≈ sulfides, and/or sulfosalts and the rest are others (arsenides, arsenites, oxides, silicates, etc.) in terms of chemical nature (Bligh 2012; Mandal and Suzuki 2002). According to Grund et al. (2012), the majority of As raw materials are generally the by-products obtained from the dressing or smelting of other ores like lead (Pb), Cu, silver (Ag), or gold (Au). However, only three types of As ores, realgar, orpiment, and arsenopyrite, have been used for the production of As_2O_3 (arsenic trioxide) commercially in China. Authors like Grund et al. (2012) and Edelstein, (1985) are of the opinion that due to its higher toxicity and relatively low demand, As is often regarded as an undesirable element in mining and post-mining operations. Keeping in mind its (As) biological as well as toxicological aspects, As compounds are mainly grouped into three categories: (a) gaseous (arsine gas), (b) organic (methylated or other derivatives of As), and (c) inorganic (arsenic trioxide and pentoxide salts of arsenous acid and arsenic acid, sulfides, halides, and arsenides, etc.) (WHO 2000; Grund et al. 2012).

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Cadmium (Cd) is a silver-white colored substance, a nonessential heavy metal, and a group IIB member of the periodic table with mass number 112.48. It occupies 48th in the chemical periodic table. The main characteristic feature of Cd that it is easily soluble in water. Cd is a potentially toxic trace element for living organisms (both plants and animals). Cd was found in the earth's crust along with zinc, copper ores, and lead. Cd is found mostly in the oxidation state +2 in the natural ecosystem but the oxidation state of +1 form is rarely found in the ecosystem. Generally, Cd concentration in the earth's crust ranges from 0.1 to 0.5 ppm but sedimentary rocks may accumulate higher levels. Phosphorites and marine phosphate have been reported with Cd concentration more than 500.0 ppm (Jackson and Macgillivray 1995). The concentrations of cadmium in natural water and groundwater is less than <1.0 μg L^{-1}. The concentrations of cadmium on surface soil will be dependent on various factors like its mobility, geochemistry, and magnitude of contamination from fertilizer and atmospheric deposition. The concentration of cadmium in air is not less than 0.8 μg/day (JECFA 1986).

According to the US EPA standard, the maximum level of cadmium is 0.005 mg/L for drinking water, 0.01–0.7 mg Kg^{-1} for soil, 20 mg Kg^{-1} for hazardous waste screening criteria, 0.5 mg L^{-1} for livestock water quality, 0.2 mg L^{-1} for surface water, and 85 mg Kg^{-1} for land application of sewage sludge.

1.1 Arsenic in Agro-Environment

Arsenic enters the environment via natural and through anthropogenic means. According to the findings of some premier organizations like the Center for Disease Control and Prevention (CDC), As is a toxic element and further secures the top position in the Priority List of Hazardous Substances under the CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) assessed by the ATSDR (Agency for Toxic Substances and Disease Registry) (Richardson 2017). In terms of chemical forms found in the terrestrial environment, compounds like As pentoxide, arsenate, dimethylarsinic acid, monomethylarsonic acid, trimethyl arsine oxide, arsanilic acid A, and arsenobetaine, possess pentavalent arsenic or As^{5+}. Compounds of trivalent arsenic (As^{3+}) dimethylarsinous acid, monomethylarsonous acid, As trioxide, arsenite also exist besides the chemical forms of pentavalent arsenic (Hoffmann et al. 2018). In the water-soil-agricultural (plant) system, inorganic As (arsenite and arsenate) comprises the largest fraction among the various forms of As. Focazio et al. (2000) found that there is a significant difference in the median As content of groundwater of developed countries (USA) and the developing countries of South East Asia. In the USA, the median As content is approximately 1.0 μg L^{-1}, whereas in the developing countries greater As content is often noticed. Authors like Meharg (2004) and Santra et al. (2013) are of the opinion that due to extensive irrigation with As-rich groundwater, crops accumulate As in significant manner and transmitted in to the next tropic level by humans. Assessing the potential threat associated with As on humans, several organizations have proposed different guideline values or permissible limits for As. Among the leading organizations, WHO (World Health Organization) suggested 10.0 µg As L^{-1} as the Maximum Contaminant Level (MCL) for As; USEPA (United States Environmental Protection Agency) recommended 0.01 mg L^{-1} as permissible limit for As content in potable water (Nachman et al. 2017).

There is no doubt that arsenic contamination is not restricted to groundwater sources. Instead, it spreads to the soil. Arsenic contamination in agricultural soil (arable land) has become a serious environmental hazard (Mishra et al. 2014). Once contaminated with a hazardous substance like As the health or productivity of the soil is compromised. It was found that As can interfere with the native soil's microbial population as well as interact or influence other closely associated flora including crops as soil acts as the sink for As (Pena-Fernandez et al. 2014; Mandal et al. 2014) (Table 1).

Table 1 Various sources and their relative contributions in As buildup in environment

Sectors	Sources	Chemical forms	Life span (Years)	References
Wood industry	Manufacturing decks, fences, and landscaping. Highway and marine uses. Poles	Wolman salts, Chromated copper arsenate (CCA).	10–40	Khan et al. (2006a, b), and Shibata et al. (2007)
Metal industry and electronics	Pb – alloys for batteries, ammunition Cu – alloys and in electronic products	Metallic form for enhancing battery's endurance and corrosion resistance. As and its compounds used for improving bullet's sphericity, as well as for improving copper alloy's corrosion resistance and tensile strength	5–20	Nassar et al. (2012)
		High-purity metallic arsenic and gallium arsenide used in various semiconductors, integrated circuits, and other electronics components	12+	
Agriculture	Insecticides, herbicides, plant desiccants, animal feed	Mainly As_2O_3 and its derivatives	≤1	Grund et al. (2012)
Glass manufacturing	Fining agent, bleaching (decolorizing) agent, In glass ceramics	As_2O_3 used for removing air bubbles. Arsenic acid to reduce dusting. As compounds employed for controlling crystal growth rate.	5–20	Loebenstein (1994)
	In production of red glass	As_4S_4 for its red color	10	

1.2 Cd in Agro-Environment

Variable amount of Cd released to the environment from natural and anthropogenic activities. Normally, Cd contributes to the atmosphere naturally through volcanic eruptions, forest fires, windblown dust, and sea spray. Weathering of the parent rocks also adds Cd to the environment (Liu et al. 2013). Mafic and ultramafic rocks are also found to have the highest content of Cd and with the weathering of this kind of rocks a major amount of Cd is released into the environment (Shah et al. 2010). Black shale, also known as carboniferous shale, contains more or less 100 mg kg^{-1} Cd and increases its content in soil (He et al. 2005). More or less 10% of Cd is contributed to the environment by natural sources. The other 90% ($5.6–38 \times 10^6$ kg $year^{-1}$) is attributed to anthropogenic activities such as use of phosphate fertilizers, fossil fuel combustion, metallurgical works, wastes from cement factories, sewage sludge, municipal waste and industrial wastes, mining, smelting, and metals ore processing (Khan et al. 2018). It is extensively used in batteries, pigments, plastic stabilizers, solar panels, and steel plates to resist corrosion. On the other hand, Cd is also found in polyvinyl chloride (PVC) plastic manufacturing, alloys, solders, rubber and textile factories, fungicides, motor oil (Khan et al. 2018). The British Geological Survey reported Cd production worldwide to be approximately 24,900 metric tons in 2015 (Brown et al. 2017). In 2016, Cd production worldwide was 23,000 metric tons excluding USA (US Geological Survey 2017). Mining activities also contribute Cd to soil as dust in near mining site fields. Cd also occurs in all types of zinc ores as a guest element because of both elements having similar geochemical properties. Industrial processes and vehicular emission are the main sources of Cd contamination in urban soil (Khan et al. 2016b). A major contribution of Cd, around 67%, comes from the production of metallic Cd electroplates used in nickel-Cd batteries (Greenwood and Earnshaw 2012). Nriagu and Pacyna (1988) reported that the total estimation of Cd contributed to the soil worldwide through different anthropogenic activities source was 5–38 × 106 kg $year^{-1}$ with 12% agriculture and animal wastes including fertilizers. Among different sources of Cd, the use of phosphate fertilizer is one of the major contributors of Cd to agricultural soil (Martens and Smolders 2013). In phoshphate fertilizers, it was reported that the amount of Cd present range from 0.1–120 kg (Nziguheba and Smolders 2008). In addition, the application of contaminated manure also adds Cd to agriculture soil. Sewage sludge also contributes a considerable amount of Cd to agriculture soil. France (16.7 mg kg^{-1}), Belgium (7.61 mg kg^{-1}), and China (7.43 mg kg^{-1}) report the high impact of Cd contamination in soil.

2 As and Cd Stress on Agricultural Crops

There is much literature available describing the consequences of exposure to As on different plant species. Adverse effects of As (various forms and in varied concentrations) including (1) inhibition of germination and seedling growth; (2) reduction in yield; (3) reduction in photosynthesis; (4) considerable As load; (5) decrease in essential mineral content in edible parts, and (6) grain quality traits of rice have been reported so far (Moulick et al. 2016a, b, 2017, 2018a, b, c, d; Shaibur et al. 2006, 2008). Beside these, having a pronounced phytotoxic impact on various crops a significant amount of As content can be also seen in various crops. Besides rice grain, a wide range of rice-based products (including infant food) are also found to have a considerable amount of As (Moulick et al. 2016a; 2018d; Carbonell-Barrachina et al. 2012; Signes-Pastor et al. 2016a, b; Sun et al. 2009). A large number of reports also suggest that a significant amount of As can also be found in crops like *Mentha aquatica* (mint)*, Lycopersicon esculentum* (tomato), *Cucumis melo* (melon), *Zea mays L.* (corn), *Pisum sativum* (pea), and even in tree species like *Pinus halepensis* (pine) (Tu et al. 2003; Szakova et al. 2009). Whereas, speaking of As induced toxicity upon transmission and associated hazards on consumers rice can be considered as a best studied crop so far. As a result of cultivating crops in As contaminated agroecosysytems located arround the world, a significant amount of As is found to enters into the food chain and then transmitted in to the next tropic level (Moulick et al. 2016a, 2018d; Santra et al. 2013). Being a staple food for more than half the world population, rice is also considered the primary entry route of arsenic (Chung et al. 2014). Authors like Melkonian et al. (2013) and Gilbert-Diamond et al. (2013) have confirmed that a significant correlation exists among the urinary As content, skin lesson as well as among rice intake after examining more than 18000 Bangladeshi individuals. Later, Banerjee et al. (2013) found that consumption of 500 g of cooked rice having As content ≥200 mg Kg^{-1} on a daily basis promotes genotoxicity in human.

Cd induces negative effect on plant growth. It significantly reduces shoot dry mass in cucumber at lower concentration of 0.05 μM in hydroponic culture. Cd also disturbed mineral nutrient uptake by cucumber (Tack et al. 1998). The application of 2 and 10 μM Cd in pepper varieties showed that root length, root tip and area, fresh weight of root, stem, and leaf were drastically reduced as compared to control (Huang et al. 2015; Xin and Huang 2014; Leon et al. 2002). The same was reported in other plants such as *Solanum tuberosum* L. (Hassan et al. 2016), *Brassica olerace* (Jinadasa et al. 2016), *Raphanus sativa* (Varalakshmi and Ganeshamurthy 2013), and *Glycine max* (Wang et al. 2016a). Photochemical efficiency, chlorophyll content, and photosynthetic intensity are regarded as sensitive indicators for plant growth under Cd stress (Chen et al. 2008). Many have reported that Cd is found to be capable of inhibiting the photosynthesis process. Cd cannot synthesize chlorophyll and binds to proteins, ultimately damaging the photosynthesis apparatus, especially decreasing light harvesting complex II and photosystems I and II. Cd also inhibits certain enzymes of the calvin cycle (Shanying et al. 2017). Reduction of starch grain, intact grana, and reduced size of chloroplast were found in *Sedum alfredii* (Jin et al. 2008), *Pisum sativum* (Sandalio et al. 2001), *Picris divaricate* (Ying et al. 2010), *Solanum taberosum* (Hediji et al. 2010), and *Glycine max* (Shamsi et al. 2014). Under Cd stress, the uptake and accumulation of different mineral nutrient in plants may be greatly influenced (Zhi et al. 2015; Li et al. 2016c). Zn^{2+}, Mg^{2+}, Fe^{2+}, Ca^{2+}, Mn^{2+}, and Si^{2+} compete with Cd for exchange site in soil. Metwally et al. (2004) reported that Phosphorus (P), Calcium (Ca), Manganese (Mn), Zinc (Zn), Sulfur (S), Potassium (K), and Boron (B) were reduced in the presence of 5.0 mg Kg^{-1} Cd in soils in both sensitive and tolerant varieties of *Pisum sativum.* At lower concentration of Cd (1.12 mg kg^{-1}), an interesting result was found: Mn content was decreased but Fe and Zn content increased in *Glycine max* (Zhi et al. 2015). Zn, Mn, Ca, and K content in aerial part of *Solanum tuberosum* seedling was decreased under Cd stress in hydroponic conditions (Bertoli et al. 2012). Jinadasa et al. (2016) reported that in the presence of excess amount of Cd, there was significant reduction of Mn, Zn, K, Cu, Fe, and Ca content in root, leaves, and stem of *Brassica olerace* compared to the control. Similar kind of result was found in other plants such as potatoes, tomato, and lettuce (Khan et al. 2016a). Some reverse results were also found. Under Cd stress (1, 2.5, and 5 mg kg^{-1}) enhanced the accumulation of mineral nutrients including K, P, Mg, Ca, Fe, and Zn in *Allium fistulosum* L. can be seen (Li et al., 2016c). When Cd is present in soil, it can induce water stress in plants by reducing stomatal conductance, transpiration rate, and relative leaf water content (Dominguez et al. 2011). As a result, there is physiological damage from reduction in the intercellular space and chloroplast and also cell enlargement (Sandalio et al. 2001). Fernandez et al. (2013) mentioned that Cd also influences plasma membrane permeability as a result of reduction in content of water in plants. In *P. eurphratica*, loss of water and wilting was found under Cd stress (Polle et al. 2013). *Sorghum biocolor* (Kuriakose and Prasad 2008) and *P. eurphratica* (Siddiqui et al. 2009) seeds show reduced water imbibition under Cd stress. Sun et al. (2013) mentioned that in the presence of Cd, *P. eurphratica* shows clear shrinkage of cytoplast, i.e., water content imbalance. Cd inhibits root hair growth in plants, which ultimately reduces the surface area of water absorption by plants (Gouia et al. 2003). A report mentions that Cd can lead to change of anatomical and structural features of cells and result in a negative effect on plant growth and development (Kupper et al. 2000). Shah and Dubey (1993) reported low mitotic index, cell division, cell proliferation, and chromosomal aberration in various plant species under Cd stress. Ali et al. (2013) found at high concentration of Cd (500 mM), cell wall break, plasmolysis, undeveloped mitochondria, and lack of endoplasmic reticulum in *Brassica* species root tip cell. Similar types of results were found in *Gossypium hirsutum* (Daud et al. 2009). In case of genetic variation, many reports mention that Cd directly interrupted the structure and function of DNA (Cambier et al. 2010). Sun et al. (2013) observed that Cd induced the loss of cell viability accompanied with DNA fragmentation and condensation of chromatin in *P. eurphratica*.

Hyperaccumulator plants can be defined as those plants that are capable of growing in highly contaminated soils and that accumulate higher concentrations of heavy metals in various tissues. These plants accumulate 10–500 times higher than normally plants (Chaney and Oliver 1996). Hyperaccumulator plants used to absorb heavy toxic metals by using their roots and then transport them into the shoot and thus help to store these metals whereas, a fraction of the heavy toxic metals gets volatilized too (Doty et al. 2000). Kramer (2010) mentioned that heavy metals accumulation in aerial parts ranges from 1000 to 10,000 mg Kg^{-1} in plants shoot, which is an indicator of a hyperaccumulator plant species. But in the case of Cd, the accumulation is lower (100 mg kg^{-1}) in plants due to its highly toxic nature. Based on two factors used to distinguished from hyperaccumulator and non-hyperaccumulator plants are bio-concentration factor (BCF) and translocation factor (TF) value of >1.0 and it's tolerance property should not have display any toxic symptoms (Rascio and Navari-Izzo 2011). However, most Cd hyperaccumulator plants have shown slow growth rate and low biomass yield with short life span. This type of hyperaccumulator plants has low biomass production as the significant portion of their metabolic energy gets involved in adapting them under heavy metal contaminated environment (Kabata-Pendias and Pendias 2001). Some interesting facts about non-hyperaccumulator plant species are: rapid growth, high biomass yield, and desired Cd-accumulating capacity. Plants like *Brassica juncea, Cynodon dactylon, Typha latifolia* etc. were generally used as Cd hyperaccumulators.

3 Factors Affecting As-Induced Phytotoxicity in Agroecosystem

Organic matter (often referred to as organic carbon) originates from decomposed matter (including a wide range of plant and animal products and by-products). Organic carbon usually plays a crucial role in modulating As dynamics in the rice agroecosystem (Tareq et al. 2013). During cultivation of rice paddy, organic carbon is generally added to soil in the form of manure prepared from mainly cow dung and other sources such as rice straw, husk, etc. In most cases, especially in the case of transplanted rice, seedlings are generally uprooted (from seed bed) and then transplanted manually into actual paddy fields and harvested after maturation (Said-Pullicino et al. 2016). Transplanted rice generally leads to the creation of DOC (dissolved organic carbon) in paddy soils, aided by flooded conditions as the uprooted seedlings are transplanted in well-ploughed soil with greater amount of manure (organic carbon) than in the seed bed. Speaking of the mechanism describing the role of organic carbon modulating As-induced phytotoxicity in paddy field soil, two theories can be considered. According to Mladenov et al. (2015), first the organic carbon gets absorbed onto Fe^{3+} (through a ligand exchange), which in turn has to compete with inorganic As species (As^{5+} and As^{3+}) in order to predominate in the competition for active adsorption sites. Thus, As-contaminated paddy field soil enriched with organic matter often facilitates the mobility as well as the phytoavailability. According to another view, advocated by authors like Chen et al. (2016) and Mladenov et al. (2009), organic matter in soil solution facilitates the microbial reduction (Fe^{III} reducing microbes) and stimulates the release of As^{3+} in a greater amount to the soil solution. Findings of authors like Williams et al. (2011) and Radloff et al. (2007) indicated that organic carbon shares a significantly high affinity with As and thus favors the formation of organo-As complexes. Rahaman et al. (2011) further showed that the application organic matter vermicompost along with water regime management, and later sue of poultry manure into As - contaminated paddy field soils resulted to a reduction in total As concentration in rice tissues. Whereas, authors like Fu et al. (2011) contradict the earlier trend of relationship among As and organic carbon, with respect to grain As content, forwarded by Rahaman et al. (2011)

Soil acidity and redox potential are the two important soil properties that modulate As phytoavailability and toxicity to field crops like the rice plant. Generally, rice has been cultivated using intense irrigation during *boro* season (winter) or even during *kharif* (monsoon season) with occasional irrigation to provide a semi-aquatic environment. In the pore water of paddy field soil (which are often submerged or extensively irrigated) are associated with relatively low pH (acidic) and inorganic As species predominates under this condition. Findings of Ahmed et al. (2011) suggest that the acidic soil pH favors the As accumulation and translocation in grain. The authors further added that soil pH and grain As content also share a significant positive correlation. A comparatively alkaline environment with pH >7 promotes building up of negative surface charges which later stimulates the desorption of As^{5+} and As^{3+} into the soil solution, in close contact with root system. When the paddy field soil pH lies in the range of 2–11 along with oxidising situation, $H_2AsO_{4\_}$ and $HAsO_4^{2-}$ are the two most prevalent As species. When pH reaches beyond 12 in reducing condition, arsenous acid converted into $H_2AsO_{3^-}$ and $HAsO_3^{2-}$ depending upon the environment Bissen and Frimmel (2003). Smedley and Kinniburgh (2002) have suggested that mechanisms closely associated with soil environments like formation of root plaques, the reductive dissolution of metal hydro (oxides) and soil microbial flora facilitated various reduction and oxidation process further determines the intensity of As toxicity to the plants.

