

Review

Aluminum stress signaling in plants

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Aluminum (Al) toxicity is a major constraint for crop production in acidic soil worldwide. When the soil pH is lower than 5, Al³⁺ is released to the soil and enters into root tip cell ceases root development of plant. In acid soil with high mineral content, Al is the major cause of phytotoxicity. The target of Al toxicity is the root tip, in which Al exposure causes inhibition of cell elongation and cell division, leading to root stunting accompanied by reduced water and nutrient uptake. A variety of genes have been identified that are induced or repressed upon Al exposure. At tissue level, the distal part of the transition zone is the most sensitive to Al. At cellular and molecular level, many cell components are implicated in the Al toxicity including DNA in nucleus, numerous cytoplasmic compounds, mitochondria, the plasma membrane and the cell wall. Although it is difficult to distinguish the primary targets from the secondary effects so far, understanding of the target sites of the Al toxicity is helpful for elucidating the mechanisms by which Al exerts its deleterious effects on root growth. To develop high tolerance against Al stress is the major goal of plant sciences. This review examines our current understanding of the Al signaling with the physiological, genetic and molecular approaches to improve the crop performance under the Al toxicity. New discoveries will open up new avenues of molecular/physiological inquiry that should greatly advance our understanding of Al tolerance mechanisms. Additionally, these breakthroughs will provide new molecular resources for improving the crop Al tolerance via molecular-assisted breeding and biotechnology.

Introduction

Aluminum (Al) toxicity is a serious factor limiting crop productivity in acid soil. Al is one of the major constituents of soil and it dissolves in the soil in various ionic forms among these Al³⁺ is the most toxic form. Apart from Al³⁺ cation, Al has the potential to form various hydroxy-Al and polynuclear species in solution.

When the soil pH drops below 5.0 Al³⁺ is solubilized in the soil. Approximately 70% of soil in world is problem soil contaminated with acid, alkali, heavy metals etc. However acid soil is the most frequently encountered limiting production of most of the world's staple food. It has been estimated that approximately 50% of the arable land is negatively impacted by the Al toxicity due to acidic soil. Considerable measures must be taken to overcome this problem.

Occurrence of Aluminum

Aluminum (Al) is a member of boron group of chemical element with atomic number 13. In the earth crust it is the most abundant metallic element and third most abundant of all element (after oxygen and silicon). The Al release from soil minerals under acidic conditions occurs as Al(OH)²⁺, Al(OH)³⁺ and Al(H₂O)³⁺ that commonly effect on Al toxicity.¹

An Overview of Al Toxicity in Plant

The most easily affected region of Al toxicity is the root in plant. The Al toxicity is due to the inhibition of root growth. Root elongations a process of cell division, but Al phytotoxicity block the mechanism of cell division. As a result of this root become stunted and brittle, root hair development is poor and the root apices become swollen and damage.² Al causes extensive root injury leading to poor ion and water uptake.³ The root apex i.e., root cap, meristem and elongation zone is highly sensitive to Al and accumulates Al very easily. As a result it attracts greater physical damage than the mature region of the root tissue. Primary toxic effects of Al are localized to the distal transition zone in the root tip.⁴ In this root zone meristematic cells exit the division phase and prepare for F-actin dependent rapid cell elongation.^{5,6} Cell division in the meristem and cell elongation in the elongation zone is inhibited by the primary effects of Al occurring in the adjacent transition zone, in which these processes are less active.

Al is so reactive that there are many potential Al binding sites including the cell wall, the plasma membrane surface, the cytoskeleton and nucleus that could target of injury. Al strongly binds to the cell wall of root epidermal and cortical cells.⁷ The extent to which Al can bind to the cell wall components depends on the density of negative charges and ultimately determines the cation exchange capacity (CEC). In addition to rapid accumula-

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tion of Al in the cell wall and apoplast of the root apex, Al rapidly accumulates in the plasma membrane as well the symplasm of sensitive plant affecting many processes of root growth.⁸⁻¹⁰ Plasma membrane is rich in phospholipids, representing sensitive target of the Al phytotoxicity.