Under submerged conditions, acidic soil with low redox potential or Eh favors soil microbes (sulfur-reducing microbes) to stimulate the conversion of SO_4^2 (sulfate) to S^{-2} (sulfide). This process plays a vital role in As mobilization in paddy field soil (Jia et al. 2015; Wilkin et al. 2003). According to Burton et al. (2014) and Fisher et al. (2008), as a result of reduction of SO_4^2, the S^{-2} promotes the immobilization of As^{3+} by allowing precipitation as either iron sulfide or arsenosulfide complex. Bostick et al. (2005) reported that, under low soil pH (acidic) As^{3+} and S^{-2} after reacting, forms thioarsenite complex, later in solid phase this complex gets precipitates and thus minimize the effective As content and later phytoavailability too. Although there are only a handful of studies reporting the interactive aspect among inorganic As and SO_4^2. Burton et al. (2014) reported that As and SO_4^2 interaction in paddy filled soil concluded that a reduction in As^{3+} and ferric ion is correlated with microbes-mediated SO_4^2 reduction. The author has the opinion that the reduction in As^{3+} and ferric ion may have contributed to the co-precipitation of As by forming insoluble complex of As and Fe and thus becoming less phytoavailable.

Similar to Sulfur (S), Manganese (Mn) also shares an inverse relation with As in rice field soil. Findings of Tani et al. (2004) and Hossain et al. (2008) indicate the role of soil microbes and Mn-plaque on rice root and their role in reducing As content in rice tissues, respectively. The authors further explained that the reduction in As content in rice tissues, attributed the microbial influenced (stimulation) MnO_2 mediated oxidation of As^{3+} and sequestrations of As respectively. Lafferty et al.

(2011) reported the ability of MnO_2 to decrease movement of As in soil–water interface (in paddy field) by either (1) facilitating the absorption of As^{5+} or (2) by influencing the conversion of As^{5+} to As^{3+}. Authors like Pan et al. (2014) and Smedley and Kinniburgh, (2002) are of the opinion that apart from S and Mn, Fe (among other soil elements) is another key factor that determines As mobility and phytoavailabilty to rice plant. When compared with the other two elements (S and Mn) reports suggest that Fe (as FeOOH, through reductive dissolution) in soil–water interface modulates the mobility of As^{3+} in a favorable manner, as a result rice plants become more prone to As-induced phytotoxicity and greater accumulation of As (Huang et al. 2012; Yamaguchi et al. 2011). However, contrasting views also exist regarding the role of Fe in As mobilization in As-contaminated paddy field. Under submerged condition in paddy field, the presence of Fe-plaque on rice root surface leads to sequestration of As species by either being coprecipitated or being absorbed at the FeOOH boundary, thus minimizing the phytoavailabilty of As (Jain et al. 1999; Youngran et al. 2007).

Besides the various cations (i.e., other elemental forms) present in paddy field soil, evidences also indicate that anions or ionic species are also found to modulate the As phytoavailability and toxicity to rice plants. Among the anions, the role of Si (silicon) as fertilizer, $Si(OH)_4$ or silicic acid have been investigated in detail. Authors like Li et al. (2009) found that when Si was used in the form of SiO_2 in As-rich paddy soil in comparatively greater amounts, a noteworthy reduction in As content was observed in rice plants. The authors have the opinion that when Si is applied in higher doses in soil, it competes with As^{3+} for the transporter, and greater than the prescribed dose of SiO_2 when applied actually encourages the uptake of $Si(OH)_4$ instead of As^{3+}. Along with the role of Si, the role of P (phosphorus) as PO_4^3 or phosphate has also been addressed well. Authors like Sharma et al. (2014) and Cao et al. (2003) have reported that being a structural analogue of As $^{5+}$, application of PO_4^3 (which promotes the absorption of As $^{5+}$ in soil) was found to significantly reduce As content in rice plant.

4 Factors Affecting Cd-Induced Phytotoxicity in Agroecosystem

Soil pH is a major factor for controlling heavy metal availability (Barančíková et al. 2010). In fact, pH factor is the key factor for controlling uptake of heavy metals by plants. However, Kukier et al. (2004) have mentioned that pH factor is not the most important factor for controlling uptake of heavy metals. Seuntjens et al. (2004); Amini et al. (2005), and Basta et al. (2005) found that pH influences the uptake of Cd in soil solution. Kuo et al. (2004), Sappin-Didier et al. (2005), and Tsadilas et al. (2005) report that the decreasing pH in soil enhances the Cd content in plants. Tudoreanu and Phillips (2004) state that Cd is indirectly proportional to pH due to its speciation that varies with pH, the concentrations of free ions concentration, etc.

to a certain level of toxic effect and can be stated as a function of pH alone (Lofts et al. 2004). Adams et al. (2004) performed an experiment in 162 grain samples of *Triticum aestivum* and 215 of *Hordeum vulgare* under different pH conditions and found that pH most significantly influenced the Cd content in grains.

Soil organic matter (OM), texture, salinity, redox potential, and nutrient status are also influential in Cd uptake by plants. Organic matter plays the most important role in pH-dependent negative charge in soils where soil is able to hold cationic metals. Depending on whether organic matter is soluble or insoluble, it plays different roles in controlling Cd uptake by plants. Recent research has most emphasized on Cd mobility and bioavailability in both agricultural and environmental fields. Soil texture is one of the important factors for bioavailability of heavy metal in soil. Beyer and Cromarite (1987) reported that clay-containing soil significantly increases the availability of heavy metals, as a result of which harmful effects were found in living organisms. Sandy soil bears large size of pore along with low retention capacity as compared to loamy soil. (Rashid and Ryan 2004; Martens and Reed 1991). Transfer of heavy metals from soil to plants is influence by soil texture. Treder and Cieslinski (2005) mentioned that plants cultivated on sandy soil have been found to have the highest concentrations of heavy metals as compared to clay soil.

Soil aging is also another factor that affects the controlling of Cd uptake. Lock and Janssen (2003) mentioned that aging is a long-term chemical process; the bioavailability of Cd in soil can reduced with time, with little or no reduction of Cd in the soil. Different plants exhibit variability in their growth, reproduction, and occurrence for the survival under the heavy metal stressed condition. The mechanisms of heavy metals' uptake by different plant species are not the same, so the toxicity level of heavy metals varies from plant species to species (Garty 2001; Zechmeister et al. 2003). Plant species is also a factor that controls heavy metals' uptake by plants.

5 Mitigation Strategies

5.1 Soil Amendments or Nutrient Management

Proper management of mineral nutrients in growth media could control cadmium accumulation in plants. Some particular individual mineral nutrients have been discussed that significantly decrease the cadmium uptake in different plant tissues. Application of mineral nutrients (Fe, S, P, and Si) as fertilizers or modulating its doses in soil can decrease the As accumulation and mobilization into edible plant parts by reducing its uptake and its subsequent translocation in field crops in a significant manner. Iron (Fe) being an essential mineral nutrient for plants plays a vital role in reducing the As absorption in rice. With external application, Fe facilitates the deposition of Fe-oxide or promotes the formation of Fe-plaque in rice roots and thus reduces As uptake as well as increases the coprecipitation of Fe and As. Due to the formation of a barrier, i.e., Fe-plaque, which consists of mostly siderite (5%), goethite (32%), and ferrihydrate (63%), the phytoavailability of As gets reduced on the root surface, as a result the subsequent translocation is also decreased (Liu et al. 2004; Nath et al. 2014). Lu et al. (2010) carried out an intense field study regarding the status of P in rice shoots across the rice fields of China and found that P content can effectively reduce As content in grain. Neupane and Donahoe, (2013) found that coapplication of Ca along with P can stimulate the formation of Ca-P complex that can effectively decrease As content in rice grain. Lee et al. (2016) explained the possible mode of action that can be taken into account to understand the antagonistic behavior among P and As that finally restricts As mobility and uptake by rice plant. The authors proposed that due to having structural and behavioral similarity, a competitive aspect among P and As on absorption pattern can be seen that further reduces the translocation of As from root to shoot.

Proper management of nitrogen (N) practices could ameliorate cadmium toxicity in plants. Larsson Jonsson and Asp (2011, 2013) reported that the application of nitrogen form in growth medium and pH strongly affected the cadmium accumulation in plants. Increasing the concentrations of N from 60 to 120 and finally upto 240 Kg N Kg^{-1} were found to reduced Cd accumulation in potato tubers. The application of ammonium sulfate increased the cadmium uptake concentrations in tubers compared to other forms. It is due to present of ammonium sulfate which actually decrease the pH of the soil (Larsson Jonsson and Asp 2011). Konotop et al. (2012) reported that supply of nitrogen decreased cadmium uptake by soybean plants and increased tolerance capacity against cadmium stress, where the oxidative stress decreased and the activity of CAT enhanced.

The use of phosphorus fertilizers for the purpose of decreasing cadmium toxicity in plants has been well documented (Rizwan et al. 2016b, c). Phosphorus plays an important role in cadmium uptake by plants (Wang et al. 2014). The high level of phosphorus present in the soil decreases the Cd content in root and shoots of cabbages. Phosphorus interacts with cadmium to form a cadmium-phosphorus complex and, bonding to the cell wall fractions, might be involved in the P-mediated lower cadmium uptake and translocation in cabbage (Qiu et al. 2011). However, this study also mentioned that the application of phosphate in the soil with the intension to reduce Cd uptake may vary with different species and genotypes as they adapted different mechanism to cope up the Cd induced toxicity.

Zinc (Zn) and Cd have similar oxidative status and they compete with each other during metals' uptake and translocation in plants. Cd concentrations decrease in cucumber seedlings through foliar spray and seeds soaked in Zn solution. The application of combination of foliar Zn and red mud in cucumber shoot shows decreasing Cd concentrations (Yang et al. 2011). The application of Cd and Zn (combined) in hydroponic solution increases the concentration of Cd and Zn in shoots and roots of bean plants (Chaoui et al. 1997). Tang et al. (2016) mentioned that the application of foliar Zn in plants under Cd stress condition might reduce the Cd concentrations. Lower dose of Cd and Zn concentrations applied in the soil of tomato, increased the concentration of Zn and decreased the Cd concentration (Mohammad and Moheman 2010). However, tomato growth was not hampered under Cd stress due to higher concentrations of Zn in plants. Application of Zn helps plants under Cd contaminated soils to reduce Cd content.

Iron (Fe) also is an essential micronutrient to plants for growth and development. Tomato seedlings were grown in hydroponic culture with different concentrations of Fe. Under Fe limiting condition, the pH of the nutrient solution significantly reduced as compare to control, which facilitates the Cd uptake by seedlings. The author's advice that Fe deficiency have changed the root exudation as a result enhancing the Cd availability in growth medium (Bao et al. 2010). The previous reports has been mentioned that Fe could reduce the Cd concentration in plants but identified the effect form of Fe to reduce the Cd uptake by plants.

Silicon (Si) is the second abundant element in soil but it is not an essential element for plants. However, a number of research works have mentioned that the application of Si on plants makes them tolerant against biotic and abiotic stresses (Adrees et al. 2015; Rizwan et al. 2016a; Rahman et al. 2017). Findings of Detmann et al. (2012) revealed that supplementation of Si results in significant modulation of primary metabolism, promotes amino acid remobilization in rice. There is evidence that suggests that in the presence of Si, a noteworthy reduction in arsenite (As) uptake and translocation was observed in rice. Antagonistic behavior of Si against As may be attributed to the common mode of uptake facilitated by NIPs or nodulin-26 like intrinsic proteins (Ma and Yamaji 2006; Raab et al. 2007; Bogdan and Schenk 2008). Though monosilicic acid is the only phytoavailable chemical form of Si (Epstein 2009), findings of Matsumoto et al. (2015) and Fleck et al. (2013) indicate that application of silica gel (@10.0 g Kg^{-1} as soil supplement can effectively reduce As content in aboveground biomass. Similar to Si, P, and Fe, S is also an essential element required for optimum plant growth. S plays an important role in minimizing As accumulation and subsequent translocation in plants (Muñoz-Bertomeu et al. 2009; Zhang et al. 2011b ; Dixit et al. 2015). Authors like Finnegan and Chen (2012) found that adequate S content (supplemented) becomes essential for plants to survive in As-contaminated environment. Song et al. (2010) observed that upon exposure to stressors like As, plants start producing ligands and phytochelatins (GSH and PC, respectively), generally facilitated by adequate S supply. The sulfhydryl of GSH and PCs facilitates the conversion of arsenate to arsenite and finally modulates the localization into the vacuoles. Supplementation with S (@ 5 mM) has been found to promote the As concentration in roots by stimulating thiolic ligand synthesis and subsequent increase in As complexation in roots, thus minimizing As translocation from roots to shoots (Duan et al. 2011; Dixit et al. 2015). The Si can also ameliorate Cd-induced toxicity in many plants like *Orzya sativa* (Chen et al. 2019), *Triticum aestivum* L. (Keller et al. 2015), tomato (Wu et al. 2015), cucumber (Feng et al. 2010), and cotton (Farooq et al. 2016). The exogenous application of Si to growth media enhances the growth, biomass, and photosynthesis, where it reduces chlorosis and protects chloroplast under Cd stress, in cucumber seedlings. While enzymes associated with nitrogen metabolism are also reported to be protected by Si under Cd stress in Cucumber (Feng et al. 2010). Si added into nutrient solution enhanced the growth and biomass of cucumber and tomato under Cd stress and significantly reduced oxidative stress and Cd accumulation by plants. Total organic acid content was increased in tomato leaves with Si application, whereas it was decreased in cucumber leaves and roots (Wu et al. 2015). This showed that Si involvement in enhancing Cd tolerance in tomato and cucumber is associated with different physiological responses of each of these plants. Similar result was found in two pakchoi cultivars; with the exogenous application of Si in aqueous media enhancing the tolerance and also decreasing the MDA and H_2O_2 content along with enhancing the antioxidant enzymes activities under Cd stress. Song et al. (2009) observed that Si also help to enhance the ascorbic acid, glutathione, and non-protein thiol content compared to Cd stress with or without. With the application of lower dose of Si (50 Kg^{-1}) in Cd-contaminated soil, Si did not change the soil pH and the accumulation of Cd increased in shoot and bulbs compared with Cd-stressed plants (Wu et al. 2016). The mentioned report showed that Si could alleviate Cd toxicity and decrease the Cd concentration by plants and become tolerant to Cd stress but its responses varied within varieties and cultivars. Most of the experiments were conducted in laboratory condition, hence there is a lack of soil or field studies regarding Si.

Se or selenium is an essential micronutrient for animals. The essentiality of Se for plants is still under debate, though supplementation of Se in small amount on alleviating various abiotic stress is well established in various plants. In the recent past, a few in vitro studies were carried out to investigate the consequences of priming rice seeds with Se on germination, seedling growth, and cultivation of Se-primed rice seedlings in As-contaminated environment, as an alternative mitigation option for the first time. Findings of Moulick et al. (2016a, 2017) suggest that priming rice seeds with Se before sowing can promote germination, seedling growth in both soil-based and soil-less condition by facilitating modulation in As-induced redox imbalance and minimizing As uptake. Whereas, cultivating Se-primed rice seedlings in As-contaminated pot soil, a contrasting As accumulation pattern noticed among Se primed and unprimed plants at maturity. It was found that that by facilitating As uptake in root but significantly reduce As translocation into the above ground portion the Se primed rice plants minimizes the As content in brown rice (Moulick et al. 2018a, b). Their findings also suggest that in the Se-primed seedlings beside restricting as translocation, the supplemented Se also promotes uptake sand translocation of other essential minerals such as Fe, Mn, Zn etc (Moulick et al. 2018c). At lower doses of Se, the growth and development of plants are enhanced but Se becomes toxic for plants and animals at high concentrations (El-Ramady et al. 2014). Many have reported that Se has great potential to reduce cadmium uptake and ameliorate the cadmium toxicity in plants (Hawrylak-Nowak et al. 2014). It has been noted that the exogenous application of Se reduces cadmium contents and enhances the growth, chlorophyll content, and photosynthetic activities in cucumber seedlings under cadmium stress. Se also influences the stress response related protein in cucumber seedlings. Application of Se and zinc reduced cadmium concentrations in cabbage and lettuce and the mineral status of plants was also better (He et al. 2005). In Garlic (Sun et al. 2010), broccoli (Pedrero et al. 2007), pepper (Mozafariyan et al. 2014), *Brassica juncea* (Ahmad et al. 2016), *Helianthus annus* (Saidi et al. 2014) it was found that Se induced plant growth, enhanced biomass, and reduced cadmium uptake under cadmium stress condition.

Pretreatment of seeds with 2% $CaCl_2$ helps to increase the shoot and root dry weight and lengths, total chlorophyll content and also soluble proteins content, and reduce oxidative stress induced by cadmium stress condition in faba bean (Issam et al. 2012). The exogenous application of K and Ca under Cd stress condition revived the morphological characteristics of root in soyabean, such as longer root length. It is found that due to the presence of Ca which help in reducing absorption of Cd by roots whereas it was also found that K did not have influence in cd uptake (Yang and Juang 2015). The exogenous application of Mg in Hoagland solution helps in enhancing the growth and reducing the cadmium uptake by plants as compared with cadmium-treated spinach seedling (Kashem and Kawai 2007). The application of S in the growth medium increases the biomass and mineral nutrient status in lettuce under cadmium stress condition (Matraszek et al. 2016). Sulfur also enhances the antioxidant enzymes activities under cadmium-treated spinach seedlings, as result reducing the oxidative stress.

5.2 Role of Soil Microorganism

Soil microorganisms used to detoxify As species through sorption at their extracellular surface which have uronic acids, proteins and amino sugars with a hydrogen bonding potentials. By employing the specific extracellular components (such as uronic acids, proteins and amino sugars) having high affinity to form hydrogen bonding and thus facilitating the absorption of As at their extracellular surface. Soil microbes (*Bacillus sp., Rhodococcus sp., Halobacterium sp.*) usually detoxify various forms As (Huang et al. 2014; Williams et al.2013; Yang et al. 2012). Whereas, the AMF or arbuscular mycorrhizal fungi also found to protect the crop plants from As induced adverse effects by down regulating the mRNA expression of OsLsi1 and OsLsi2 genes, as a result translocation of As also gets decreased in aerial part (Chen et al. 2012).

In recent years, many researchers have worked on the interaction of microbes, plants, and heavy metals. Microbes play a crucial role in plant growth and in counteracting heavy metals. Many researchers have stated that microbes could affect Cd uptake by plants (Khan et al. 2014; Kumar et al. 2015). Many microbes (bacteria and fungi) are reported to ameliorate Cd from contaminated soils. The application of arbuscular microrrhizal fungi (*Funneliformis mosseae, Glomus versiforme, and Rhizophagus intraradices*) on Cd-contaminated soils helped reduce Cd concentration in shoot of pakchoi as compared with control. The AMF helped to increase the soil pH, electrical conductivity, and metal content in organic matter and proportional Cd in the postharvest soil (Zhipeng et al. 2016). The inoculation of *G. versiforme* and *F. caledonium* in Cd-contaminated soil incremented P acquisition and biomass of plants and decremented the Cd content in both root and shoot in Cucumber. Among *G. versiforme* and *F. caledonium*, *G. versiforme* showed significant increase in the translocation from root to shoot of Cd as compared to *F. caledonium*, where it reduced the bioavailability of Cd and soil pH compared to control

(Hu et al. 2016). *Glomus* spp. are applied in different plants under Cd-contaminated environment and this AMF is shown to enhance the tolerance capacity in plants against Cd. *G. intraradices* in *Thlaspi praecox* (Vogel-Mikus et al. 2005) and *Salix viminalis* (Bissonnette et al. 2010), *G. versiforme* in *Solanum nigrum* (Liu et al. 2015), *G. etunicatum* in *Zea mays* (Huang et al. 2009), *G. geosporum* in *Aster tripolium* (Carvalho et al. 2006) are a few examples for *Glomus* spp. interacting with different plants under Cd stress. Similarly, other species are also reported like *Rhizophagus irregularis* in sunflower (Hassan et al. 2013), *Funneliformis mosseae* in *Oryza sativa* (Li et al. 2016a) and sunflower (Hassan et al. 2013). The inoculation of endophytic fungus in tomato plants results in reduction of Cd accumulation in root and mineral nutrition status compared with control under Cd stress condition (Khan et al. 2014).