Al can alter the function of plasma membrane by interacting with the lipid thus inducing lipid peroxidation. Al can bind principally to phospholipids within the membrane. Several reports have been described the Al mediated interference with membrane lipid, as a result of which there is an increase in the highly toxic reactive oxygen free radicals. More over due to Al toxicity there is change in the membrane potential and this change in membrane potential is directly correlates with changes in the membrane surface potential i.e., zeta potential. In one sentence the shifting of plasma membrane potential to Al-induced depolarization. Calcium uptake has been strongly affected due to Al toxicity. Cytoplasmic Ca^{2+} is known to regulate many processes in cell growth and metabolism. The disruption of cytoplasmic Ca^{2+} homeostasis is another mechanism hypothesized to cause Al injury.^{9,11,12} The Al-dependent disruption of cytoplasmic Ca^{2+} homeostasis may be directly or indirectly involved in the inhibition of the cell division or root elongation. Al might disrupt Ca-dependent metabolism by maintaining Ca^{2+} levels in the cytoplasm or by preventing Ca^{2+} transients from occurring altogether. The evidence supporting this hypothesis is indirect at best. For instance, callose (I-3-P-glucan) synthesis in plants requires an increase in Ca^{2+} , and several polyvalent metal cations, including Al, so it induces callose synthesis in roots within 30 min.¹³ This phenomenon of callose synthesis shows a rapid link between Al stress and changes in $[\text{Ca}^{2+}]$. Calcium uptake rapidly recovers when Al is removed from the solution. Calmodulin (CaM) plays a pivotal role in cellular metabolism and there is some evidence that interactions between Al and CaM could be an important cause of cell toxicity.

According to Sivaguru et al.⁴ Al induced the accumulation of callose in the plasmodesmata of root cell in wheat, thus blocking the cell to cell trafficking. Plant cell requires dynamic cytoskeleton based network for proper functioning of cell differentiation and cell division. Al toxicity disrupts the structure of cytoskeleton. In addition to this microtubules and actin filaments are also the target of Al. Some evidences were reported which suggested the physiological injuries due to the Al^+ toxicity. In two recent studies,^{14,15} it was proved that Al induced the decrease in the chlorophyll content and photosynthetic rate. The impact of Al toxicity in photosynthesis is indirect. Due to Al toxicity there is disturbance in the chloroplast architecture. Moreover, there is decrease in photosynthesis due to reduction of electron transport in photosystem II (PSII).

Al inhibited the efflux of H^+ from barley roots.¹⁶ Decrease activities of K^+ , Mg^+ and ATPase of plasma membrane were scored due to Al stress. Increase in the ATP and PPI dependent H^+ pumps of the tonoplast membrane of barley. In the nucleus, binding of Al to DNA or to chromatin could condense DNA molecules and inhibit the cell division by reducing its capacity to provide a viable template for transcription.¹⁶ Al has been shown to accumulate in the symplast.¹⁷ The nuclei of the root tip cells shows accumulation of Al within 30 min of Al treatment in a sensitive genotype.¹⁸

The mitochondrial activity was repressed in cultured tobacco cell and pea roots treated with Al and this is followed by inhibition of respiration, depletion of ATP and production of reactive oxygen species at later stages.¹⁹ It is proved that exposure to Al could affect production of reactive oxygen species (ROS) in plants because Al stress causes peroxidation of lipids in the plasma membrane, the effect that could be due to ROS and Al induces the expression of several genes encoding antioxidative enzymes such as glutathione S-transferase, peroxidase and superoxide dismutase (SOD). Long-term treatment of green gram (*Vigna radiata*) with Al resulted in greatly increased levels of peroxide and lipid peroxidation in the leaves.²⁰ The imposition of biotic and abiotic stresses can give rise to further increases in ROS levels. Metals, including Al, are known to act as catalysts in ROS production and to induce oxidative damage in plants.¹⁹⁻²² Large number of swollen mitochondria with many vacuoles, structural disturbances of the plasma membrane, and pre-apoptotic nuclear structures were some of the characteristic features of Al treated tobacco cells, confirming that Al signaling follows the mitochondrial pathway of cell death.²⁴

Al toxicity affected severely the mitochondrial respiratory functions and altered the redox status studied in vitro and also the internal structure, which caused finally cell death in tobacco cells.²³ Increase in the vascular and total cell volume with out the change in the nuclear volume has been observed due to 24 hour of Al treatment. A marked increase in the surface area of Golgi complex and endoplasmic reticulum was identified under Al stress. Plant cells are well equipped with complex non enzymatic antioxidants such as ascorbate, glutathione, tocopherol and carotenoid, and with enzymatic antioxidants such as catalase, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase (SOD), mono dehydro ascorbate reductase, dehydro ascorbate reductase, glutathione-S-transferase (GST) and glutathione reductase, which help to detoxify the ROS.^{21,24} Signal transduction is also affected by Al, a key signal transduction enzyme designated as phospholipase C (PLC) is inhibited by Al toxicity. This suggests that Al may interfere with the phosphoinositide signaling pathway.²⁵