5.3 PGPRs

Plant growth promoting rhizobacteria (PGPRs) are microbes that are able to ameliorate/reduce the heavy metals toxicity in plants along with enhancing growth and development. *Methylobacterium orzyae* strain *CBMB20* and *Burkholderia sp.* strain *CBMB40* are two PGPRs found in *Oryza sativa*. These two PGPRs help in the decrement of the Cd and nickel concentrations in tomato root and also reduce the Cd translocation to shoot in tomato, whereby enhancing plant growth under heavy metal stress condition (Madhaiyan et al. 2007). Under Cd stress condition, the application of PGPRs exogenously resulted in increment of plant growth, chlorophyll content, biomass, and mineral nutrients in *Glycine max*. Guo and Chi (2014) mentioned that these two PGPRs also help in increasing uptake of iron and decreasing the Cd content in *Glycine max*. *Pseudomonas* sp*., RJ10*, and *Bacillus* sp. *RJ16* are some Cd-resistant strains of bacteria that increase the concentration of Cd and lead in the aboveground tissue as compared to control in tomato (He et al. 2009).

5.4 Exogenous Application of Plant Growth Regulators

The application of PGRs could enhance plant tolerance to Cd stress through different mechanisms in plants. Salicylic acid (SA) is an endogenous phenolic plant growth regulator and plays a key role in plants in plant physiological processes. Studies conducted over the last few years have proved that SA becomes tolerant against abiotic stress, especially metal stress. Under Cd-stressed condition, SA-pretreated plants show enhanced growth, biomass, and chlorophyll content (Zhang et al. 2011a; Roychoudhury et al. 2016). Pretreatment of plant seeds with SA helps to reduce oxidative stress, which indicates that the level of MDA, H_2O_2, and proline content reduces in plants under Cd stress (Noriega et al. 2012; Koc et al. 2013). SA also enhances antioxidant enzymes activities in plants under Cd stress as compared to control and Cd-treated plants (Zhang et al. 2011a; Tamas et al. 2015). *Glycine max* (Noriega et al. 2012), *Hordeum vulgare* (Tamas et al. 2015), *Triticum aesativum* L. (Shakirova et al. 2016), *Vigna radiate* L.Wilczek (Roychoudhury et al. 2016), *Phaseolus aureus* and *Vicia sativa* (Zhang et al. 2011a), *Zea mays* (Szalai et al. 2013), *Oryza sativa* cv. Longai (Panda and Patra 2007) are some examples of how SA plays an important role against Cd stress in different plants. Abscisic acid (ABA) is popularly known as a stress hormone, which helps in tolerating metal stress including Cd stress (Pompeu et al. 2017). Many reports mention that ABA pretreatment of seedlings leads to decrement of the activities of SOD, CAT, POD, APx, GSH, and ascorbic acid in roots of *Vigna radiate* L. Wilczek under Cd-stress condition (Li et al. 2014). Exogenous application/pretreatment of seeds of ABA induces Cd content in *Oryza sativa* L. (Hsu and Kao 2003; Uraguchi et al. 2009) and *Brassica napus* (Meng et al. 2009).

Nitic oxide (NO) is a signaling molecule that regulates the growth and development processes. NO is also known to reduce oxidative stress under heavy metals including Cd stress (Piterkova et al. 2015). Several reports have concluded that the exogenous application of NO induces growth and biomass, and improves photosynthesis under Cd stress compared with respective control and Cd alone (Wang et al. 2016b). In addition, NO also has melioration mineral nutrient status, enhancing the antioxidant enzymes activities and reducing alternation of structure in leaves under Cd stress (Tran et al. 2013). *Cucumis sativus* L. (Yu et al. 2013), *Vigna radiate* (Nahar et al. 2016), *Oryza sativa* L (He et al. 2014), and *Cynodon dactylon* L. (Pers) (Shi et al. 2014) are few examples of nitric oxide (NO) protecting the plants against Cd toxicity.

5.5 Agronomic Interventions

To date, several intervention strategies have been investigated to find a suitable option in order to reduce transmission of As through field crops. Among the field crops, rice is considered as an efficient As accumulator due to its greater capability to translocate As from root to shoot (called translocation factor), when compared with the other crops. Besides, the prevalence of rice cultivation in “As - Hot Spot Areas” scattered throughout the world, especially in Asia, along with greater impact of rice in their respective economies, has added to the significance of rice (Moulick et al. 2016a, 2018d).

In order to minimize the accumulation and transmission of As through the rice plant and grain, one has to understand the various factors modulating As accumulation in the rice plant. The factors can be divided into four broad catagories: (a) soil and soil-related factors (such as pH, conductivity, available N:P:K, organic matter, presence of As, and other cationic and/or anionic species); (b) agronomic practice, i.e., the way in which farmers cultivate rice, whether by broadcasting the seeds directly to the field itself (DSR rice) or transplanting rice seedlings, cultivate rice by adopting SRI (systemic rice intensification) method or by any other method along with irrigation and fertilizer management; (c) concentration of As in irrigation water and mode of irrigation; (d) biological aspect including variety (hybrid/high yielding or local) as well as some associated factors such as previous experience of the variety to As stress, presence of other stressor (biotic), and the growth stage during which the variety experienced As stress.

Among the agronomic mitigation options tried for minimizing As content in grain, water management practices have a considerable share: aerobic rice cultivation (Xu et al. 2008; Sarkar et al. 2012), sprinkler irrigation and periodical irrigation (dry and wet condition) practices (Spanu et al. 2012; Barla et al. 2017), rice cultivation with raised beds (Duxbury and Panaullah 2007); intermittent or periodically flooding (Somenahally et al. 2011; Sarkar et al. 2012).

Many studies have been done on the application of different amendments for the ameliorating of contaminated soils (Huang et al. 2011; Li et al. 2016b; Ultra et al. 2016; Yin et al. 2016). The application of single or combined amendments helps to significantly reduce the availability of heavy metals in soils. The application of limestone, calcium, magnesium, phosphate fertilizer, silicon fertilizer, Chinese milk vetch, pig manure, and peat in copper and cadmium contaminated soils significantly reduce the concentration of copper and cadmium in rice roots, respectively (Lee et al. 2009). Ning et al. (2016) found that the application of 1% pulverous slag enhanced the soil pH and increases the silicon concentrations in the soil, and results in significantly decreasing the concentration of cadmium, copper, and zinc concentrations in rice tissue compared to control. Yang et al. (2016) reported that the application of rapeseed cake in the contaminated soil enhanced the growth of plants and also the formation of iron plaque on the rice root surface, which perhaps reduced uptake of cadmium by plants. The foliar application of silicon or potassium-rich fertilizer, which is absorbed rapidly, modified the antioxidant enzymes activities such as SOD, CAT, and POD and increased some mineral nutrients in plants, as a result of which rice became tolerant against cadmium (Feng et al. 2013; Wang et al. 2016b). Therefore, the foliar application of Si amendment (Cui et al. 2017; Yu et al. 2016, 2017) and Se amendment (Cui et al. 2018) helps to remediate soil contamination problems in paddy fields. Comparing Si-amendment and Si-amendment, the foliar application of Si-amendment is a more efficient method than Se-amendment of heavy metals remediation for contaminated soil.

Many studies have been reported the application of zinc in contaminated soil. This could enhance the grain yield and also growth on the basis of different types of zinc-containing fertilizers, mode of application, and responses of genotypes of maize and rice (Fahad et al. 2015). Among the various types of zinc treatment, rubber ash was applied in the cadmium-contaminated soil, enhancing the growth and decreasing the cadmium content in both crops (Fahad et al. 2015). Different concentrations of zinc were applied in cadmium-contaminated soil, resulting in enhancing the grain yield of yellow lupin depending on the manner of the dose (Brennan and Bolland 2014).

The foliar application of zinc helps to reduce cadmium content in many crops plants (Lv et al. 2019; Wang et al. 2018). For example, Saifullah et al. (2016) found that the foliar application of Zinc in wheat increases the grain yield and reduces the cadmium content in wheat plants. In case of brown rice, foliar spray of Zinc (3–5 g L^{-1}) significantly decreases cadmium concentration and enhances the zinc content (Lv et al. 2019; Wang et al. 2018). A field study showed that the spraying of $ZnSO_4$ on leaves has significantly reduced the concentrations of cadmium on rice grains in most cultivars, but has increased cadmium concentrations in some cultivars (Suo et al. 2012). Phosphorus (P) and Manganese (Mn) are also essential nutrients for plant growth and development. The foliar spray of Mn or P can reduce cadmium concentrations in plants (Zhang et al. 2011c; Zhou et al. 2013). Zhang et al. (2011c) reported that the spraying of 0.3% of KH_2PO_4 significantly reduced the cadmium concentrations of *Capsicum annuum* L. from contaminated soil where the foliar spray of $MnSO_4$ significantly decreased the cadmium concentrations in wheat grain (Zhou et al. 2013).

6 Conclusion

Arsenic- and cadmium-induced phytotoxicity in crop plants and its subsequent transmission to the food chain is an alarming issue. At present, the adverse effects associated with consuming As and Cd rich food either directly or indirectly have made the situation worse than we can imagine. Although considerable research has been carried out throughout the world, the complexity associated with mitigating the toxicity of the abovementioned toxicants is truly a challenge. So a comprehensive and collaborative research work among national and international research agencies to devise some suitable mitigation option(s) that are not only farmer-friendly but can be also broadly applicable across species are required. This chapter is a small effort to summarize the complexity and possibilities to mitigate As- and Cd-induced stress and its transmission.

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Plant–Microbe–Metal (PMM) Interactions and Strategies for Remediating Metal Ions

Rahul Mahadev Shelake, Rajesh Ramdas Waghunde, and Jae-Yean Kim

1 Introduction

Metals ions are an integral part of several key enzymes in all kingdoms and play a crucial role in central biological processes such as nitrogen fixation, photosynthesis, and respiration (Chandrangsu et al. 2017). All six classes of enzymes consist of metalloenzymes that are nonfunctional without metal cofactors (Table 1). The magnitude and necessity of metal ions in proteome of each organism is not the same and varies with the size of proteome (Waldron and Robinson 2009). Even though some organisms possess higher quantities of metal ions in their bodies, depending on their habitat, it does not imply they require those quantities of metal ions. Thus, only an appropriate concentration of bioavailable metal ions is essential for survival and reproduction of each species (Mahadev et al. 2013). In addition, bioavailability of all metal ions in excess can be harmful to cell growth and hamper physiological processes. In addition, some of the metal ions are always toxic and detrimental to living cells. Some examples include Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg), generally recognized as heavy metals (density higher than water, i.e., <5 g/cm^3) (Shelake et al. 2018). To retain the appropriate concentrations of each metal ion, living systems have evolved metal homeostasis mechanisms that not only regulate the bioavailable concentrations inside the cell but also help in metal ion import, acquisition by enzymatic machinery, and storage or export of excess amount of metal ions. In plant species, the amount of each toxic metal tolerated by plants is

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Table 1 Representative examples of metalloenzymes from all six classes

Enzyme Class	Reaction	Example	
		Enzyme	Cofactor
EC 1 (Oxidoreductase)	Electron transfer	Alchohol dehydrogenase	Fe, Zn
EC 2 (transferase)	Group transfer	Methionine S-methyltransferase	Mg, Zn
EC 3 (hydrolase)	Hydrolysis	Urease	Ni
EC 4 (lyase)	Join group by double bond	RuBisCO	Mg
EC 5 (isomerase)	Convert specific group to isomeric form	Xylose isomerase	Mg
EC 6 (ligase)	Joins two molecules by a chemical bond	E3 ligase	Zn

EC enzyme commission number

not the same and varies depending on the metal type and plant species (Van der Ent et al. 2013). Surplus amounts of metal ions affect the plant physiology by replacing key components in biomolecules, blocking enzymatic activities or altering the functioning of cellular pathways via stress signaling mechanisms, leading to reactive oxygen species (ROS) production in plant body.

Microbes cohabit with plants and continuously interact with each other in nature. Microbes form a symbiotic, commercialistic or parasitic association with plants, and thus, the investigation of plants and associated microbes, i.e., plant microbiota or microbiome, is gaining increasing attention in recent studies (Shelake et al. 2018). In addition, newer aspects of PMM studies include growth stimulation through metal hormesis, i.e., enhanced growth of plant by minimal dose of metal ion (Poschenrieder et al. 2013). This chapter is an overview of various metal homeostasis mechanisms in plants and microbes, factors affecting plant–microbe–metal (PMM) interactions and recent techniques for studying PMM interactions. Further, we summarize the sources responsible for higher bioavailable metal concentrations leading to pollution and potential use of plant–microbe metaorganism for metal remediation for higher crop productivity, and preserving sustainable environment.

2 Metal Homeostasis Mechanisms

The development of oxygenic photosynthesis and molecular oxygen released in the process has had a major impact on both the geosphere and the biosphere (Blindauer 2008). Hence, the evolution of photosynthetic cyanobacteria and the oxygenation status of oceans seem closely related and directly affect the speciation of metals. Most of the studies about evolution of metal ions and their utilization propose that the presence of oxygen decreases availability of some metal ions by reduction process, such as Iron (Fe), Cobalt (Co), Nickel (Ni), and Manganese (Mn).

Simultaneously, availability of some metal ions significantly increases, e.g., Zinc (Zn), Copper (Cu), and Cadmium (Cd). This scenario suggests that these changes in chemistry and bioavailability of metals might be responsible for biological evolution to a considerable degree, including that of the evolution of metal homeostatic machineries (Blindauer 2008; Mahadev et al. 2013).

Maintenance of an optimal bioavailable amount of each metal ion is mediated by the coordinated efforts between metal uptake, storage and efflux processes to fulfil the metal requirement of the living cell defined as metal ion homeostasis (Waldron and Robinson 2009) or simply called as metallostasis (Capdevila et al. 2017). Recent advancements in molecular biology and genome sequencing methods enable sequencing data of whole genome for several organisms that are available in the public domain, such as the NCBI database (Shelake et al. 2016). Bacteria and plants have evolved different sets of homeostasis mechanisms, which are discussed in the following section.

2.1 Bacteria

Bacterial metallostasis mechanisms comprehensively studied because of the tug-of-war for acquiring nutrients, comprising metal ions between bacterial pathogen and mammalian host (Capdevila et al. 2017; Zygiel and Nolan 2018). The metallostasis machinery in bacteria commonly can be divided into three categories depending on their functions (Ma et al. 2009). The first set of machinery includes the membrane transporters involved in uptake and efflux of metal ions across the cell membrane. Transport systems include proteins positioned on cell membranes (depending on species, it may include outer, inner or plasma membranes and additional lipopolysaccharide layers) that facilitate metal transport and release into the cytosol, e.g., ATP-binding cassette (ABC) transporters, P-type ATPases, and cation diffusion facilitator (CDF) proteins. The second category includes metal-chelator proteins (metallochaperones) and metal-storage proteins, both involved in maintaining intracellular concentrations. Metal-storage proteins, e.g., metallothioneins, differ from metallochaperones as their main role is in assisting cells to evade metal toxicity, whereas metallochaperones transfer metal ions to acceptor proteins, mostly apoenzymes, through intracellular trafficking (Capdevila et al. 2017).

The third category consists of metal-responsive transcription factors, also called metal-sensing proteins or metalloregulators. Based on names of founding members, metal-sensing proteins in bacterial systems are classified into seven major families (Giedroc and Arunkumar. 2007; Osman and Cavet 2010). These seven families consist of CsoR–RcnR, MerR, CopY, ArsR–SmtB, DtxR, Fur and NikR, which are further divided into three broad classes on the basis of their mode of regulating the transcription process (Fig. 1, modified and described in details by (Osman and Cavet 2010)). These family members differ in their metal preferences, mode of action, and in their structural features.

A

Founding Member	Additional Representives	Metals sensed
Fur (ferric uptake regulator)	Zur, Mur, Nur	Fe, Zn, Mn, Ni
DtxR (diphtheria toxin regulator)	Ide, MntR, ScaR, SirR, TroR	Fe, Mn, Cd, Zn
NikR (nickel-responsive regulator)	None	Ni
MerR (mercury responsive regulator)	ZntR, PbrR, CueR, GolS, CoaR	Hg, Zn, Cd, Pb, Cu, Au, Ag, Co
ArsR–SmtB (arsenical regulator, Synechococcus metallothionein)	CadC, AztR, ZiaR, CmtR, CzrA, MntR, KmtR, BxmR	As, Sb, Bi, Zn, Cd, Pb, Co, Cu, Ag
CsoR–RcnR (copper-sensitive operon regulator, regulation of cobalt and nickel repressor)	None	Cu, Ni, Co
CopY (copper-P-type ATPase regulator)	None	Cu

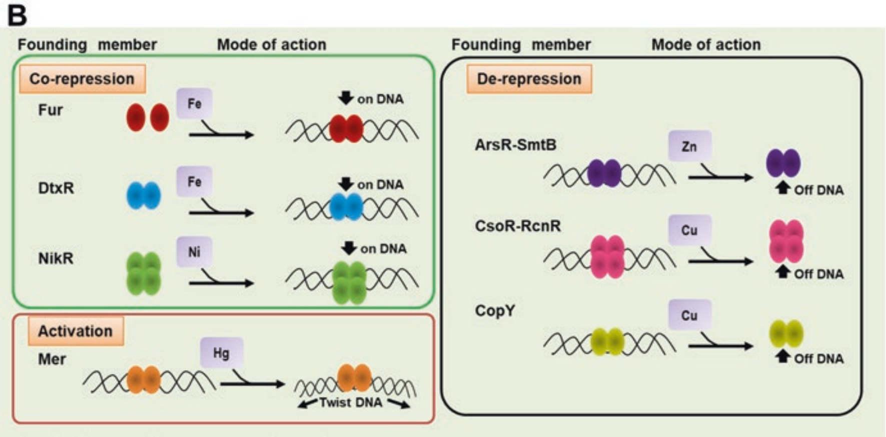

Fig. 1 Classification of bacterial metal-sensing proteins. (**a**) The seven major families of metal-sensing proteins also known as metal-responsive transcriptional regulators described with founding member, additional representative, and the metal sensed by each family member. (**b**) Three broader classes of bacterial metal-sensing proteins based on their mechanism of gene regulation

2.2 *Plants*

Plant systems have evolved more complex metal homeostasis pathways than prokaryotes. Different cell organelles in plants (e.g. mitochondria and chloroplasts) require metal ions (Mn, Cu, Zn, and Fe) for their regular activities (Bashir et al. 2016). Therefore, plant consists of various metallostasis mechanisms for metal uptake, translocation, sequestration, speciation, storage and toxic metal tolerance (Fig. 2). A recent review by Andresen et al. (Andresen et al. 2018) summarized updated protein network of trace metal metabolism in plant species. Plants consist of specialized proteins for each metal, and sometimes groups of metal ions possessing similar physicochemical properties often share metallostasis pathways. Plants obtain most of the minerals including metal ions via roots. ATPases and ABC transporters mediate primary active metal transport. Transporters, natural-resistance-associated

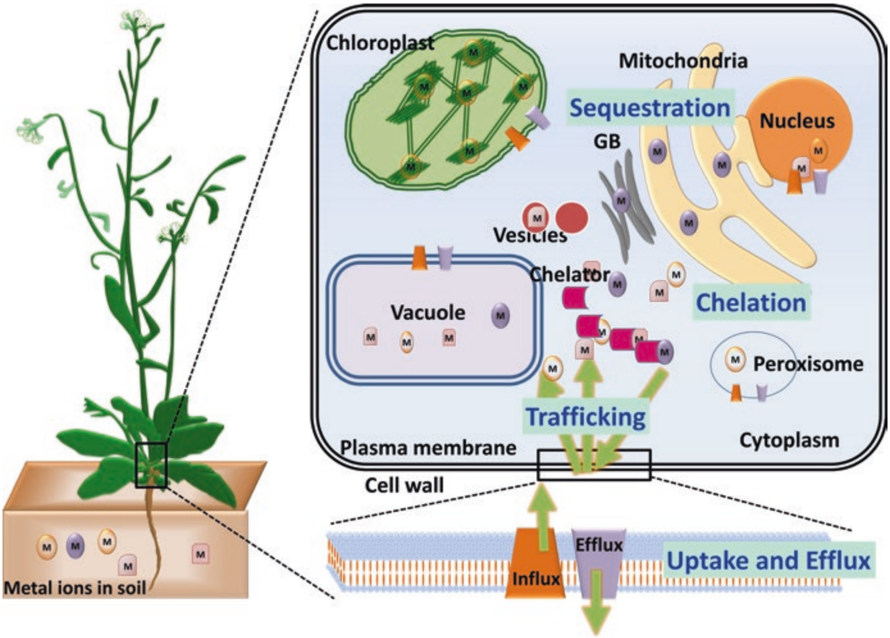

Fig. 2 Representative model describing metal homeostasis in plants

macrophage proteins (NRAMPs), ZRT/IRT-like proteins, and CDF proteins mediate the secondary active metal transport in plant systems. Another way for metal export and import across the cytosol occurs via exocytosis and endocytosis, respectively.