Al Toxicity and Tolerance Mechanism

Over the past decades many laboratories around world have focused their efforts on identifying and characterizing the mechanisms employed by plants that enable them to tolerate toxic levels of Al in acid soils. This research revealed that there are two main classes of Al tolerance mechanism. Some are those that operate to exclude Al from the root apex and other are those that allow the plant to tolerate Al accumulation in the root and shoot symplast. As proposed by Taylor,¹² the tolerance strategies identified can be separated into those in exclusion of Al from the root apex and mechanisms that allow the plant to tolerate Al within cells. A wealth of studies provide very strong evidence that Al^+ tolerant genotypes of wheat, corn, sunflower, soybean and common bean, among other exclude Al from root by exertion of organic acids that chelate Al.²⁷⁻³¹ The study of these tolerance mechanisms in plant become an interesting and essential topic of research. Production of organic acid (OA) plays a vital role in the mechanism of Al detoxification. Activation of organic acid efflux occurs rapidly

with any measurable delay after exposure to Al in several plants including wheat, in which it is well studied.^{30,32} Of the organic acid, citrate has the highest binding activity for Al followed by citrate, malate and succinate.³³ Rice bean roots can specifically release citrate to alleviate Al toxicity.³⁴ In order to determine the key step involved in the Al-stimulated citrate efflux, several anion channel inhibitors and citrate carrier inhibitors as well as a protein synthesis inhibitor were used. The results indicated that de novo protein synthesis (possibly of the citrate carrier and anion channel themselves) rather than citrate biosynthesis is the critical step leading to citrate efflux in roots.

There is strong evidence that malate exudation from wheat and citrate exudation from corn roots in response to Al occurs by activation of an anion channel located in the plasma membrane.^{15,30,35} Al might directly bind and then activate a membrane protein or an associated receptor, or it might indirectly activate the channel via cytosolic components. The two most important families of channel proteins are the chloride channel family and a subset of the ATP-binding cassette (ABC) protein super family. In yeast (*Saccharomyces cerevisiae*), Pdr12, an ABC protein, assists the carboxylate efflux. In cowpea, root cap mucilage was shown to bind to Al and the mucilage removal increases the Al sensitivity of root,³⁶ Henderson and Ownby³⁷ correlated the amount of mucilage produced by wheat root to Al tolerance and suggested that mucilage aided in forming a diffusion barrier to Al or concentrated organic acids that chelated Al. The mucilage from maize roots has been shown to bind Al,³⁸ but did not give satisfactory protection of roots from Al toxicity. This lack of protection is due distance between site of formation of mucilage and the Al sensitive zone i.e., distal part of the transition zone (DTZ).

The Al tolerance of canola (*Brassica napus*), *Arabidopsis thaliana*, tobacco (*Nicotiana tabacum*) and alfalfa (*Medicago sativum*) have been reported to be enhanced by increasing organic acid biosynthesis through overexpression of citrate synthase or malate dehydrogenase genes derived from plants or bacteria. Other potential mechanism of Al exclusion has been identified than organic acid (OA) efflux, this mechanism is the exudation of phenolic compounds. Phenolics, which are characterized as organic compounds containing one or more hydroxylated aromatic rings, represent a broad range of plant compounds including alkaloids, flavonoids, terpenoids and glycosides. They reportedly form strong complexes with Al³⁺ at neutral pH and were implicated in internal Al detoxification in tea and other Al-accumulating species.³⁹ For the better understanding of Al tolerance mechanisms, genes which are conferring the tolerance should be studied. Many studies must be done because in this field physiological as well as molecular level of study is essential. With respect of genetic analysis of Al tolerance, the work has been done in cereals especially among members of the Triticeae (e.g., wheat, rye). Among these the tolerance gene in wheat has been first focussed. The ALMT1 gene encoding a malate transporter from wheat (*Triticum aestivum*) can confer Al tolerance in transgenic tobacco cells. Delhaize et al.⁴⁰ generated transgenic barley (*Hordeum vulgare*) plants expressing the ALMT1 gene to exude malate and withstand Al stress. In wheat, the most extensively studied Al-resistant sources all have the Brazilian

ancestor, Polyssu, in their pedigrees.⁴¹ BH 1146 and Atlas 66 have been used widely in inheritance and gene expression studies^{42,43} and they both can be traced back to Polyssu although Atlas 66 was developed in the USA. More recently, a Chinese wheat landrace, FSW, was found to have Al resistance similar to Atlas 66, but FSW has a different haplotype pattern for the markers derived from ALMT1.^{60,62} Inheritance of Al resistance in wheat has been well studied. A major QTL on 4DL has been identified in wheat cultivars BH 1146, Atlas 66 and Chinese Spring.⁴³ Markers are available for screening this QTL in wheat materials.⁴⁴ In addition, diagnostic markers for ALMT1 gene were reported^{44,45} and also mapped on the 4DL QTL region of Atlas 66.³⁸