Primarily, plants produce two cysteine-rich polypeptides, i.e., phytochelatins (PCs) and metallothioneins (MTs), the main components that play a major role in tolerance against metal ion toxicity. The synthesis processes of PCs (manufactured by enzymatic procedures) and MTs (produced as proteins and encoded by DNA) are not the same but both possess similar functions (Joshi et al. 2016). Structural and functional aspects of both the compounds, PCs and MTs, are reviewed in our previous work (Shelake et al. 2018).

3 Factors Influencing Plant–Microbe–Metal (PMM) Interactions

Even though most studies focus on analyzing the effect of several factors on metal compositions in soil and plant samples, less attention is given to understand the correlation between factors altering metal bioavailability and its effects on plant–microbe interactions. Mostly, natural phenomena and anthropogenic activities determine the metal availability to plant and microbes, thus determining the nature and fate of the PMM interactions.

3.1 Metal Bioavailability and Speciation

The terms, metal bioavailability and metal speciation, are roughly defined and varies accordingly. Metal bioavailability is the metal fraction at the definite environmental unit in specific time point, either accessible or possible to make available for microbial or plant uptake from the ecosystem (Giller et al. 1998; Olaniran et al. 2013). Metal speciation is the documentation and measurement of the different forms or phases of metal ions exist in ecosystem. Therefore, total metal concentrations may not define the toxicity to biological systems but metal speciation and bioavailability decide the PMM interactions.

Due to the use of inconsistent methods and parameters for investigating the metal availability, speciation, and toxicity, it seems impractical to measure the bioavailable concentrations of each metal ion precisely to plant or microbe (Olaniran et al. 2013). Complexity of microbial diversity in soil, soil composition, plant-root exudates and plant–microbe interactions add more muddle while measuring dynamicity of bioavailable metal concentrations in soil. Natural processes, such as weathering of rocks, volcanic eruptions, and release of toxic gases, forest fire, and wind-dust towards agricultural areas from deserts under specific environmental conditions influences the bioavailable concentrations of each metal ion to plants and microbes. It also affects the total metal content in a specific land unit (Srivastava et al. 2017).

3.2 Edaphic Factors

One of the most studied factors affecting metal bioavailability comprises soil properties, e.g., organic matter content, pH, cation exchange capacity, clay, etc. (Rieuwerts et al. 1998). Metal solubility in soil solution generally reduces with an increasing pH, consequently decreasing the plant's metal uptake (Reboredo et al. 2019). The soil organic carbon (SOC) is a good indicator of soil health (Zornoza et al. 2015) andincludes a diverse mixture of organic materials, e.g., humus, microbial cells, charcoal, plant–animal residues, graphite, and coal (Shelake et al. 2019). Metal pollution affects the soil's microbial activities, thereby reducing the turnover rates of SOC and nitrogen (N) due to reduction in total plant biomass (Zhou et al. 2016). These detrimental effects of metal pollution on microbial diversity and SOC vary with climate, soil type, soil pH, and ecosystem types (terrestrial or agro-ecosystem). Also, soil formed from natural material, timespan of weather process, the kind of parental source, physico-chemical properties of soil, type of natural or agricultural vegetation, and the climatic changes tend to have considerable impact over the occurrence of metal compositions in soils (Arunakumar et al. 2013).

3.3 Human Activities

Human activities greatly affect the chemical forms of heavy metals in soils and consequently disturb the microbial diversity. Mining and smelting of metal ores, wastewater disposal from industries, and livestock grazing and manure causes increased amount of bioavailable heavy-metal content at the Earth's surface in urban and agricultural soils (Pourret et al. 2016; Srivastava et al. 2017). Some agricultural management practices, such as irrigation with metal-contaminated water bodies, and fertilizer applications also affect the metal redistribution in soil, plant, and microbes. Some chemical forms of pesticides (fungicides, insecticides, and herbicides) either directly consist of metals or alter the metal bioavailability in soils. A recent study reported higher metal content in agricultural soils and edible crops such as pumpkin, lettuce, spinach, common purslane (*Portulaca oleracea*), and potatoes collected from Terras da Costa, Portugal (Reboredo et al. 2019). The soil samples showed higher quantities of Mn, Cu, Fe, As, Zn, and Pb because of intensive application of chemical pesticides and fertilizers.

4 Techniques to Explore PMM Associations

4.1 Traditional Methods

Plant–microbe–metal interactions are generally studied using cultivation techniques in the laboratory, under greenhouse conditions, or directly in smaller agricultural lands. Such investigations provide valuable data on plant–microbial interactions, tolerance or accumulation levels of metals by plants, and microbial diversity, primarily in the rhizosphere (Prosser 2002). However, these techniques have some limitations and bias because of culture-media-controlled environmental factors (temperature, water, fertilizer, humidity, etc. (Singh et al. 2004).

4.2 Modern Tools

Modern biotechnology tools, for example, rRNA gene sequencing, polymerase chain reaction (PCR)-based techniques together with DNA fingerprinting techniques (DDGE—denaturing gradient gel electrophoresis), fluorescence in situ hybridization (FISH), terminal restriction fragment analysis (T-RFLP), and amplified rDNA restriction analysis (ARDRA) offer truthful evidence about the microbial species involved in PMM associations (Singh et al. 2004). Omics tools like ionomics (changes in uptake of metal ion), transcriptomics (gene expression changes), proteomics (protein expression changes under metal stress), and metabolomics (changes in metabolite composition) provide molecular framework and further expand the PMM knowledge (Basu et al. 2018). Additionally, mass spectrometry (MS) techniques in combination with next-generation sequencing (NGS) address metal-associated plant–microbe interactions and other aspects of metallostasis and metal remediation. The recently emerged genome editing (GE) technique has huge potential to alter the genes involved in the metallostasis pathways of plants or microbes in vivo. Such GE studies together with NGS and MS contribute detailed information on PMM associations.

5 Metal Pollution and Remediation Strategies

Metal toxicity and pollution are a major concern for the environment and human health. Metals are not biodegradable like some organic pollutants, and hence difficult to remove from polluted sites (Etesami 2018). Owing to these characteristics, bioavailable metal ions accumulated in the soil enter the food chain through edible plants and drinking water (El-Kady and Abdel-Wahhab 2018). Metal remediation can be defined as the removal of toxic metal ions from the environment using diverse tools and techniques. Selection of the most suitable technique for metal remediation is complicated due to economic, ethical, environmental, technological, and social considerations as well as impact on human health (Diep et al. 2018). Thus, there is a need to develop alternative techniques so that choice can be made depending on the suitability and applicability under defined conditions.

6 Applications of Plant–Microbe Interactions for Metal Remediation

6.1 Natural Hyperaccumulators

Phytoremediation, i.e., metal remediation by plants involves many mechanisms, such as phytostabilization, phytovolatilization, phytoextraction, and phytodegradation (Basu et al. 2018). Plant species that accumulate metals to remarkably high amounts in their bodies, which may be several (hundreds or thousands) times higher than the amount of metal in most plant species, are known as hyperaccumulator plants (Van der Ent et al. 2013). Criteria to categorize a plant species as hyperaccumulator are well defined in recent times and minimal-threshold levels accumulated in foliar dry weight varies for each metal ion (Reeves et al. 2018). When plants grow in the natural habitat, the metal content in dry weight of leaf tissues is higher than 100 μg/g cadmium, selenium, thallium; 300 μg/g chromium, cobalt, copper; 1000 μg/g rare earth elements, arsenic, nickel, lead; 3000 μg/g zinc; and 10,000 μg/g manganese, and are recognized as hyperaccumulator species. Hyperaccumulator plants showing the highest accumulation of heavy metals are listed in Table 2 adopted from Reeves et al. (2018).

Table 2 List of hyperaccumulator plants showing the highest accumulation of heavy-metal ions

Plant	Metal (threshold in μg/g)	Reference
Arabidopsis halleri	Cd (>1000)	Stein et al. (2017)
Pteris vittata	As (>100)	Ma et al. (2001)
Aeolanthus biformifolius	Cu (>300)	Malaisse et al. (1978)
Haumaniastrum robertii	Co (>300)	Brooks (1977)
Virotia neurophylla	Mn (>10,000)	Jaffré (1979)
Berkheya coddii	Ni (>1000)	Mesjasz-Przybyłowicz et al. (2004)
Noccaea rotondifolia subsp. cepaeifolia	Pb (>1000)	Reeves and Brooks (1983)
Dicranopteris linearis	La and Ce (>1000)	Shan et al. (2003)
Astragalus bisulcatus	Se (>100)	Galeas et al. (2006)
Biscutella laevigata	Ti (>100)	LaCoste et al. (1999)
Noccaea caerulescens	Zn (>3000)	Reeves et al. (2001)

A database of known hyperaccumulator plants is useful and may provide a common platform for relevant information on specific plant types for further research (Reeves et al. 2018). A recently developed online database—Global Hyperaccumulator Database (www.hyperaccumulators.org)—provides free access to everyone. It consists of detailed information about distribution, analytical data, collection records, ecology, taxonomy, and literature citing the species' characterization.

6.2 *Engineered Plants*

Some modern techniques like plant tissue culture (PTC) as model systems (cell suspensions, callus, and hairy root culture) in bioremediation studies are frequently used due to several benefits (Doran 2009). The PTC techniques are advantageous in identifying efficacy of plant species in metal uptake without interference of other nutritional or environmental factors. On the other hand, it is also a major limitation of using data from PTC technique in metal remediation studies. Biochemical parameters are strictly monitored in PTC whereas microbial diversity and mixture of metal contaminates are beyond the human control in open-field experiments.

Recently, the engineering of plants for metal remediation has been reviewed and discussed by several groups (Fasani et al. 2018; Ojuederie and Babalola 2017; Wani et al. 2018; Yadav et al. 2018) and some examples of different plants reported are summarized in Table 3. Tools such as biotechnology, genetic engineering, and genome editing, help in the identification of novel genes, pathways, and obtaining systematic knowledge of plant-microbial metal tolerance at the molecular level (Wani et al. 2018). The transporters across the membrane of vacuole play a crucial role in Cd and As sequestration and detoxification inside the plant cell (Zhang et al. 2018a). Targeting vacuolar transporters could be a promising biotechnological approach to engineer plants free from metal contamination.

Table 3 Engineered plants and plant–microbes associations showing potential in metal remediation or metal-stress tolerance

Plant	Gene/microbe	Metal	Reference
Engineered plants			
Tobacco	*AtPCS1/CePCS1*	As	Gielen et al. (2017)
Petunia	*RsMYB1*	Cu, Zn, Mg, Cr	Ai et al. (2018)
Rice	*TaPCS1*	Cd	Mayerová et al. (2017)
Sedum alfredii	*ATPase 3 (HMA3)*	Cd	Liu et al. (2017)
Arabidopsis	*NnPCS1*	Cd	De Araújo et al. (2017)
Poplar	*TaPCS1*	Pb, Zn	Gong et al. (2017)
Rice	*HsCIPKs*	Hg, Cd, Cr, Cu	Pan et al. (2018)
Tomato	*HsfA1a*	Cd	Cai et al. (2017)
Arabidopsis and poplar	*PtABCC1*	Hg	Sun et al. (2018)
Canola	*OsMyb4*	Cu and Zn	Raldugina et al. (2018)
Arabidopsis	*VsCCoAOMT*	Cd	Xia et al. (2018)
Arabidopsis	*VsPCS1*	Cd	Zhang et al. (2018b)
Rice	*HvYS1*	Zn, Mn, Cu	Banakar et al. (2017)
Arabidopsis	*TpNRAMP5*	Cd, Co, Mn	Peng et al. (2018)
Arabidopsis and tobacco	*MnPCSs*	Zn, Cd	Fan et al. (2018b)
Arabidopsis	*MsYSL1*	Cd	Chen et al. (2018)
Arabidopsis	*BpPMSR3*	Cd	Han et al. (2018)
Arabidopsis	*OsPRX38*	As	Kidwai et al. (2019)
Plant-microbe associations			
Sedum alfredii Hance	*Pseudomonas fluorescens* Sasm05 and IAA	Zn, Cd	Chen et al. (2017)
Rice	Several rhizosphere bacterial genera	Cd	Wang et al. (2019)
Rice	Sulfate-reducing bacterium (SRB1-1)	Cd, Pb	Shan et al. (2019)
Rice	Alishewanella sp. WH16-1	Cd	Shi et al. (2018)
Pennisetum purpureum	*Klebsiella variicola*	Pb	Das and Osborne (2018)
Rice cultivars	Several rhizosphere bacterial genera	Cd	Hou et al. (2018)
Robinia pseudoacacia	Several rhizosphere bacterial genera, arbuscular mycorrhizal fungi		Fan et al. (2018a)
Wheat	*Enterobacter ludwigii* CDP-14	Zn	Singh et al. (2018)
Eichhornia crassipes (Mart.) Solms	Consortia of *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, *Actinomyces*	As	Kaur et al. (2018)
Sunflower and tomato	*Pseudomonas* sp. (strain CPSB21)	Cr	Gupta et al. (2018)
Rice	*Pseudomonas aeruginosa*, *Bacillus cereus*	Cd, Pb	Nath et al. (2018)
Wheat	*Ralstonia eutropha* Q2-8	Cd, As	Wang et al. (2018)
Soybean	*Kocuria rhizophila*	Multi-metal	Hussain et al. (2019)
Mung bean	*Acinetobacter lwoffii*	As	Das and Sarkar (2018)

6.3 Plant–Microbe Interactions in Metal Remediation or Metal-Stress Tolerance

The network of mechanisms contributing to metal tolerance or avoidance in PMM interactions, promotion of plant growth, and the possible modes of PMM interactions in metal remediation are described in Fig. 3 (Shelake et al. 2018). The mechanisms of metal detoxification are generally classified into two broad groups. The first group contains the mechanisms that help plants avoid metal stress through higher sequestration of necessary nutrients or greater production of biomass. The second group comprises the strategies that help plants tolerate metal stress by any of the processes of metal detoxification (bioaccumulation, translocation, immobilization, mobilization, etc.). Beneficial PMM interactions for heavy-metal stress and tolerance have been reported in previous studies (Rajkumar et al. 2012; Shelake et al. 2018). Recent studies on beneficial PMM interactions for heavy-metal stress and tolerance published during the last couple of years are summarized in Table 3. Utilizing the natural ability of PMM interactions is a promising way to develop novel metal-remediation strategies in agricultural soils.

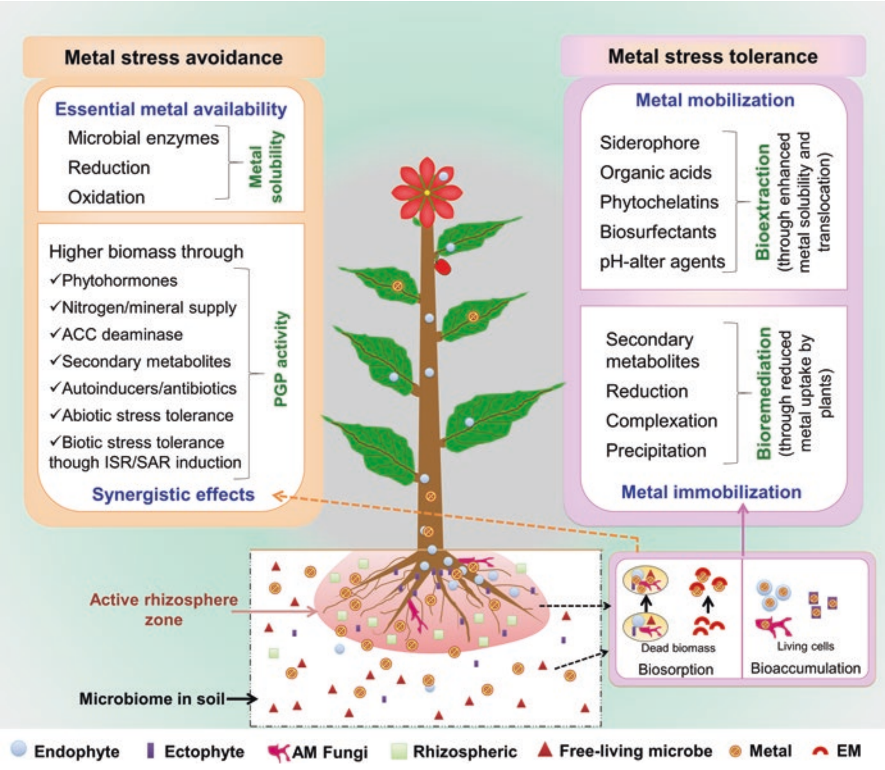

Fig. 3 Advantageous plant–microbe–metal (PMM) interactions. (Adapted from (Shelake et al. 2018) with permission)

Endophyte microbes and plant roots secrete several chemical compounds (inorganic and organic), thereby modifying the bioavailable metal concentrations to plant and microbiome in the surrounding (Rajkumar et al. 2012). Shift in chemical forms of metal occurs due to diverse geochemical-biophysical mechanisms like metal chelation, metal transformation, metal immobilization, metal solubilization, metal precipitation, volatilization, translocation, etc. (Waghunde et al. 2017). This way is safer than using engineered microbes or transgenic plants, with fewer environmental and ethical concerns.

7 Conclusions and Future Prospectives

In this chapter, we provided an overview on the basics of metal homeostasis mechanisms in microbes and plants. We addressed the factors affecting the metal bioavailability to microbes and plants, including metal speciation, edaphic and anthropogenic factors. Moreover, synopsis about traditional and modern techniques to study PMM interactions and its use in metal remediation strategies are helpful to understand novel facets of PMM interactions. Modern approaches for metal remediation involve the application of chemical, transgenic, and synthetic nanoparticles, which are costly or unpredictable in terms of their effect on nature and human health in the longer term. This does not imply elimination of modern methods such as nanoparticle, chemical, or transgenic technologies. Appropriate combination of bioremediation tools will help effective application in metal-remediation technology. Thus, exploiting PMM interactions through the use of metal-resistant bacteria in metal remediation or their use to confer tolerance or to enhance uptake capacity to plants will deliver promising technologies for a toxic-metal-free and sustainable environment.