However, some studies demonstrated that more than one gene might be involved in Al resistance of wheat. Berzonsky⁴² reported that Al resistance in Atlas 66 was determined by a complex genetic mechanism involving several genes. Near-isogenic lines containing a single Al resistance gene from Atlas 66 show only partial Al resistance, providing indirect evidence to support this assumption. Further study of the near-isogenic lines suggested that at least two genetic loci might contribute to Al resistance in Atlas 66.⁵⁴ More recently, Zhou et al.⁴⁶ reported a minor QTL for Al resistance on chromosome 3BL of Atlas 66, in addition to the major QTL on 4DL. The two genes BnALMT1 and BnALMT2 from rape (*Brassica napus*) show homology to ALMT1 from wheat and shows Al tolerance. Low level of tolerance mechanism by five genes i.e., Arabidopsis blue copper-binding protein gene (*AtBCB*), tobacco GST (*parB*), tobacco peroxidase gene (*NtPox*), a tobacco guanosine diphosphate-dissociation inhibitor gene (*NtGDI*) and F9E10.5, has been defined. Al-tolerance genes in the moderately tolerant wheat Chinese Spring are located in chromosome arms 6AL, 7AS, 2DL, 3DL, 4DL and 4BL and in chromosome 7D. In self-incompatible rye, the long arm of chromosome 4 contains a major Al resistance locus called Alt3.⁴⁷ In rice, also Al tolerance mechanism has been extensively studied. Many varieties of rice has been characterised for QTL and Twenty-seven QTLs important for Al tolerance, as estimated by relative root growth, were identified in the five studies. Rice chromosome 3 (linkage block 3C) is homologous to triticeae 4L; genetic markers linked to Al tolerance loci common with wheat, barley. The two genes *WAK1* (wall associate kinase) from *Arabidopsis thaliana* and wali3, wali5 and wali61 (protease inhibitors), and part of plant Asn synthetases (wali7); respectively, confers tolerance for Al stress. Triticale is a synthetic wheat/rye hybrid that is largely grown on acid soils in Europe, South America and Australia.⁴⁸ Its Al tolerance is considered to be inherited from rye. A short arm of chromosome 3R carries genes necessary for Al tolerance. Using wheat-rye addition lines, major genes influencing Al tolerance in rye were located on chromosomes 3R, 4R, and the short arm of 6R.⁴⁹ The Al-induced genes encoding proteins that function to overcome oxidative stress e.g., glutathione S-transferase, peroxidase, blue copper-binding protein, phenylalanine ammonia lyase, 1,3-(β -glucanase, or cysteine proteinase) has been previously reported. Altolerance is genetically controlled by few major genes.^{50,51} But the research reports on the Al tolerance in oat are very few. Genetic studies made in Brazil indicate that Al in oat is controlled by one or two dominant genes with

the tolerance genotype carrying AlaAla. In addition, expression of these Al-induced genes in transgenic *Arabidopsis* plants conferred Al tolerance.⁵² Basu et al.⁵³ reported that transgenic *Brassica napus* overexpressing *MnSOD* gene acquires an Al resistance phenotype.

The identification of stress-regulated genes provides new tools to reduce Al stress. Among the candidate genes regulated by Al stress, several could play a role in alleviating phosphate deficiency and provide energy to fight oxidative stress. Nutrient deficiency, and especially phosphate, occurs in the presence of Al due to the precipitation of Al phosphate.³⁶ The mechanism of expression of two *GST* genes in *Arabidopsis* *AtGST1* and *AtGST11*, under Al stress was elucidated by Ezaki et al.⁵⁴ An approximately 1-kb DNA fragment of the gene was fused to a β -glucuronidase (*GUS*) reporter gene (*pAtGST1::GUS* and *pAtGST11::GUS*) and introduced in *Arabidopsis thaliana* and significant tolerance to Al stress has been observed. The constructed transgenic lines showed a time-dependent gene expression to a different degree in the root and/or leaf by the Al stress. The *pAtGST1::GUS* gene was induced after a short Al treatment (maximum expression after a 2-h exposure), while the *pAtGST11::GUS* gene was induced by a longer Al treatment (approximately 8 h for maximum expression). Since the gene expression was observed in the leaf when only the root was exposed to Al stress, a signaling system between the root and shoot was suggested in Al stress. Regulation of Al tolerance by alternative oxidase (AOX) in tobacco has been observed through an overexpression approach.⁵⁵ Recently, two genes *STAR1* and *STAR2*, were identified by Huang et al.⁵⁷ which are responsible for Al tolerance in rice. Both, *STAR1* and *STAR2* are expressed mainly in the roots and are specifically induced by the Al exposure.

Endocytosis and Endocytic Vesicle Recycling as Primary Target of the Al Toxicity?