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Phytostabilization of Heavy Metals: Understanding of Principles and Practices

A. M. Shackira and Jos T. Puthur

1 Introduction

The use of green plants in the restoration of contaminated sites is generally termed as phytoremediation, a technique that evolved during the last decades of the twentieth century (Salt et al. 1995). Even though the term "phytoremediation" was coined in the earlier 1990s, the concept of using plants to clean up contaminated environments dates back to 300 years. The first plant species documented for the bioaccumulation of heavy metals were *Thlaspi caerulescens* and *Viola calaminaria*, and the credit for this goes to Baumann (1885) at the end of the nineteenth century. Later in 1935, Byers reported that plants coming under the genus *Astragalus* are capable of accumulating up to 0.6% Se in the tissue. One decade later, Minguzzi and Vergnano (1948) identified some plants which are able to accumulate nickel (Ni) in shoots. Later on, Rascio (1977) reported the potential of *Thlaspi caerulescens* toward zinc (Zn) accumulation and tolerance.

The idea of using plants to remove metals from contaminated soil was further developed by Utsunomiya (1980) and Chaney (1983), and the first field trial on Zn and cadmium (Cd) phytoextraction was conducted by Baker et al. (1991). Following Baker till date, there were enormous reports on the heavy metal remediation technique using a number of plants (Salt et al. 1995; Clemens 2001; Suresh and Ravishankar 2004; Lone et al. 2008; Lutts and Lefévre 2015; Devi et al. 2016). These findings throw light to several similar studies, and now a number of plants have been identified as metal tolerators or as metal accumulators.

Phytoremediation in its sense is a very broad technique including several kinds of remediation alternatives (rhizofiltration, phytoaccumulation, phytoexcretion, phytostabilization, and phytovolatilization), and plants should be selected as per the requirement of the site. When an area is only slightly contaminated, plants with a

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strong ability to take up metals from soils can be utilized. These accumulated metals can either be stored inside the plant tissue, a process termed phytoaccumulation, or may exclude from the tissue, processes termed phytoexcretion and phytovolatilization. However, when dealing with more heavily polluted sites, like sites for the disposal of mine tailings, plants that do not transport the metals to the shoots, but instead bind them in the root or the rhizosphere, are preferred. This approach is termed phytostabilization (Wong 2003; Zeng et al. 2018).

Phytostabilization is a less invasive, low-cost phytotechnology which uses green plants to stabilize the toxic metal ions within the root or near the rhizosphere. It is now widely accepted as a means to decontaminate and restore the physical, chemical, and biological characteristics of the contaminated soils (Bolan et al. 2003; Kumpiene et al. 2008). Phytostabilization is not actually physically removing the contaminants from the soil, but rather causes the deactivation and immobilization of the potential ions, thereby preventing the further movement of the same to the food chain. The term phytostabilization thus refers to the use of pollutant-tolerant plants for mechanical stabilization of polluted land in order to prevent bulk erosion, reduce airborne transport, and leach pollutants (Fitz and Wenzel 2002; Boisson et al. 2016). It is mostly used for the remediation of soil, sediment, and sludges (USEPA 2000) and depends on roots' ability to limit contaminant mobility and bioavailability in the soil. The main objectives of phytostabilization have been summarized by Vassilev et al. (2004) as follows:

(i) To change the trace element speciation in the soil aiming to reduce the solubility and exchangeable fraction of these elements
(ii) To stabilize the vegetation cover and limit trace element uptake by crops
(iii) To reduce the direct exposure of soil-heterotrophic living organisms to pollutants
(iv) To enhance biodiversity by limiting the metal mobility

2 Phytostabilization Criteria

Phytostabilization can be applied to fields with varying level of contamination and also with different soil texture, i.e., features like soil pH, salinity, and metal levels, and contaminant types may vary from field to field. Thus, phytostabilization efficiency can be enhanced only through the careful selection of the appropriate plant species and also of the applied amendments particular to the field selected (Berti and Cunningham 2000). There are thus two major components in the phytostabilization process to be considered: the plant itself and the amendments added to the system.

2.1 Selection Criteria of Plants for Phytoremediation

For the effective phytostabilization process, selection of plant is a crucial step, and some important criteria must be considered before selection. Plants characterized as suitable candidates for phytostabilization must be native to the contaminated area and be tolerant to other stress factors like drought, salt, and metal and also must limit the metal accumulation to the shoot (Mendez and Maier 2008). In other words, plants that over-accumulate toxic trace metals in their roots, excluding or limiting translocation to shoots, can be regarded as efficient phytostabilizers (McGrath et al. 2002; Maestri et al. 2010). Moreover, it was suggested that plant species with high bioconcentration factor or BCF_{root} (>1) and low translocation factor or TFs (<1) could be considered as a potential candidate for the phytostabilization (Yoon et al. 2006; Meng et al. 2013; Shackira and Puthur 2017). Additionally plants used for phytostabilization need to exhibit tolerance to multiple metals and metalloids present in the sediments (Fitz and Wenzel 2002).

To meet the complete objectives of phytoremediation (i.e., to decrease water and soil pollution), the selected plants should also be native to the region that needs to be depolluted, grow quickly, and have dense root and shoot systems preventing heavy metal dispersion by water and/or wind erosion (Berti and Cunningham 2000; Freitas et al. 2004; Ali et al. 2013). Besides the plants must possess a large quantity of propagules (Henson et al. 2013) and should preferentially disperse by seeds to allow the implementation of phytostabilization on a large scale (Mench et al. 2006; Bert et al. 2008). It has been also reported that the reclamation methods for phytostabilization require elevated seed production, which usually results in a more continuous vegetation cover (Mendez and Maier 2008; Mench et al. 2010). Finally, species need to have the potential to promote soil development process by a long-term succession in the polluted areas (Mendez and Maier 2008).

To conclude, selection of a plant species for phytostabilization of heavy metals should take into consideration the following features:

(a) Plants should be tolerant to the soil conditions.
(b) Plants must grow quickly to set up a ground cover.
(c) Plants should have dense rooting systems.
(d) Plants must be easy to establish and to maintain under field conditions.
(e) Plants must have a relatively long life or be able to self-propagate (Berti and Cunningham 2000; Mendez and Maier 2008).

2.2 *Soil Amendments*

The rate of phytostabilization can be amplified when used in combination with certain soil amendments which facilitate trace metal immobilization in the soil, and this remediation strategy is known as aided phytostabilization (Alvarenga et al. 2009). The final aim is to reduce metal mobility in the substrate, and numerous organic and inorganic materials have been used to reduce solubility and bioavailability of heavy metals. These include liming material, phosphate, compost, biosolids, zeolite, and aluminosilicates such as bentonite, fly ash, etc. (Bolan et al. 2003; Kumpiene et al. 2008; Mench et al. 2010). Inclusion of such amendments in the substrate will then improve the nutritional status of the rooting medium, facilitate plant establishment in

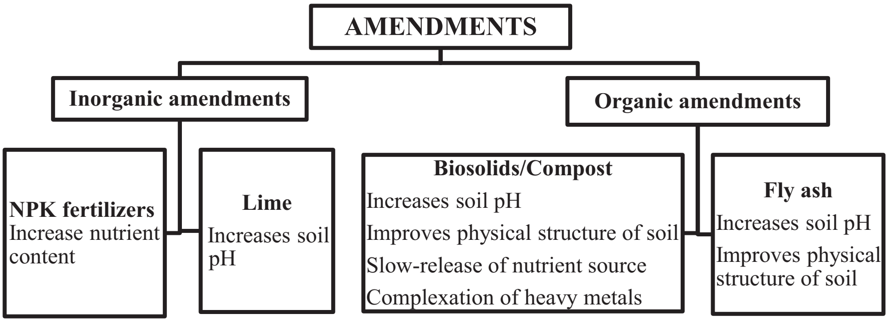

Fig. 1 Various organic and inorganic amendments used for increasing the rate of immobilization of toxic metal ions in the phytostabilization process

a more vigorous manner, supply plant nutrients in a slow-release form, and reduce metal leaching to the belowground level (Kumpiene et al. 2008).

Incorporation of organic additives to the contaminated soils induces numerous changes in the chemical (e.g., pH, organic acids, soil solution composition) and biological (e.g., microbial diversity) properties of soil (Perez-de-Mora et al. 2006; Stanczyk-Mazanek and Sobik-Szoltysek 2010). Amendments added to the soil thus convert the soluble and pre-existing high-soluble solid phase forms to more stable solid phases resulting in a reduced biological availability and plant toxicity of heavy metals. A decrease in the mobile trace element pool in soil promoted by amendments allows the settlement and the growth of vegetation as well as ecosystem restoration on highly contaminated sites (Ruttens et al. 2006). Specific functions of organic and inorganic amendments used in phytostabilization process are listed in Fig. 1.

3 Mechanism Underlying Phytostabilization

During phytostabilization, plants accumulate metals within root tissue or near the rhizosphere and are able to reduce the mobility or bioavailability of metals by stabilizing it in the substrate and/or by accumulating within the root. The higher accumulation of metals in roots may further decrease the mobility of metals in sediment (Nedjimi and Daoud 2009). The basic mechanism underlying phytostabilization depends on various factors like microorganisms present in the rhizosphere, root exudates, cell wall binding of metal ions, chelation of metal ions by metal-binding molecules, and their eventual sequestration into the vacuoles. The basic mechanism underlying phytostabilization includes the trace element mobility in the rhizosphere and is controlled by various soil factors such as pH, organic matter, texture, redox potential, and temperature and also by microbes (Chaignon and Hinsinger 2002). Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction (Ghosh and Singh 2005).

3.1 Role of Microorganisms

Under natural conditions plant roots are exposed to a large number of different microorganisms which play crucial roles in the recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests and pathogens (Filip 2002). Association between plant and microbes may be specific or non-specific, i.e., in specific association, plants provide carbon source that helps bacteria to reduce the toxicity of the contaminated soil, and in non-specific association, various metabolic activities of plants increase the growth of microbial community, which then degrades the contaminants entrapped in soil (Kushwaha et al. 2015). Thus, the microorganisms (bacteria and mycorrhiza) living in the rhizosphere of plants not only actively contribute to change the metal speciation, but they can also assist the plant in overcoming phytotoxicity, thereby aiding the revegetation process (van der Lelie et al. 1999).

It has been reported that the addition of bacteria like *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, *M. arabinogalactanolyticum*, *Alyssum murale*, etc. to the soil significantly increased the phytoavailability of heavy metals including Ni by reducing the soil pH, thereby facilitating phytostabilization (Abou-Shanab et al. 2003). Similarly, *Brassica napus* plants were inoculated with different kinds of bacteria like *Pseudomonas chlororaphis*, *Azotobacter vinelandii*, *Bacillus mucilaginosus*, and *Microbacterium lactium* for the increased tolerance toward various heavy metals (Wu et al. 2006; Ma et al. 2009; He et al. 2010). Different *Glomus* spp.-mediated phytostabilizations of heavy metals like Zn and Cd in *Zea mays* L. have been reported earlier by Janeeshma (2015).

3.2 Complexation with Root Exudates

Exudates secreted by roots affect the solubility, mobility, and phytoavailability of metal ions which play crucial role in phytostabilization (Colzi et al. 2012). About 12–40% of the photosynthates transported from the leaves to the roots are released into the rhizosphere during plant development naturally. These exudates may include various kinds of sugars, polysaccharides, organic and amino acids, peptides, and proteins depending upon the plants (Lin et al. 2003; Hinsinger et al. 2006). Root exudates may be grouped into two, high-molecular-weight (e.g., mucilages including polysaccharides, polyuronic acid, and ectoenzymes) and low-molecular-weight (e.g., organic acids, sugars, phenols, and various amino acids including nonprotein amino acids, such as phytosiderophores) compounds. Root exudates play a significant role in the process of phytostabilization by enhancing the accumulation, stabilization, or volatilization of contaminants from soil (Kushwaha et al. 2015).

In addition to the above facts, root exudates constitute an efficient energy sources for microorganisms present in the soil and act as ligands for binding heavy metal ions which ultimately influence the pH of the rhizosphere. The change in soil pH influences the mobilization of metals in soils and their accumulation by plant roots. Thus, roots can indeed modify the trace element mobility by changing soil pH and electrochemical potentials through element sorption in the apoplast or their rhizodeposition/complexation in the rhizosphere (Hinsinger 2001; Lombi et al. 2001; Chaignon and Hinsinger 2002).

Stabilization of toxic metal ions in the rhizosphere by root exudates without uptaking it into the root plays a crucial role in the phytostabilization process. Graminaceous plants secrete an amino acid compound, namely, phytosiderophore which can form a stable complex with iron (Fe), Cd, Zn, and copper (Cu) (Chaignon et al. 2002; Xu et al. 2005). Nair et al. (2008) have also reported the role of siderophores secreted by plant roots in arsenic (As) immobilization, thereby detoxifying the toxicity. Similarly, organic acids like oxalic acid, malic acid, citric acid, etc. secreted by wheat plants prevent the entry of Cd^{2+} into roots (Kushwaha et al. 2015). There are also reports stating that the heavy metals especially Ni- and Zn-chelating histidine molecules along with citrate accumulate in root exudates and help to reduce the uptake of the same inside the cell (Salt et al. 2000; Hall 2002).

3.3 Cell Wall Binding

From the soil, heavy metals can enter the plant root cell either by simple passive diffusion through the plasma membrane or by active metal uptake via special metal transporters. There are enormous reports stating that cell wall is recognized as one of the main compartments for heavy metal accumulation in plant roots (e.g., Małecka et al. 2008; Meyers et al. 2009; Konno et al. 2010). Plant cell walls are rich in pectin compounds, which are able to bind divalent and trivalent metal cations, and the main pectin domain in the cell wall responsible for binding metal ion is homogalacturonans (HGA) (Pelloux et al. 2007; Caffall and Mohnen 2009). The physiological advantage of metal binding with the HGA of cell wall is the metabolic inactivation of the absorbed metal ions within the apoplast itself there by reducing the toxicity (Jiang and Wang 2008).

Bringezu et al. (1999) have reported that different heavy metals were accumulated in the epidermal cell walls of heavy metal-tolerant *Silene vulgaris* ssp. *humilis*. Moreover, it has been reported earlier that lead (Pb) can bind efficiently with the carboxyl group of pectin in the cell wall and is considered as the most important reliable interaction by which a plant can tolerate Pb toxicity (Meyers et al. 2008; Jiang and Liu 2010). Moreover, increased content of various metals including Fe, Cu, Zn, and Pb have been observed in the cell walls of *Minuartiaverna* sp. *hercynica* growing on heavy metal-contaminated mine tailings (Solanki and Dhankhar 2011; Kushwaha et al. 2015). Enhanced pectin level in the roots of Cu-tolerant *Silene paradoxa* has resulted in binding of Cu at a higher rate to the root cell wall, thereby restricting Cu accumulation within the roots itself (Colzi et al. 2012).

3.4 Chelation with Metal-Binding Molecules

Metal ions once enter to the cytosol may be sequestered into the vacuole via chelation of metal ions by organic acids, amino acids, peptides, or metalloproteins, thereby providing greater resistance to the toxicity of heavy metals. The two best-characterized heavy metal-binding ligands in plant cells are the phytochelatins (PCs) and metallothioneins (MTs). These include different classes of low-molecular-weight, cysteine-rich peptides or polypeptide molecules which have a high affinity to various heavy metals and are synthesized inside the plant cell (Hall 2002).

The synthesis of PCs in plants is triggered by the presence of heavy metals. Heavy metals induce the activation of an enzyme, namely, phytochelatin synthase, which acts upon a glutathione substrate so as to produce PCs (Cobbett and Goldsbrough 2002; Suresh and Ravishankar 2004). It has been reported earlier that Pb and mercury (Hg) exposure in a halophyte *S. salsa* was found to significantly enhance the mRNA expression of *SsPCS*. *SsPCS* is the second *PCS* gene cloned from a halophyte, and it might contain a different metal sensing capability than the first *PCS* from *Thellungiella halophila* (Cong et al. 2016).

Plant MTs have been classified into Classes I, II, and III based on the arrangement of Cys residues. Most of the plant MTs are Class I proteins containing two smaller Cys-rich domains and a large spacer region devoid of this amino acid. In Class II MTs, Cys residues are distributed in a scattered manner in the entire protein sequence. Class III MTs differ markedly from Class I and II MTs and are enzymatically derived (Usha et al. 2009). Expression analysis of different metallothionein genes was studied by several groups in different plant species. Significant increase in the transcripts of AmMT2 gene in *Avicennia marina* plants was reported by Huang and Wang (2010) in response to Zn, Cu, and Pb. Similarly in *Bruguiera gymnorrhiza*, remarkable increase was found in the transcript level of BgMT2 in response to Zn, Cu, and Pb in leaves (Huang et al. 2011). Chen et al. (2014) studied the metallothionein gene (KoMT2) expression in the leaves of *Kandelia obovata* seedlings exposed to Cd stress and the expression levels of the gene was found to be increased. The overall mechanism underlying phytostabilization has been elucidated in Fig. 2.

4 Pros and Cons of Phytostabilization

Phytostabilization, the approach of using green plants for in situ stabilization/decontamination of metal wastes, is a feasible alternative to other costly remediation practices. As mentioned earlier, plants play an important role in phytostabilization by protecting the soil surface and also by physically stabilizing the soil with dense root systems to prevent erosion. Plant roots also help to minimize water percolation through the soil, further reducing contaminant leaching to the belowground part (Berti and Cunningham 2000). In addition, plant roots can also provide surfaces for sorption

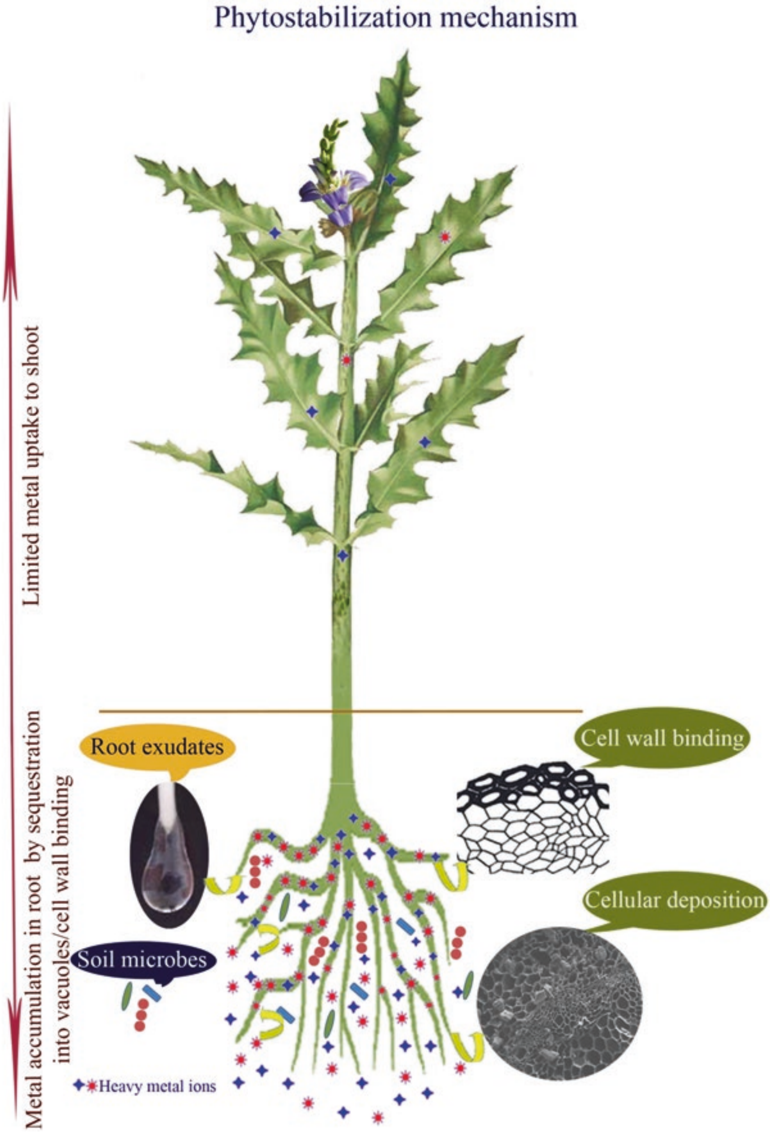

Fig. 2 General mechanisms of phytostabilization of heavy metals

or precipitation of trace metal elements, thereby playing an important role in groundwater protection and reduction of metal dispersion (Ruttens et al. 2006).

Phytostabilization is employed to treat polluted soils involving the establishment of a persistent plant cover that prevents pollution spread by erosion, water percolation, leaching, and wind dispersal of toxic dust (Berti and Cunningham 2000; Wong 2003). Phytostabilization can also diminish the metal bioavailability by their precipitation to less soluble compounds (Wong 2003). Vegetative stabilization improves the chemical

Table 1 Major advantages and limitations of phytostabilization technique

Sl no.	Advantages	Limitations
1	Disposal of polluted biomass is not required	The contaminants remain in place
2	Effective immobilization reduces leaching and bioavailability of toxic metal ions	It is useful at sites with shallow contamination and is not much effective at sites where the contaminants reaches deep in the soil
3	The presence of plants with dense root system reduces soil erosion	The vegetation and soil may require long-term maintenance to prevent rerelease of the contaminants and future leaching
4	Does not destroy or remove soil organic matter, soil microorganisms, and soil texture	Vegetation may require extensive fertilization or soil modification using amendments
5	It has a lower cost and is less disruptive than other more vigorous soil remedial technologies	Contaminant stabilization might be due primarily to the effects of soil amendments, with plants only contributing to stabilization by decreasing the amount of water moving through the soil
6	Revegetation enhances ecosystem restoration and renders the site aesthetically pleasing	The root zone, root exudates, contaminants, and soil amendments must be monitored to prevent an increase in metal solubility and leaching
7	Vegetation provide physical stability to the soil	Plants that accumulate heavy metals in the roots and in the root zone typically are effective at depths of up to 24 inches

and biological properties of the site through an increase in organic matter content, cation exchange capacity, and biological activities (Lutts and Lefévre 2015). Furthermore, this method of using appropriately selected plant species can improve the soil parameters and affect the fertilization methods (Reevers et al. 2007).

However, this technology does not achieve a complete cleanup of the contaminated soil, but rather changes the mobility of potentially toxic elements by either reducing concentrations in the soil water/matrix or by reducing re-entrainment of toxic particulates following the development of a stable and permanent vegetation cover. Sometimes, the use of soil additives is required for the physical and chemical immobilization of toxic metal ions in the process of phytostabilization. Thus, even though phytostabilization offers a great deal in limiting the bioavailability of metal ions, it cannot be sometimes suggested as a perfect remedial measure especially in the sites where stabilization is not much possible. Major advantages and disadvantages of phytostabilization are listed in Table 1.

5 Phytostabilization: The Most Affable and Affordable Technique

Phytostabilization seems to be a more comprehensive practice since this technique offers an aesthetically pleasing and environmental-friendly approach with an affordable economic feasibility. Remediation of polluted soil through phytostabilization is nowadays being applied by a number of researchers who have estimated the actual cost of the technique implemented in a field to be less as compared to other remediation techniques.

5.1 Cost-Effectiveness of Phytostabilization

Phytostabilization has been recognized as an adaptable, eco-friendly, and cost-effective strategy for the restoration of a functional ecosystem which is highly polluted. In general, the in situ inactivation of metals through phytostabilization may be an economically realistic and cost-effective remediation alternative, especially for vast industrial sites, dredged sediment dumps, and other dumping grounds where due to the huge volumes of material to be treated, excavation or landfilling is impractical and especially cost-inefficient (Vassilev et al. 2004). In general, the economics of any phytoremediation technique like phytostabilization are characterized by two considerations: the potential for application and the cost comparison to conventional treatments. Before going to practice phytostabilization in a larger field, one has to consider the whole system costs that may include the following:

(i) **Design costs:**
    (a) Site characterization
    (b) Work plan and report preparation
    (c) Treatability and pilot testing

(ii) **Installation costs:**
    (a) Site preparation
    (b) Soil preparation (physical modification: tilling, chelating agents, pH control, drainage)
    (c) Infrastructure (irrigation system, fencing)
    (d) Planting (seeds, plants, labor, protection)

(iii) **Operating costs:**
    (a) Maintenance (irrigation water, fertilizer, pH control, chelating agent, drainage water disposal, pesticides, fencing/pest control, replanting)
    (b) Monitoring (soil nutrients, soil pH, soil water, plant nutrient status, plant contaminant status) (EPA 2000; Vassilev et al. 2004)

The actual costs of the phytostabilization process cannot be practically listed accurately and may vary strongly with specific site conditions, contaminant type, plants selected, distance to target level, scale of operation, etc. However, Cunningham et al. (1995) have made an attempt to calculate the actual costs of the process. According to him, for phytostabilization, cropping system costs have been estimated at US\$200–\$10,000/ha, equivalent to US \$0.02–US \$1.00/m^3 of soil, assuming a 1 m root depth (Cunningham et al. 1995). The cost of traditional remediation techniques like physical removal or chemical stabilization of metals ranges from approximately

US\$1.50 to US\$450 per m^3 of soil entrapped with metal contaminants (Berti and Cunningham 2000; Evans and Willgoose 2000). Moreover, as an emerging remediation technology, phytostabilization can minimize this cost to an estimated US\$0.40–26 per m^3 for revegetation (Ford and Walker 2003).

5.2 *Practical Applications of Phytostabilization*

Phytostabilization can be applied at sites where other regulatory strategies of metal decontamination usually fail and have high environmental risks due to continuous erosion or leaching (Berti and Cunningham 2000). This mechanism can be used to minimize migration of contaminants in soils through absorption and accumulation by the roots, adsorption onto roots, or precipitation within the root zone of plants. Phytostabilization is a management strategy for stabilizing toxic contaminants: its purpose is to establish a vegetation cover that will reduce soil erosion, windblow of contaminated particles, and water pollution by interception of incident precipitation.

Many heavy metals including Pb, As, Cd, chromium (Cr), Cu, Zn, etc. have been efficiently stabilized by phytostabilization technique (Berti and Cunnigham 2000; Alkorta et al. 2010). Phytostabilization is functional in different plant species in order to cover the different metal content in the soil and to conserve a portion of the plant diversity of pristine areas. In order to self-sustain the phytostabilization, the use of different tolerant and native plants species is recommended because they can perform distinct functional roles in the habitat apart from decontamination. An association of several species in polluted area should also increase the soil cover percentage, combining several shoot and root characteristics and then improving erosion control. Even though till date numerous plant species have been reported to be potential candidates for phytostabilization of different kinds of lands, the two most studied sites of phytostabilization are wetlands and mining sites.

5.2.1 Phytostabilization in Wetlands

In general, the term “wetlands” refers to transition zones between terrestrial and aquatic systems with soil saturated with water for at least part of the year or covered by shallow water along with characteristic wetland plant species (Kalff 2002). Wetlands constitute a highly productive ecosystem and are often situated close to highly populated and industrial areas. As a consequence, large amounts of toxic metal pollutants are entrapped in the salt marsh ecosystems and act as important sinks for heavy metals. Thus wetlands may be regarded as crucial sites for phytostabilization.

As an ecosystem, wetlands are helpful for recovering and cycling nutrients; releasing excess nitrogen; deactivating phosphates; treating wastewater; removing toxins, chemicals, and heavy metals; etc. The extensive rhizosphere of wetland plants provides an enriched culture zone for the microbes involved in degradation. The wetland sediment zone provides reducing conditions that are conducive to the metal removal pathway. The physicochemical properties of wetlands provide many positive attributes for remediating heavy metals. These unique characteristics of wetlands promote various biogeochemical processes that are responsible for the extraordinary capacity of wetlands to retain heavy metals from a diverse range of industrial effluents including municipal, agricultural, refinery, and pulp-mill effluent (Moshiri 1993). Thus, phytostabilization with wetland plants is an eco-friendly, aesthetically pleasing, cost-effective, solar-driven, passive technique that is useful for cleaning up environmental pollutants with low to moderate levels of contamination (USEPA 2001).

Castro et al. (2009) have reported that monocotyledonous halophyte species such as *Triglochin maritima* and *Juncus maritimus* provide higher Hg stabilization than the dicotyledonous species of halophytes such as *Sarcocornia perennis* and *Halimione portulacoides*. Moreover, mangroves with high BCF_{root}, for example, *Kandelia obovata* (Liu et al. 2014), *Avicennia marina* (MacFarlane et al. 2003), *Phragmites australis* (Weis and Weis 2004), *Aegiceras corniculatum* (Wu et al. 2015), etc., are appropriate candidates for phytostabilization, retaining metallic inputs and thereby reducing transport to adjacent estuarine and marine systems. Moreover, the halophytic shrubs recommended for phytostabilization include creosote bush (*Larrea tridentata*) and desert broom (*Baccharis sarothroides*). Recently, Shackira and Puthur (2017) and Shackira et al. (2017) have reported high BCF_{root} (>1) and low TF_{shoot} (<1) in *Acanthus ilicifolius* L. at high Cd and Zn concentrations, suggesting that *Acanthus ilicifolius* is a potential candidate species for Cd and Zn phytostabilization in polluted wetlands.

5.2.2 Phytostabilization in Mine Tailings

Introduction of plants directly at mine tailings has repeatedly been attempted, but has usually failed. This is due to the fact that such impoundments offer a harsh environment with high levels of heavy metals, low levels of macronutrients, and poor substrate structure (Clemensson-Lindell et al. 1992). Extraction of metals by mining results in large volumes of wastes that have to be removed in order to avoid contamination of the environment. The problem arises primarily after extraction of nonferrous base metals like Cu, Pb, and Zn, since these are found in ores with high sulfide content. After the metals have been extracted, approximately 95% of the rock is left as finely grained sand called mine tailings, containing high levels of metal sulfides, among which pyrite (FeS) is most abundant. The acidic drainage water from mine tailings may be a source of heavy metal leakage to various water bodies ultimately entering to the food chain and cause serious threats to the ecosystem (Notter 1993).

The success of the phytostabilization and ecological succession in the mining areas depends on the knowledge of the characteristics of metals and contaminated soils, rhizosphere processes, as well as the rate of uptake, translocation, accumulation, and chelation of metals by the pioneer plants (Mendez and Maier 2008; Abreu and Magalhães 2009). The plant populations (metallophytes) inhabiting the mine sites are usually specific ecotypes which are well adapted to mining conditions as well as drought and nutritional stresses. The ecological behavior of distinct plant species occupying mining areas has been studied by several authors (Freitas et al. 2004; Batista et al. 2007; Anawar et al. 2011; Abreu et al. 2012).

A low coverage by grasses could promote the colonization and establishment of native shrub species, since the facilitation is known to be an important process in harsh environment and enhance the phytostabilization and restoration strategies (Cunningham et al. 1995; Wong 2003; Padilla and Pugnaire 2006; Shackira and Puthur 2017; Shackira et al. 2017). *Cistus ladanifer* L. is one of the spontaneous species considered promising for phytostabilization of mining areas with multi-elemental (e.g., As, Cu, Pb, Zn, etc.) contamination (Alvarenga et al. 2004; Abreu et al. 2011; Anawar et al. 2011; Santos et al. 2012). Halophytes (salt-tolerant plants) are especially valuable in phytostabilization, and *Atriplex* spp. have been reported as pioneer species inhabiting in mine tailings (Jefferson 2004). The use of plants from Fabaceae family and/or other nitrogen-fixing species within the plant community can also be advantageous in the process (Wong 2003; Ahmad et al. 2012). List of plants characterized as potential candidates for phytostabilization of various heavy metals in different soil types are summarized in Table 2.

Table 2 List of plants characterized as potential candidates for phytostabilization of various heavy metals

Plant	Metal	Site of stabilization	References
Agrostis capillaris L.	As	Wetlands	Porter and Peterson (1975), Benson et al. (1981), Symeonidis et al. (1985)
Deschampsia cespitosa (L.) P. Beauv.	As	Wetlands	Cox and Hutchinson (1980, 1981)
Silene vulgaris (Moench) Garcke	As	Wetlands	Paliouris and Hutchinson (1991)
Avicennia marina (Forsk.)	Cu and Cd	Wetlands	MacFarlane et al. (2003)
Phragmites australis (Cav.) Trin. ex Steudel	Cu and Pb	Wetlands	Weis and Weis (2004)
Lolium perenne L.	Cu	Soils	Santibáñez et al. (2008)
Atriplex halimus L.	Cd	Wetlands	Nedjimi and Daoud (2009)
Quercus ilex subsp. *ballota*	Cd	Mine tailings	Domínguez et al. (2009)
Lupinus uncinatus Schldl.	Cd	Soils	Ehsan et al. (2009)
Triglochin maritima L. and *Juncus maritimus* Lam.	Hg	Wetlands	Castro et al. (2009)
Rhizophora mucronata (Lam.)	Cu, Pb, Mn, and Fe	Wetlands	Pahalawattaarachchi et al. (2009)
Microchloa altera (Rendle) Stapf	Cu	Soils	Shutcha et al. (2010)

(continued)

Table 2 (continued)

Plant	Metal	Site of stabilization	References
Haumaniastrum katangense (S. Moore) P.A. Duvign. & Plancke	Cu	Soils	Chipeng et al. (2010)
Typha latifolia L.	Mn, Cr, As, Zn, Co, Cd, Ni	Industrial sludge	Varun et al. (2011)
Arachis pintoi Krapov. & W.C. Gregory	Cu	Mine tailings	Andreazza et al. (2011)
Salix babylonica L.	Cu	Mine tailings	Chen et al. (2012)
Ricinus communis L. cultivar Zibo No. 8	Cd	Soils	Zhang et al. (2014)
Silene vulgaris Garcke (Moench)	Cd	Wetlands	Moreno et al. (2014)
Festuca rubra L.	Zn, Cd	Mine tailings	Galende et al. (2014)
Vigna unguiculata subsp. *sesquipedalis* L.	Cd	Soils	Deivanai and Thulasyammal (2014)
Oenothera glazioviana Micheli	Cu	Mine tailings	Guo et al. (2014)
Kandelia obovata Sheue, Liu & Yong	Cd	Wetlands	Liu et al. (2014)
Aegiceras corniculatum L.	As	Wetlands	Wu et al. (2015)
Andropogon schirensis Hochst.ex A.Rich., *Eragrostis racemosa* (Thunb.) Steud., *Loudetia simplex* (Nees) C. E. Hubb., *Monocymbium ceresiiforme* (Nees) Stapf, and *Hyparrhenia diplandra* (Hack.) Stapf.	Cu	Soils	Boisson et al. (2016)
Lupinus microcarpus Sims	As	Soils	Díaz et al. (2016)
Cistus ladanifer L.	As, Pb, Cu, Zn	Mine tailings	Santos et al. (2016)
Acanthus ilicifolius L.	Cd, Zn	Wetlands	Shackira and Puthur (2017), Shackira et al. (2017)
Osmanthus fragrans Lour., *Ligustrum vicaryi* L., *Cinnamomum camphora* (L.) J. Presl., *Loropetalum chinense* var. *rubrum*, and *Euonymus japonicas cv. Aureo-mar.*	Cd	Soil	Zeng et al. (2018)
Eichhornia crassipes (Mart.) Solms., *Pistia stratiotes* L.	Cd	Water	Sricoth et al. (2018)

6 Future Perspectives

Phytostabilization programs have low installation and maintenance costs compared to other remediation options. Polluted soils which lack green vegetation are considered as susceptible to soil erosion and leaching, and hence to guarantee the successful restoration of degraded ecosystem, the use of potential phytostabilizing species which have tolerance to multiple stress conditions is highly recommended. Moreover, this technology can increase the incomes from nonproductive contaminated soils if associated with species with economic value. Although the technique, phytostabilization, has become more widely accepted nowadays, further research is needed concerning the testing of new amendments and the selection of tolerant plant species for the process.

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In Planta Synthesis of Nanomaterials for Environmental Remediation

Reshma V. Patil and Kiran D. Pawar

1 Biogenic Synthesis of Nanoparticles

Nanotechnology is an umbrella term which is used to refer to the collection of techniques, methods, and processes used in the production of goods or services by controlling shape and size of the matter at the nanoscale. An important aspect of nanotechnology is the synthesis of material with at least one dimension in the nanometer scale (1–100 nm) and the direct control over particle morphology and dimensions during formation (Fawcett et al. 2016). A recent decade has witnessed a remarkable increase in the research efforts directed toward the fabrication of nanomaterials (NMs) with controlled morphologies and useful properties (Sharma et al. 2015). The synthesis of NMs with controlled particle size, shape, and crystalline nature that could be used for various potential applications has become one of the main objectives in nanotechnology (Sharma et al. 2015). Depending on desired applications, a wide range of NMs like nanoparticles, nanowires, nanofibers, nanopowders, nanoprisms, nanoplates, quantum dots, quantum wells, fullerenes, thin films, dendrimers, and nanotubes have been developed with a variety of protocols and methods (Millstone et al. 2005). "Nanobiotechnology," an extended term, can be defined as the branch of nanotechnology that uses biological entities or their products for the design, synthesis, and characterization of nontoxic bioactive NMs and devices that can be used in biomedical and biotechnological applications (Albrecht et al. 2006). Such biosynthesized NMs are known to interact with cells and tissues at a molecular level with a high degree of specificity and biocompatibility and with a minimal toxicity. The synthesis of NMs can be done by using either of two approaches, namely, top-down approach and bottom-up approach (Nielson et al. 2009; Poinern et al. 2013) (Fig. 1). The top-down approach involves a material undergoing significant size reduction via physical or

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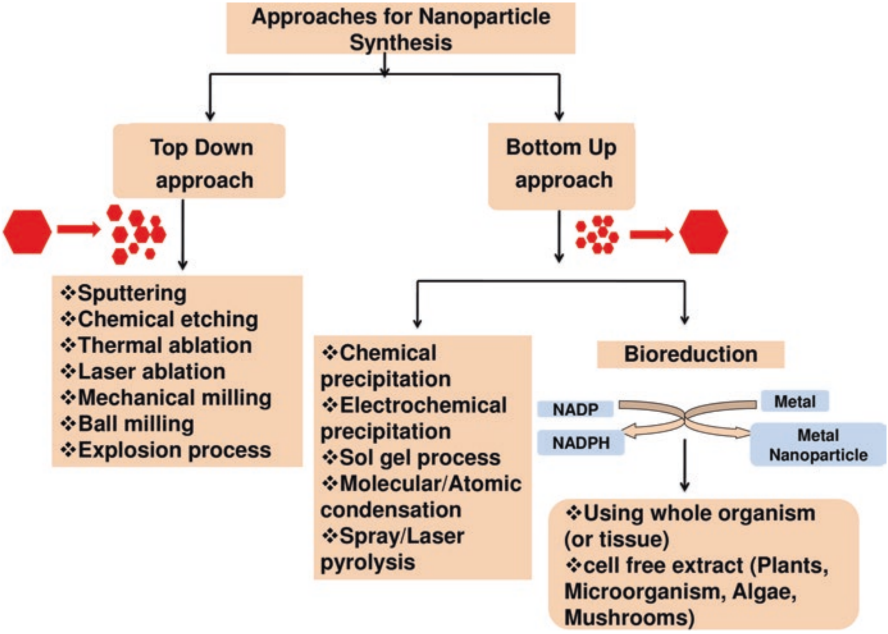

Fig. 1 The generalized flowchart of methodologies for nanoparticle synthesis. (Adapted from Mittal et al. 2013)

chemical processes. During size reduction, the resulting particle size, shape, and surface structure are heavily dependent on the technique used (Nielson et al. 2009; Birnbaum and Piqú 2011).