Any explanation of the Al toxicity in plants must deal with the fact that shoots are less sensitive than roots; and that in the roots only very small region of the root apex, the distal portion of the transition zone, is the most sensitive portion of the whole root.^{4,63-65} In other words, the primary target must be either a molecule which is expressed only in this very particular root apex zone, or a process which is accomplished very actively only in cells of this root apex zone. Recent studies highlight high rates of endocytosis and endocytic vesicle recycling in cells of the this root apex zone.^{66,67} Moreover, these cells internalize Al via endocytosis⁶⁴ and the endosomal Al affects endosomes, endocytic vesicle recycling^{64,68,69} and all the processes linked to this phenomenon including the actin cytoskeleton,^{63,68} nitric oxide (NO) production⁶⁴ and the polar auxin transport.⁶⁹⁻⁷¹ Close interactions between high Al-sensitivities, endocytic vesicle recycling, prominent NO production and signaling as well as dynamic and abundant actin cytoskeleton are characteristic not only for root cells of the transition zone^{63,64,68} but also for apices of the tip-growing cells such as pollen tubes and root hairs⁷³⁻⁷⁵ (review in ref. 76). The higher Al toxicity is also linked to higher proportion of recycling pectins including the deesterified JIM5-positive pectins and the boron cross-linked RGII pectins.⁷⁷⁻⁷⁹ As these cell wall pectins are internalized together with the PIN proteins

transporting auxin and auxin itself,⁸⁰⁻⁸² it is not surprising that the Al toxicity affects strongly the polar auxin transport in cells of the transition zone.⁶⁹⁻⁷¹ Finally, also in animals/humans, Al is neurotoxic and the Al-sensitive neurons are also active in endocytosis and endocytic vesicle recycling (reviewed in ref. 76). So rather unexpected unification of the Al toxicity phenomena in biology is possible as common features of the Al toxicity emerge for both animal and plant cells.⁷⁶

Future Directions and Conclusions

Al³⁺ solubilized in acidic soil is extremely toxic in terms of root elongation, and is believed to be the primary factor inhibiting plant growth. Therefore, intensive research has been conducted in order to ascertain the mechanisms inherent to the Al toxicity and tolerance, on scales from the global to the molecular. Many of the biological activities of the plant are altered via the Al toxicity. So through selection and breeding process strategies, it is possible to develop Al tolerant plant. Better understanding of the Al tolerance mechanisms involving internal detoxification of Al with organic acids and the sequestration of the Al-OA complexes in the vacuole will be needed. Also, deeper understanding of the role of mitochondria and biochemical mechanisms involved in Al stress signaling needs to be achieved. Designing appropriate screening method remains the most challenging aspect of developing and characterizing Al tolerant plant. Over the past decades many researches has been done for significant progress towards the goal of developing crops better suited for cultivation with Al toxicity in acid soil. Several physical aspects of the Al cytotoxicity have been uncovered. Screening assays based on Al-accumulation in root cells and excluding of Al from the root should be extensively studied for better response of plant to the Al-phytoxicity. With further identification of molecular markers linked with Al-tolerance gene it is possible to develop better Al tolerant crop. However the nutrient deficiencies associated with the Al toxicity in acid soil need to be addressed in developing new Al-stress tolerant plant lines. These technologies will prove useful in environmental cleanup procedures as well as in restoration of soil fertility. These measures in the field of research can be able to solve the problem of food scarcity due to abiotic stress and thus give food security to the malnourished population in the developing third world countries.

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Heavy metal stress and plant life: uptake mechanisms, toxicity, and alleviation

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12.1 Introduction

Heavy metals (HMs) pollution in environment occurs due to their release from natural resources such as rocks, ore minerals, volcanoes, and weathering (Szyczewski *et al.*, 2009) and various anthropogenic activities such as urban advancement, electricity generation, and mining and refinery industries (Kabata-Pendias and Mukherjee, 2007; Norgate *et al.*, 2007). HMs are transition metals, which possess atomic masses more than 0.002 kg, weight about 5 N/m³, and density greater than 5 g/cm³ (Järup, 2003; Rascio and Navari-Izzo, 2011). These metals are categorized as essential metals such as copper (Cu), zinc (Zn),