Unfortunately, size reduction also tends to introduce surface imperfections that can significantly impact the overall physicochemical properties of the fabricated NMs and therefore limit their biomedical applications (Poinern et al. 2013). The bottom-up approach builds NMs via the assembly of atoms, molecules, and smaller particles or monomers (Poinern et al. 2013; Cheng et al. 2013). In addition, many of the chemical and physical processes used in both approaches suffer from several drawbacks such as low material conversion rates, high levels of technical complexities, high energy requirements, and high cost of productions. Furthermore, many of these processes employ hazardous chemicals such as reducing agents, organic solvents, and non-biodegradable stabilizing agents. Therefore, such processes often result in toxic chemical deposition on the surface of NMs making them unsuitable for biomedical and biotechnological applications (Albrecht et al. 2006; Ai et al. 2011). Because of these drawbacks associated with physical and chemical methods of synthesis, there has been a growing interest in developing new eco-friendly synthesis technologies based on the principles of green chemistry (Duan et al. 2015). To emphasize this alternative approach, recent research has focused on using biological entities to synthesize a wide variety of NMs. In fact, nature has provided ways and insight into the synthesis of various NMs.

The process of synthesis of the NMs by living organisms is known as "biomineralization." It has now been reported in the literature that biological systems can act as the "bio-laboratory" for the production of pure metal and metal oxide NMs at the nanometer scale using biomimetic approach. The biological entities have active molecules and compounds that can act as reducing agents and stabilizing agents to synthesize NMs with diverse sizes, shapes, compositions, and physicochemical properties (Mohanpuria et al. 2008). As these processes involving biological entities use no harsh chemicals and solvent systems, they are classified as a *green synthesis*. NMs synthesized using biological systems such as bacteria, fungi, plants and their molecules, etc. are referred to as biogenic NMs. Such biogenic NMs possess the advantage of having uniform size and shape. Moreover, they have also been found to possess better stability owing to stabilization by proteins and other biomolecules from the organisms. In addition, biogenic NMs show desirable properties such as large surface area, high dispersity, good solubility, excellent biocompatibility, low cytotoxicity, ease of surface modification, excellent catalytic activities, novel photonics and optoelectronic properties, and others. Recently supported Au-NPs are extensively explored in the area of catalysis for different oxidation and reduction reactions like 4-nitrophenol, a toxic pollutant (Dauthal and Mukhopadhyay 2012). These properties of biogenic NMs make them excellent materials for wide range of applications including biomedical applications (Rai and Yadav 2013).

Various living entities such as bacteria (Shivaji et al. 2011; Stephen and Macnaughton 1999), fungi (Chan and MatDon 2013; Syed et al. 2013), plant extracts (Akhtar et al. 2013), and waste materials (Kanchi et al. 2014) have been reported as eco-friendly sources of reducing and capping agents for the biogenic synthesis of NMs.

1.1 Synthesis of Nanoparticles by Microorganisms

Among the various biological entities, microorganisms like bacteria, fungi, yeast, and algae show excellent capabilities of reducing the metal ions and therefore can synthesize NMs. During the past decade, many studies have demonstrated the reducing capabilities of microbes to transform the inorganic metal ions into metal nanoparticles. Each of these biological entities, to varying degrees, can perform as natural bio-factories for producing particular nanoparticles. The fundamental mechanism of synthesis by microbes is biomolecule-mediated reduction of metal ions to nanoparticles. Detoxification of metals by microorganisms is one of the major reasons for such synthesis of nanoparticles. To protect themselves from the toxic effects of soluble metal ions, microorganisms employ the mechanism of biomolecule-mediated synthesis of nanoparticles. To accomplish this, the microorganisms grab the target metal ions from their environment and then turn them into the element metal through biomolecules generated by the cellular activities (Sadowski et al. 2008). The four major metal detoxification strategies that subsequently lead to nanoparticle synthesis have been identified as (1) modifications in the cellular transport mechanisms to restrict entry of the toxic ions into the cell, (2) sequestration of the toxic species within the cell (intracellular sequestration) or outside the cell (extracellular sequestration), (3) activation of energy-dependent efflux pathways to eliminate the toxic species, and (4) enzyme-catalyzed oxidation or reduction of the toxic species to a less toxic form (Nalwa 2005).

Studies have found that many microorganisms can produce inorganic nanoparticles either through intracellular or extracellular routes. During biogenic synthesis, the nanoparticles may be sequestered in separate intracellular compartments inside the cells and therefore are referred to as intracellular biogenic particles. Separation of these nanoparticles requires disruption of the cells. On the other hand, some microorganisms synthesize nanoparticles outside the cell or send them outside post-synthesis. Such nanoparticles are referred to as extracellular biogenic nanoparticles. Bacteria possess remarkable ability to reduce heavy metal ions and are one of the best candidates for nanoparticle synthesis. Some bacteria have specific defense mechanism to quell stresses like heavy metals and ions. Bacteria like *Pseudomonas stutzeri* and *Pseudomonas aeruginosa* are reported to grow and survive even at high metal ion concentration (Rajasree and Suman 2012; Husseiny et al. 2007). A number of bacteria, fungi, and yeast are well-known for the synthesis of nontoxic noble nanoparticles. However, the microbial-mediated synthesis of nanoparticles is not industrially feasible as it requires expensive medium and maintenance of highly aseptic conditions. However, to improve the rate of synthesis and monodispersity of nanoparticles, factors such as microbial cultivation methods and downstream processing techniques have to be improved. The use of plants and plant products as sustainable and renewable resources in the synthesis of nanoparticles is more advantageous over prokaryotic microbes, which need expensive methodologies for maintaining microbial cultures and downstream processing. Hence, exploration of the plant systems as the potential bio-factories has gained heightened interest in the biological synthesis of nanoparticles (Velusamy et al. 2016).

1.2 Plant-Mediated Biogenic Synthesis of NMs

The use of microorganisms for biogenic synthesis is tedious as it involves maintenance of culture and scaling up. Therefore, the biosynthesis of nanoparticles using plant systems is under extensive research and is gaining importance due to its simplicity and rapid rate of synthesis with diverse morphologies (Ghosh et al. 2012). Table 1 represents some of the recent (after the year 2010) studies demonstrating the use of plants for synthesis of nanoparticles/NMs.

The knowledge of plants having ability to reduce metal ions dates back to the early 1900s. Initially this practice was restricted to whole plant extract and plant tissues only. Now it is well established that even different parts of the plant such as leaf, root, fruits, latex, seed, and stem can also be used for NM synthesis. Synthesis of nanoparticles using plants or their extracts is considered cost-effective and therefore can be used as an economic and valid alternative for the large-scale production

Table 1 Use of plants for synthesis of nanoparticles

Plants	Nanoparticle	Size (nm)	Shape	References
Mulberry	Ag	40	Spherical	Akl and Nida (2012)
Aspergillus flavus	Ag	–	–	Saha et al. (2010)
Syzygium cumini	Ag	10	–	Mittal et al. (2014)
Tea	Ag	20–90	–	Sun et al. (2014)
Aloe vera	Ag	20	–	Zhang et al. (2013)
Coleus aromaticus	Ag	40–50	–	Vanaja and Annadurai (2013)
Ocimum tenuiflorum	Ag	25–40	Spherical	Patil et al. (2012)
Euphorbia prostrata	Ag	80	Rod	Zahir et al. (2012)
Punica granatum	Ag	400	Spherical	Chauhan et al. (2011)
Loquat	Ag	5–40	Spherical	Akl et al. (2013)
Nerium oleander	Ag	67	Spherical	Suganya et al. (2013)
Castor oil, khat, and sunflower	Ag	28	–	Gebru et al. (2013)
Castus speciosus	Ag	420	Spherical	Malabadi et al. (2012)
Oryza sativa, *Vigna radiata*, *Brassica campestris*	Ag	25	Spherical	Mazumdar and Ahmed (2011)
Solanum xanthocarpum	Ag	406	Spherical	Amin et al. (2012)
Vitex negundo	Ag	30	Spherical	Zargar et al. (2011)
Memecylon umbellatum	Ag, Au	20	Spherical	Arunachalam et al. (2013)
Punica granatum	Ag, Au	25	Spherical	Ahmad et al. (2012)
Allium cepa	Au	54	Spherical	Parida et al. (2011)
Cymbopogon flexuosus	Au	200–500	Spherical	Iravani (2011)
Putranjiva roxburghii	Au	38	Spherical	Badole and Dighe (2012)
Tanacetum vulgare fruit	Au	11	Triangular	Dubey et al. (2010)
Mentha piperita leaves	Au	150	Spherical	MubarakAli et al. (2011)
Memecylon edule leaves	Au	10–45	Triangular, hexagonal, circular	Elavazhagan and Arunachalam (2011)
Murraya koenigii leaves	Au	20	Spherical, triangular	Philip et al. (2011)
Psidium guajava leaves	Au	25–30	Spherical	Raghunandan et al. (2009)
Camellia sinensis leaves	Au	40	Spherical, triangular, irregular	Sharma et al. (2007)
Coriandrum sativum leaves	Au	6.7–57.9	Spherical, triangular, truncated triangular, decahedral	Narayanan and Sakthivel (2008)

(continued)

Table 1 (continued)

Plants	Nanoparticle	Size (nm)	Shape	References
Eucalyptus camaldulensis leaves, *Pelargonium roseum* leaves	Au	5.5–7.5	Crystalline	Ramezani et al. (2008)
Cinnamomum zeylanicum leaves	Au	25	Spherical, prism	Smitha et al. (2009)
Magnolia kobus leaves	Au	5–500	Spherical, triangular, pentagonal, hexagonal	Song et al. (2009)
Diospyros kaki leaves	Au	5–500	Triangular, pentagonal, hexagonal	Song et al. (2009)
Terminalia catappa leaves	Au	10–35	Spherical	Ankamwar (2010)
Stevia rebaudiana leaves	Au	8–20	Octahedral	Mishra et al. (2010)
Mangifera indica leaves	Au	17–20	Spherical	Phillip (2010)
Azadirachta indica	Au	–	–	Thirumurugan et al. (2010)
Beta vulgaris sugar beet pulp	Au	–	Spherical, rod shaped, nanowires	Castro et al. (2011)
Nyctanthes arbor-tristis flower extract	Au	19.8	Spherical, triangular, hexagonal	Das et al. (2011)
Cuminum cyminum seed	Au	1–10	Spherical	Krishnamurthy et al. (2011)
Trigonella foneumgraecum leaves	Au	15–25	Spherical	Aromal et al. (2012)
Sphaeranthus amaranthoides leaves	Au	39	Spherical	Nellore et al. (2012)
Achillea wilhelmsii	CdO	10–35	Spherical	Andeani and Mohsenzadeh (2012)
Pinus resinosa	Pd	16–20	Spherical	Coccia et al. (2012)
Cinnamomum zeylanicum	Pd	15–20	Crystalline	Sathishkumar et al. (2009a)
Curcuma longa tuber	Pd	10–15	Spherical	Sathishkumar et al. (2009b)
Musa paradisiaca	Pd	50	Crystalline irregular	Bankar et al. (2010)
Cinnamomum camphora	Pd	3.2–6.0	–	Yang et al. (2010)
Glycine max	Pd	15	Spherical	Petla et al. (2012)
Diospyros kaki	Pt	2–12	Crystalline	Song et al. (2010)
Pinus resinosa	Pt	6–8	Irregular	Coccia et al. (2012)

(continued)

Table 1 (continued)

Plants	Nanoparticle	Size (nm)	Shape	References
Azadirachta indica	ZnO	9.5–25.5	Spherical	Bhuyan et al. (2015)
Cocos nucifera	ZnO	20–80	Spherical, hexagonal	Krupa and Vimala (2016)
Sargassum multicum	ZnO	30–57	Hexagonal	Azizi et al. (2013)
Phyllanthus niruri	ZnO	25.61	Hexagonal wurtzite, quasi-spherical	Anbuvannan et al. (2015)
Pongamia pinnata	ZnO	26	Spherical, hexagonal, nanorod	Sundrarajan et al. (2015)
Trifolium pratense	ZnO	60–70	Spherical	Dobrucka and Długaszewska (2016)
E. crassipes	ZnO	32–36	Spherical	Vanathi et al. (2014)
Ocimum basilicum L. var. *purpurascens*	ZnO	50	Hexagonal	Abdul et al. (2014)
Solanum nigrum	ZnO	20–30	Wurtzite hexagonal, quasi-spherical	Ramesh et al. (2015)
Aloe vera	ZnO	25–65	Spherical, hexagonal	Qian et al. (2015)
Gossypium	ZnO	13	Wurtzite, spherical, nanorod	Aladpoosh and Montazer (2015)
Moringa oleifera	ZnO	16–20	Spherical	Elumalai et al. (2015a)

(Huang et al. 2007). Because of low cost of production, ease of scaling up the production volume, short production time, and safety, plant-mediated biosynthesis has advantages over all other biological systems. This makes plants and their products an attractive platform for NM synthesis (Njagi et al. 2011). Plant-mediated biosynthesized NMs exhibit property such as low toxicity and high biocompatibility as bioreduction, capping, and subsequent growth of nanoparticles are mediated by functional biomolecules produced by plants (Satyavathi et al. 2010; Kumar and Yadav 2009). The recent investigation on possible biomolecules and their combinations involved in bioreduction implicated biomolecules such as enzymes, proteins, amino acids, vitamins, and polysaccharides in the plant-mediated biosynthesis (Iravani 2011). An important significance of plant extracts, in the context of synthesis of NMs, is that they act both as reducing and stabilizing agents. As different plant species and their extract produce different combinations and concentrations of organic reducing agent, the NMs synthesized using them vary greatly in terms of size- and shape-dependent physical, chemical, and biological properties. Moreover, the size, shape, and resultant properties largely depend on the physicochemical parameters of synthesis such as pH of reaction (Raveendran et al. 2003), incubation temperature (Haverkamp and Marshall 2009), reaction time (Selvakannan et al. 2004), and concentration and electrochemical potential of a metal ion (Willett et al. 2005). Owing to these effects of synthesis parameters, plants and their extract offer an opportunity for size-, shape-, and property-tunable synthesis. Thus, size- and shape-tunable biogenic nanoparticles can be synthesized by varying these synthesis parameters. Moreover, plant-mediated biogenic nanoparticles exhibit desirable properties such as large surface area, excellent biocompatibility and minimum cytotoxicity, good solubility with ease of surface modification, excellent catalytic activities, and novel photonics properties. These properties associated with plant-mediated biogenic NMs make them suitable for wide range of biotechnological and biomedical applications.

2 Mechanism of Plant-Mediated Biogenic Synthesis of NMs

The general mechanism for biogenic synthesis by living organisms remains the same. However, the specific mechanism for the plant-mediated synthesis of nanoparticles has not yet been interpreted well. To date, many theories and hypothetical mechanisms have been suggested to explain the mechanism of synthesis, especially for plant-mediated biogenic synthesis of metal nanoparticles. Generally, the plant-mediated synthesis of metal nanoparticles requires an aqueous medium and is regarded as a green process. It is very likely that for the bioreduction reaction of metallic ions, several phytoconstituents of plant extracts act synergistically (Shankar et al. 2004). During plant-mediated biogenic synthesis, the nanostructured building blocks are formed first and then assembled to produce the final nanoparticles (Thakkar et al. 2010). This requires mainly three constituents: reducing agents, stabilizing agents, and solvent medium (Vijayaraghavan and Nalini 2010). The precursor metal salts are first made into metal ions which are then reduced to atoms by means of reducing agents produced by plants. Subsequently, the atoms then nucleate into small clusters that grow into nanoscale particles.

The mechanism of plant-mediated nanoparticle synthesis by bottom-up approach is shown in Fig. 2. As biogenic metal nanoparticles remain stable for 3 months or more, it is evident that there are reducing agents as well as a stabilizing agent in the system that synthesize and stabilize the metal nanoparticles. This suggests that the phytoconstituents of plant extracts serve dual role as reducing agent and stabilizing agent. The identification of these components from the complex cellular environment is extremely challenging. The complex nature and huge variety of phytochemicals in the plant extracts make it challenging to identify and recognize specific biomolecules that act as bioreducing and stabilizing agents during biogenic synthesis. The nature of the reducing and stabilizing agents greatly varies with the plant species employed for the synthesis (Shankar et al. 2004).

2.1 Role of Plant Metabolites in Biogenic Synthesis

In general, various plant metabolites have been suggested to play an important role in the synthesis of metal nanoparticles through an electron shuttle or charge-capping mechanism (Mittal et al. 2013). The plant metabolites like proteins, sugars, organic

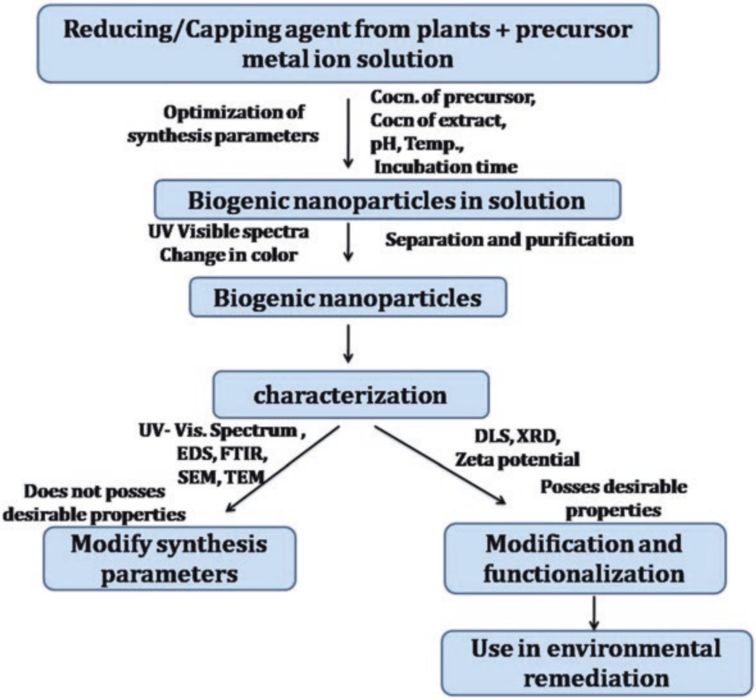

Fig. 2 Mechanism of plant nanoparticle synthesis by bottom-up approach. (Adapted from Yadav et al. 2017)

acids, phenolic acids, polyphenols, terpenoids, alkaloids, flavonoids, and tannins play an important role in the bioreduction of metal ions (Mounicou et al. 2009). The structures of plant metabolites possibly involved in nanoparticle synthesis are given in Fig. 2. Thus, prior knowledge on the presence of these plant metabolites greatly helps in selecting the source for biogenic synthesis of nanoparticles (Prakash et al. 2013).

2.1.1 Proteins and Amino Acids

Proteins and amino acids present in plants and their extract function as the reducing and stabilizing agents leading to biogenic synthesis. Numbers of studies have demonstrated the possible roles of proteins and amino acids in biogenic synthesis of metal nanoparticles (Prakash et al. 2013). Because of the complex structure of proteins, protein-mediated bioreduction leading to nanoparticle synthesis is complicated. Protein-based compounds like metallothioneins and phytochelators are considered as the key principle behind the synthesis of nanoparticles (Prakash et al. 2013).