manganese (Mn), nickel (Ni), iron (Fe), cobalt (Co), molybdenum (Mo), and selenium (Se) and nonessential metals. Essential HMs play vital regulatory roles in several cellular reactions including electron transfer and in enzyme activation, in redox reaction as well as in the synthesis of pigments (Babula et al., 2009; Fageria et al., 2009; Chaffai and Koyama, 2011), whereas nonessential metals, such as chromium (Cr), cadmium (Cd), lead (Pb), silver (Ag), mercury (Hg), and arsenic (As), have no role in any biological reaction and cause toxic impacts even at low concentrations by competing with crucial elements at protein-binding sites (Torres et al., 2008). Although when the amount of these metals increases beyond the optimum point, they cause toxicity in plants by decreasing growth, causing soil quality deterioration as well as affecting the yield with probable health effects on plants (Seth et al., 2007; Seth, 2012). Toxicity of HMs depends on the concentration, reactivity as well as their oxidation capacity (Szyczewski et al., 2009). Crops are more susceptible to these HMs, and they transport to organism through the food chain. HM-stressed plant shows alteration in cellular mechanisms and gene regulation (Hussain et al., 2004; Chaffai and Koyama, 2011; Choppala et al., 2014). These HMs generate free radicals in cells, which further cause toxicity in plants. Nonessential metals slow down the various physiological reactions through the alteration in biomolecules and in regulatory proteins or by replacement of crucial metals (Sarwar et al., 2010) as well as disturb the integrity of biomolecules and affect antioxidant defense system by generating reactive oxygen species (ROS) (Sarwar et al., 2010; Chaffai and Koyama, 2011; Choppala et al., 2014) (Fig. 12.2). Plant acquires several defense approaches to safeguard against metal toxicity such as sequestration, compartmentalization, exclusion, and inactivation by the secretion of organic ligands (Choppala et al., 2014). Besides this, plants also induce antioxidant system as well as maintain the metal homeostasis by restricting the metal bioavailability.

Cadmium (Cd) is categorized as a toxic HM that contaminates the agricultural and mining industries (Foy et al., 1978). It naturally presents in the environment such as in soil with an average value more than 1 mg K/g (Peterson and Alloway, 1979). Chlorosis, leaf rolls, and stunting growth of plants are the usual symptoms of cadmium toxicity (Table 12.1). Cadmium caused harmful impacts on plant productivity and development by affecting stomatal opening, transpiration, and photosynthesis (Gabielli and Sanità di Toppi, 1999) (Fig. 12.1). It also decreased the nitrate absorption and transportation from root cell to stem shoot by reducing the activity of nitrate reductase in the shoot (Hernandez et al., 1996). It also alters the permeability of plasma membrane and reduces the water conduction (Barcelo et al., 1986; Poschenrieder et al., 1989; Costa and Morel, 1994) (Fig. 12.1). Chromium is in the list of most commonly occurring elements in the earth. Cr is an important industrial pollutant that is found in two oxidation forms: Cr(III) and Cr(VI). Cr(III) is insoluble and less mobile, whereas Cr(VI) being highly soluble and easily available to plants (Cary, 1982). Chromate is easily transported across the plasma membrane, and when it enters the cell, it reduces to Cr(III). These forms are highly toxic to plants and cause negative impacts on plant growth and development (Shanker et al., 2005). Due to chromium toxicity, plant shows stunted growth, wilting of tops, chlorosis as well as damage of root and shoot (Sharma et al., 2003) (Table 12.1). Chromium causes ultrastructural changes in the thylakoid, which leads to reduction in photosynthesis (Ali et al., 2013) (Fig. 12.1). Copper is an essential nutrient for the plant, and its concentration is low that is about 20–30 ppm in normal soils and sediments (Nriagu, 1979; Salomonsand and Förstner, 2012) and

TABLE 12.1 Negative impacts of deferent heavy metals on plants.

Heavy metals	Plant	Negative impacts on plant	References
As	<i>Brassica</i>	Decreased plant growth as well as affected the root vascular cylinder diameter and the height of epidermal cell	de Freitas-Silva et al. (2016)
	Rice	Deformed root anatomy and caused lower root-specific surface area	Deng et al. (2010)
	Mung bean	Cause reduction in root elongation by inducing oxidative stress due to enhanced lipid peroxidation but not H ₂ O ₂ accumulation	Singh et al. (2007)
	Common bean	Induced growth inhibition associated with anomalies in anatomical structure, reduction in pigment composition, increased level of reactive oxygen species, and also affected the antioxidant enzyme	Talukdar (2013)
Cd	<i>Avicennia marina</i>	Cadmium mainly accumulated in the root, caused anatomical changes such as decreased cross-sectional area of xylem and the central cylinder area and decreased width of epidermis	Zhang et al. (2013)
	<i>Brassica juncea</i>	Caused structural changes in root, stem, and leaf, altered physiological and morphological characteristics	Sridhar et al. (2005)
	<i>Merwillia plumbea</i>	Toxicity resulted in hypodermal periderm development in young root part and also the protective suberized layer	Lux et al. (2010)
	<i>Arachis hypogaea</i>	Inhibition in net photosynthetic rate as well as reduction in stomatal conductance and altered leaf structure, decrease in transpiration rate	Shi and Cai. (2008)
Cu	<i>Origanum vulgare</i>	Cause anatomical changes such as disrupted epidermis, cortex of large cells with folded walls Cytological alterations occurred such as metamorphosis of the amyloplast	Panou-Filotheou and Bosabalidis. (2004)
	<i>Myriophyllum alterniflorum</i>	Differentially affected physiological parameters such as pigment contents, osmotic potential, and proline content cell and influence membrane integrity of young leaf under increased MDA content	Delmail et al. (2011)
	Bean	Showed modification in the cell wall of various tissues such as abnormal cell wall thickening in endodermis, reduction in the water absorption by plant, enhanced phenylalanine ammonia lyase	Bouazizi et al. (2010)
	<i>Bruguiera sexangula</i>	Percolated hypodermal and stealer region, xylem and phloem deformation	Gupta and Chakrabarti. (2013)
Cr	Rice	Decreased length of epidermis and stomatal frequency, adversely affecting chloroplast as well as deformed integrity of xylem and phloem, decreased plant growth, photosynthetic pigment, and protein	Tripathi et al. (2012)
	<i>Phaseolus vulgaris</i>	Reduced germination percentage, radicle growth as well as plant growth and photosynthetic pigment	Zeid (2001)

(Continued)

TABLE 12.1 (Continued)

Heavy metals	Plant	Negative impacts on plant	References
	<i>Mentha aquatica</i>	Collapsed root cap and statolith, loss of tissue organization, increase in intercellular spaces in mesophyll, less development of chloroplast and starch granules	Bianchi et al. (1998)
	<i>B. juncea</i>	Causes growth retardation, reduced number of palisade and spongy parenchyma, clotted deposition of vascular bundles, increased number of vacuoles along the vascular bundle	Han et al. (2004)
Fe	<i>Canavalia rosea</i>	Altered nutrient uptake, changed external morphology of lateral root, deformed pericycle and cortex	Siqueira-Silva et al. (2012)
	<i>Ipomoea batatas</i>	Reduced stomatal density, radicle cell showed mitochondrial impairment, decreased nutrient uptake, and increase in antioxidative enzymatic activities	Adamski et al. (2012)

MDA, Malondialdehyde.

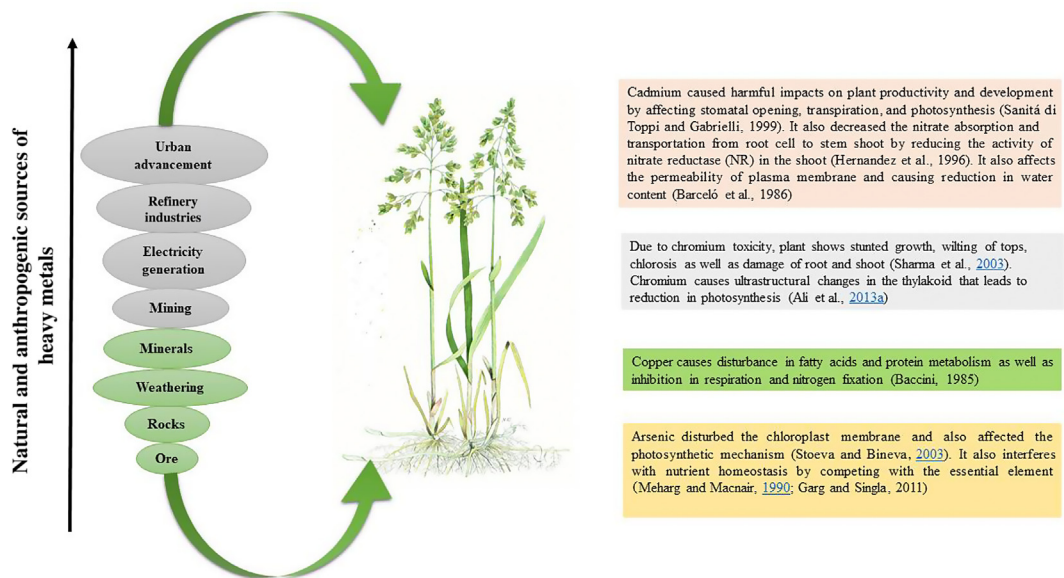


FIGURE 12.1 Source and toxicity of heavy metals.

below 2 ppb in natural waters (Baccini, 1985). But at higher concentration, it causes detrimental impacts on plant tissue and plant physiology and biochemistry such as disturbance in fatty acids and protein metabolism as well as inhibition in respiration and nitrogen fixation (Table 12.1). Iron (Fe) is the fourth recorded abundant metal in the Earth's crust, which usually presents in well-aerated soil in the Fe^{3+} and Fe^{2+} forms. It works as an electron acceptor and donor in the various electron transport chains of photosynthesis and

respiration (Fig. 12.1). It also acts as a limiting factor for the biomass production. Arsenic (As) is toxic metalloid and broadly disseminated in the environment, it predominately occurs in the form of As(V) and As(III) (Tripathi et al., 2007). At higher concentration, both forms interact with the metabolic processes and inhibit the plant growth, which leads to plant death (Table 12.1). Arsenic disturbs the chloroplast membrane and also affects the photosynthetic mechanism (Stoeva and Bineva, 2003) (Table 12.1). It also interferes with nutrient homeostasis by competing with the essential element (Meharg and Macnair, 1990; Garg and Singla, 2011) (Fig. 12.1).