Metallothioneins (MTs) are small ubiquitous proteins that play a major role in the regulation of physiologically important ZnII and CuI ions and also protect against xenobiotics, heavy metal ions like CdII and HgII, and reactive oxygen species (ROS). MTs bind to metal ions with high affinity mainly via thiolate groups of their numerous cysteine residues. Plant MTs are generally separated by long Cys-free peptide stretches of 15–40 residues length (Peroza et al. 2013). It is assumed that protein molecules facilitating the formation of nanoparticles from metal ions display high reducing activity and high potential for attracting metal ions to the regions of a molecule that are responsible for reduction but that their chelating activity is not excessive (Glusker et al. 1999). This work by Glusker and coworkers also demonstrated that the amino acid sequence of a protein can greatly affect the size, morphology, and amount of nascent nanoparticles. Amino acids can bind to metal ions through the amino and carbonyl groups of the main chain or through side chains, such as the carboxyl groups of aspartic and glutamic acid or a nitrogen atom of the imidazole ring of histidine. Other side chains binding metal ions include the thiol (cysteine), thioether (methionine), hydroxyl (serine, threonine, and tyrosine), and carbonyl (asparagine and glutamine) groups (Glusker et al. 1999). The investigation of the reducing and binding capability of 20 amino acids with metal ions of Au revealed that the reduction capability of a peptide depends on the presence of certain reducing amino acid residues (Tan et al. 2010). Moreover this study discovered that the activity of amino acids may be regulated by neighboring residues with different Au^0 binding strengths. Further the net charge of the peptide was found to effect the nucleation and growth of the Au nanoparticles. This investigation established that tryptophan is the strongest reducing agent for Au ions, whereas histidine is one of the strongest binding agents for Au ions. Recently, another study has demonstrated that ionization of the phenolic group in tyrosine leads to the formation of the semiquinone structure of tyrosine which subsequently reduces the metal ions of silver (Roy et al. 2014). Tryptophan was also reported to have reductive properties, via the release of an electron during conversion of the tryptophan residue to a transient tryptophyl radical (Selvakannan et al. 2004). Once amino acids are polymerized into the peptide chain, their individual ability to bind and reduce metal ions may change. For example, the formation of the peptide backbone changes the functionality of the R-carbon of carboxylic acids and amines of some amino acid residues since they move to a form inaccessible for interaction with metal ions. However, free side chains of amino acids can still participate in the binding and reduction of metal ions. The suitability of side chains for this interaction may change depending on the amino acid sequence, which could affect the accessibility of individual groups. The work by Tan et al. (2010) explained in detail how the amino acid sequence may affect the protein's ability to chelate and/or reduce metal ions. It was found that synthetic peptides, composed of amino acids capable of effective binding of metal ions, and of amino acids possessing high reducing activity, had lower reduction parameters than expected. It was suggested that the strong sequestration of metal ions to the peptide was inhibitory to their subsequent reduction by reducing amino acids. It was also found that peptides containing amino acids that weakly bind metal ions such as leucine, phenylalanine, and proline were ineffective in reducing tetrachloroauric acid anions, probably because of their inability to retain metal ions close to the reduction sites. For example, a synthetic peptide

GASLWWSEKL rapidly reduces metal ions to form a large amount of small nanoparticles less than 10 nm in size, whereas replacement of N- and C-terminal amino acid residues in a peptide (SEKLWWGASL) leads to a slower reduction reaction that results in the formation of larger nanospheres and nanotriangles about 40 nm in size. These studies indicate that the peptides and proteins present in plant extracts play a very important role in determining the shape of nanoparticles and affect the overall yield of nanoparticles (Glusker et al. 1999).

2.1.2 Sugars

Several studies have indicated that the sugars present in plant extracts can also induce the formation of metal nanoparticles. It is known that monosaccharides such as glucose (linear and containing an aldehyde group) can act as reducing agents. Monosaccharides containing a keto group, e.g., fructose, can act as antioxidants only when they have undergone a series of tautomeric transformations from a ketone to an aldehyde. Moreover, the reducing ability of disaccharides and polysaccharides depends on the ability of any of their individual monosaccharide components to adopt an open-chain form within an oligomer. Such an open-chain form provides access of a metal ion to an aldehyde group. For example, the disaccharides maltose and lactose have reducing ability when one of their monomers assumes an open-chain form. Sucrose, in contrast, has no ability to reduce metal ions because glucose and fructose monomers are linked in such a way that the open-chain form is not available. It was found that glucose is capable of participating in the synthesis of metal nanoparticles of various morphologies, whereas fructose mediates the synthesis of monodispersed nanoparticles of gold and silver (Panigrahi et al. 2004). Glucose was also noted to be a stronger reducing agent than fructose, because the antioxidant potential of fructose is limited by the kinetics of tautomeric shifts (Panigrahi et al. 2004).

2.1.3 Alkaloids and Organic Acids

Plant systems are capable of producing a diverse range of secondary metabolites upon exposure to various stress-inducing conditions. Various secondary metabolites such as organic acids and alkaloids are reported as bioreducing agents for the fabrication of different metallic nanoparticles. Tamuly et al. (2014) demonstrated the metal-reducing potential of pedicellamide, an alkaloid isolated from *Piper pedicellatum* and used for the synthesis of silver nanoparticles. Pedicellamide was reported to exert metal-reducing potential through the release of reactive hydrogen. The presence of ascorbic acid, along with other plant metabolites in *Citrus sinensis* peel extract, was envisaged as an effective reducing agent for green synthesis of Ag nanoparticles (Konwarh et al. 2011). Tautomerization of benzoquinone derivatives was reported to contribute to the metal-reducing capability of the *Cyperus* sp. of mesophyte genera. Similarly, in hydrophyte genera, compounds such as protocatechuic acid, catechol, and ascorbic acid of *Hydrilla* sp. have been reported to release reactive hydrogen. This reactive hydrogen contributed to the bioreduction of metallic Ag ions. In xerophyte genera, pyruvic and malic acid produced during the redox reaction of glycolytic pathway in *Bryophyllum* sp. leads to the reduction of metallic Ag ions (Jha et al. 2009). The keto-enol tautomerization of emodin in xerophytes (anthraquinone derivative) is also known for the reduction of metal ions.

2.1.4 Phenolic Acids

Phenolic acids which belong to the family of polyphenols contain a phenolic ring and an organic carboxylic acid function. Antioxidant activities of these compounds are attributed to the metal-chelating ability of highly nucleophilic aromatic rings (Wang et al. 2007). The polyphenolic compounds are capable of chelating many types of metallic ions such as Pt^{4+}, Au^{3+}, Pd^{2+}, Ag^{+}, etc. through their dense ortho-phenolic hydroxyl groups (Mohsen and Ammar 2009). Therefore, these compounds act as reducing agents for the fabrication of metallic nanoparticles. Various types of plant phenolic acids, namely, gallic acid (Huang et al. 2010), caffeic acid (Aromal et al. 2012) and, ellagic acid (Edison and Sethuraman 2012), have been implicated in bioreduction for the synthesis of metallic nanoparticles. Supporting these conclusions, few researchers have directly utilized the metal-reducing potential of gallic acid for the reduction of metal ions. Aromal and coworkers found that the hydrogen released during the transformation of caffeic acid to ferulic acid is responsible for the bioreduction of Au ions (Aromal et al. 2012). Because of additional conjugation in the propanoic side chain, caffeic acid displays strong reducing activity. This facilitates the electron delocalization by resonance between the aromatic ring and the propanoic group. The metal-reducing potential of a few other polyphenolic compounds, such as lignan (phyllanthin), flavonoid glycosides (apiin) (Kasthuri et al. 2009), and tannin (bayberry tannin) (Huang et al. 2010), is also reported in the literature.

2.1.5 Flavonoids

Flavonoids are water-soluble plant secondary metabolites that contain 15 carbon atoms. This group of secondary metabolites mainly consists of six major subgroups, namely, anthoxanthins, flavanones, flavanonols, flavans, anthocyanidins, and isoflavonoids. Flavonoids are presumed to be the key bioreducing constituents of aqueous plant extracts. The molecular oxygen scavenging (antioxidant or reducing) potential of flavonoids is directly related to their ability to donate electron or hydrogen atoms (Zhou et al. 2010; Pietta 2000). The presence of various functional groups in flavonoids makes them capable of bioreduction and nanoparticle synthesis. It has been postulated that the tautomeric transformation of flavonoids from the enol form to the keto form causes the release of a reactive hydrogen atom that can reduce metal ions to form nanoparticles. For example, it is believed that the transformation of flavonoids luteolin and rosmarinic acid from the enol form to the keto form in *Ocimum basilicum* (sweet basil) extracts plays a key role in the synthesis of silver nanoparticles from Ag ions (Ahmad et al. 2010). Moreover, the internal mechanism of the conversion of ketones to carboxylic acids in flavonoids is likely to be involved in Au^{3+} ion reduction. Interestingly, some flavonoids are able to chelate metal ions with their carbonyl groups or π-electrons. Quercetin is one of such flavonoid that shows very strong chelating activity, because it can chelate at three positions involving the carbonyl and hydroxyls at the C3 and C5 positions and the catechol group at the C3' and C4' site. These groups chelate various metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Pb^{2+}, and Co^{2+}. The presence of such mechanisms indeed explains the ability of flavonoids to be adsorbed onto the surface of a nascent nanoparticle. This probably means that they are involved in the stages of initiation of nanoparticle formation (nucleation) and further aggregation, in addition to the bioreduction stage. Moreover, isolated flavonoids and flavonoid glycosides have the ability to induce the formation of metal nanoparticles. The ability of isolated flavonoids to synthesize metal nanoparticles was demonstrated by the work of Kasthuri et al. (2009). These researchers isolated apigenin glycoside called apiin (apigenin glycoside) from the extract of *Lawsonia inermis* (lawsonite thornless, henna) and used for the synthesis of anisotropic gold and quasi-spherical silver nanoparticles with an average size of 21–30 nm (Kasthuri et al. 2009). A FTIR analysis revealed that apiin was attached to the nanoparticles through a carbonyl group.

2.1.6 Terpenoids

Terpenoids (isoprenoids) which belong to the category of terpenes are a large and diverse class of naturally occurring low-molecular-weight organic compounds synthesized by plants. The presences of terpenoids in the plants are responsible for typical aroma, taste, and color of various plant species. The hydroxyl functional groups of terpenoids are reported with reducing ability that can be used for plant-mediated synthesis of nanoparticles. The hydroxyl functional groups of terpenoids (citronellol and geraniol) present in *P. graveolens* leaf extract were found to reduce Ag^{+} ions that subsequently led to Ag nanoparticle synthesis (Shankar et al. 2004). Later, Safaepour et al. (2009) also proved the role of geraniol in the synthesis of Ag nanoparticles. Similarly, terpenoid such as linalool, eugenol, and methyl chavicol present in the bark of *C. zeylanicum* bark extract was reported with metal-reducing potential (Sathishkumar et al. 2009c). Singh et al. (2010) suggested that the eugenol of *S. aromaticum* extract acted as a bioreducing agent for the fabrication of Au nanoparticles and Ag nanoparticles. Eugenol transforms itself to its anionic form because of the proton-releasing ability of the hydroxyl group of eugenol. Furthermore, the reducing power of eugenol is significantly improved because of the inductive effect induced by the electron withdrawing methoxy and allyl functional groups present at the para and ortho positions of the hydroxyl group. The two electrons released simultaneously during the reaction are responsible for the reduction of metal ions (Fig. 3).

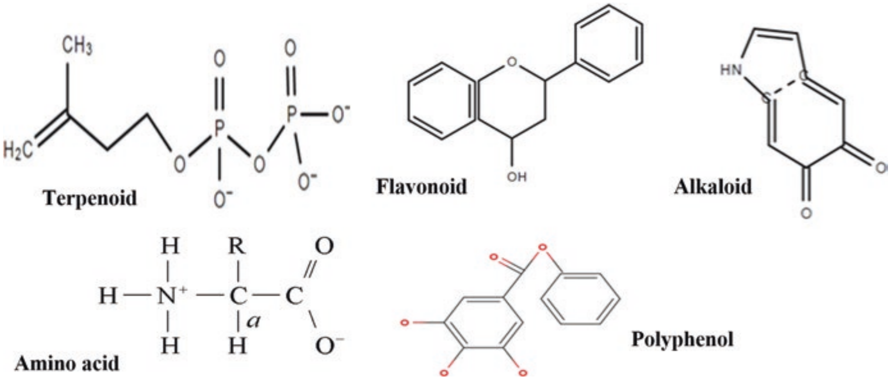

Fig. 3 Possible chemical constituents of plant extracts responsible for the bioreduction of metal ions (Dubey et al. 2009)

3 Nanotechnology for Environmental Remediation

Population growth, rapid industrialization, and long-term droughts have resulted in the spread of a wide range of pollutants in surface and groundwater system (Chong et al. 2010). The major contaminants include heavy metals, inorganic and organic pollutants, and many other complex compounds (Li et al. 2011). Moreover, the rapid increase in the use of nitro-organic compounds in the manufacturing of pharmaceuticals, plasticizers, dyes, fungicides, pesticides, and explosives has resulted in their continued release in the environment (Tafesh and Weiguny 1996). Likewise, textile industries produce large volume of colored dye effluents which are toxic and non-biodegradable. These dyes create severe environmental pollution problems by releasing toxic and potential carcinogenic substances into the aqueous phase (Pouretedal et al. 2009). The widespread use of organic dyes in the cosmetics, textile, paper, food, and pharmaceutical industries also causes serious pollution problems through industrial effluent. Because of the high stability of dye molecules, it is very difficult to degrade or remove them from industrial effluent, and therefore, they remain in water and soil surfaces for longer periods. These anthropogenic compounds are highly perilous when released into the environment and are known as “priority pollutants” by the USEPA (United States Environmental Protection Agency). In light of these hazardous impacts of toxic pollutants, it becomes imperative to remove them as they are harmful not only to human beings but also to the ecology and environment (Pang et al. 2011). Many in situ and ex situ methods of remediation technologies have been developed and are in practice to treat soil, leachate, wastewater, and groundwater contaminated by such pollutants (Rao et al. 2007). Wastewater treatment processes like photocatalytic oxidation, adsorption/separation processing, and bioremediation have been tried and tested. But factors like efficiency, operational method, energy requirements, and high cost have restricted their usability (Huang et al. 2006; Zelmanov and Semiat 2008). A combined approach involving nanotechnology and biotechnology could overcome this limitation. In the past two decades, NMs have been used as an alternative to existing treatment materials due to their efficiency, cost-effectiveness, and eco-friendly nature (Dastjerdi and Montazer 2010). Among the numerous applications of nanotechnology, remediation of environmental pollution using NMs and nanomaterial-based products is one of the rapidly emerging areas. NMs such as Au nanoparticles, Ag nanoparticles, iron, titanium dioxide, silica, zinc oxide, carbon nanotube, and dendrimers have distinctive physical and chemical properties and can be used to treat air, water, and soil pollution. The removal of environmental contaminants such as heavy metals and organic and inorganic pollutants from contaminated sites using nanomaterial synthesized by plant, fungi, and bacteria is an eco-friendly approach for environmental cleanup. Recently, many research studies have successfully demonstrated the use of plant-mediated biogenic nanoparticles for removal and degradation of many environmental pollutants. Few of such recent studies that reported the use of plant for biogenic synthesis of nanoparticles which can be used in environmental remediation have been presented in Table 2.

3.1 Mechanism

There are various possible mechanisms employed by different NMs in environmental remediation. The mechanism employed depends on the type of NMs, their physical and chemical properties, and targeted pollutants. The unique properties like high reactivity and strong sorption help NMs penetrate deeper which make NMs suitable for application in water/wastewater treatment. When the bulk material is brought down to nanoscale, surface area per unit mass of a material increases; hence, a larger amount of the material can come in contact with surrounding materials, and this affects the reactivity. In addition, NMs show quantum effect; therefore, less activation energy is required to make the chemical reactions feasible. Surface plasmon resonance is another phenomenon exhibited by nanoparticles which can be used for the detection of toxic material. As far as shape and size are concerned, various metallic and nonmetallic NMs of different shapes and sizes can be used for environmental cleanup (Rizwan et al. 2014).

Also NMs are known to diffuse and penetrate into contaminated zone where microparticles cannot reach, and they have higher reactivity to redox-amenable contaminants. NM such as oxide-coated Fe0 can form weak and outer-sphere complexes with contaminants such as carbon tetrachloride (CT). Oxide coating increases the reactivity, and hence, through electron transfer, CT can be broken down into methane and carbon monoxide, whereas benzoquinone by trichloroethene and other chlorinated aliphatic hydrocarbons can be broken down into chemicals with lower toxicities (Nurmi et al. 2005). Another important mechanism of removing industrial waste is the use of light energy (electromagnetic radiation). The light energy and particles sensitive to this energy mineralize waste which subsequently leads to its removal from solution. Because of its high stability, low cost, and safety toward both humans and the environment, NM like titanium dioxide (TiO_2) is considered as

Table 2 Use of plant-synthesized nanoparticles for environmental remediation

Type of nanoparticle	Plant species used	Pollutant removed/degraded	Functional group identified	Possible biomolecule involved	References
Zinc	*Vitex trifolia* L.	Methylene blue	Terpenoid	Vitrifolin A	Elumalai et al. (2015b)
	Ferulago angulata	Rhodamine B	Polyphenols	–	Mehr et al. (2017)
	Citrus paradisi	Methylene blue	Flavonoids, limonoids, and carotenoids	–	Brajesh et al. (2014)
	Catharanthus roseus	Phenol red	Secondary metabolites	–	Aasaithambi et al. (2016)
Iron	*Camellia sinensis*	Bromothymol blue	Polyphenols		Hoag et al. (2009)
	Orange peel	Chromium	Starch, cellulose, hemicelluloses, and lignin	–	Lopez-Tellez et al. (2013)
	Tea extract	Methylene blue, methyl orange	Polyphenols	–	Shahwan et al. (2011)
	Oolong tea extract	Water purification	Polyphenol/caffeine	–	Huang et al. (2014)
	Sorghum sp. (hybrid sorghum)	Bromothymol blue	Polyphenols	–	Njagi et al. (2011)
	Eucalyptus globulus	Removal of chromium	Polyphenols	Oenothein B	Madhavi et al. (2013)
	Eucalyptus	Azo dye, acid black 194	Polyphenols	–	Wang (2013)
	Pomegranate	Hexavalent chromium	–	–	Rao et al. (2013)
	Banana peel ash and *Colocasia esculenta* leaves	Graphene oxide	Polyphenols	–	Thakur and Karak (2014)
Gold	*Sesbania drummondii*	4-Nitrophenol	Alkaloids and terpenoids	–	Powell and Smith (1981)
	Sesbania drummondii	Lead	Alkaloids	–	Sahi et al. (2002)

Type of nanoparticle	Plant species used	Pollutant removed/degraded	Functional group identified	Possible biomolecule involved	References
Copper	*Fortunella japonica*	4-Nitrophenol	Proteins/biomolecules	–	Suman et al. (2017)
	Carica papaya	Coomassie brilliant blue R-250	–	–	Sankar et al. (2014)
	Pyrus pyrifolia	Methylene blue	–	–	Sundaramurthy and Parthiban (2015)
	Ferulago angulata	Rhodamine B	Polyphenols	–	Mehr et al. (2017)
Silver	*Convolvulus pluricaulis*	Methyl orange, 2,4-dichlorophenol (2,4-DCP)	Alkaloids, phenolic/glycosides/triterpenoids/steroids	–	Shadakshari et al. (2016)
	Azadirachta indica	Crystal violet	Chloride, alkene, carboxyl, and hydrogen bond	–	Satapathy et al. (2015)
	Allium sativum	Poly aromatic hydrocarbons	Enzyme (organosulfur)	Allicin	Abbasi et al. (2014)

an ideal semiconductor for photocatalysis. The primary criteria for an efficient semiconductor photocatalyst is that the redox potential of the charge couple, i.e., e−/h+, lies within the band gap domain of the photocatalyst (Carp et al. 2004). A photocatalyst is characterized by its capability to adsorb simultaneously two reactants, which can be reduced and oxidized by a photonic activation through an efficient absorption. Photocatalysis is a well-known process and is mostly employed to degrade or transform (into less harmful substances) organic and inorganic compounds like dyes and metal complexes. If the oxidative energy level of the excited dye/complex compound is favorable (i.e., more negative) with respect to the conduction band level of semiconductor, the dye molecule can transfer the electron to the conduction band of the semiconductor. A prerequisite for this electron transfer is a low quantum yield of the redox process catalyzed by the dye/complex molecule (Fan and Bard 1979). The injected electron reacts with surface-adsorbed O_2 which produces H_2O on protonation leading to the reduction of the organic molecule (in the presence of a redox couple) or of dye itself (in the absence of a redox couple) (Carp et al. 2004).

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