12.2 Sources and metal bioavailability

HMs enter the environment by natural as well as anthropogenic activities and transport over long distance in agro-ecosystem (Shahid et al., 2015; Saher and Siddiqui, 2016). HMs discharge naturally in the environment via the natural process (Szycczewski et al., 2009). Several anthropogenic activities are responsible for HMs that contaminate soil, air, and water, and these origins reach up to several times more than the natural emissions (Chmielewska and Spiegel, 2003). These anthropogenic activities having industrial activities such as sewage, mining and waste processing (Kabata-Pendias and Pendias, 1984; Tanhan et al., 2007), commercial fertilizers, power units, and several developmental industries (Wu et al., 2004). Due to these anthropogenic activities, HMs increase in the environment, cause serious threat to food security for the growing world population (Ayangbenro and Babalola, 2017), and also have detrimental impacts on ecosystems (Harguinteguy et al., 2016). Nowadays, mineral sources, abstraction, and utilization of various minerals in several industrial processes have imposed threats in the form of HM pollution (Li et al., 2014; Goix et al., 2015; Niazi and Burton, 2016). Numerous cases of atmospheric contamination such as release of persistent and harmful HMs that were absorbed by the atmospheric dust particles, organic pollutants, and biodegradation are the main sources of HMs in the environment (Norouzi et al., 2016). The absorption of HMs depends mainly upon the availability of these metals regulated through the different factors (Benavides et al., 2005). Metals retain in the soil in the form of various chemicals in equilibrium due to soil properties (Chaney, 1988).

Soils have HMs mostly in three forms: by absorption from mineral particles, through the complexation with humus or via the precipitation reactions. Only a small amount of metal is sufficient for the plant uptake (Walton, 1994). Usually the soil metal bulks are not available for transport into the roots (Lasat, 2002). Plants acquire extremely specific route to arouse metal availability in the soil as well as to increase absorption into the root cells (Römheld and Marschner, 1986). The significant roles of root exudates have been reported in the attainment of various nutrient metals. For instance, some grasses have been of roots exudates organic acids (OAs), that are known as siderophores such as mugineic and avenic acids, which considerably enhance the soil-bound iron bioavailability (Kanazawa et al., 1994) and possibly zinc (Cakmak, 1996a,b). Though in dicot iron acquisition is facilitated through the acidification of rhizosphere via the efflux of H^+ ion from roots, but due to acidic environment, ferric iron reduces to ferrous that is easily absorbed through the plant cell (Chaney et al., 1972; Bienfait et al., 1982). Metal availability also causes impact on the plant and microbial activities. Some bacterial sp. release biosurfactants such as

rhamnolipids, which make hydro-labile pollutants more soluble in water (Volkering et al., 1997). Plant also releases some lipophilic compounds that are known for increasing pollutant solubility or they can also promote microbial populations that are able to produce biosurfactants (Siciliano and Germida, 1998). Availability of metals affected by the metal chelators such as siderophores, OAs, and phenolics released by the plant and bacterial cells that liberate metal ions from soil and make the metals easily available to them (Pilon-Smits, 2005).

12.3 Consequences of heavy metals in plants

HMs affect the environment in several ways on different levels. It influences the organisms both positively and negatively depending on the type of metals and its concentration. On the positive aspect, HMs serve as essential micronutrients; and on negative aspect, it induces the severe toxicity (Kafka and Puncocharova, 2002). Similarly, in the plants, toxic HMs may lead to chlorosis, necrosis, phenotypic changes, and damage to plant organs (Benzarti et al., 2008). Moreover, they potentially affect physiology and biochemical structure of plant and could also hinder the growth parameters and ultimately the cell death in the plant (Popova et al., 2009). The growth reduction occurs due to decrease in photosynthetic rate, chlorophyll content, and breakage of cellular membranes. Moreover, alteration in the cell molecules and organelles occurs by the generation of ROS (Ekmekçi et al., 2009). Metal phytotoxicity increased oxidative stress that causes toxicity and affects antioxidant defense system. Burst of free radicals in plant cell is a stress marker, but these reactive radicals also behave as a messenger in signaling (Pourrut et al., 2013). HMs in contact with plants cause an inequity between ROS generation and their removal, therefore physiological changes occur (Jonak et al., 2004). HM-induced overgeneration of ROS, which leads to apoptosis as a result of which membrane peroxidation, damage to RNA, DNA, and key enzymes inhibition, and protein oxidation occur in plants (Flora, 2011; Shahid et al., 2014). Therefore to prevent their damage to cells caused by HMs plant established a defense mechanism with the help of phytochelatin (PCs), metallothioneins (MTs), and sulfur compounds (Hossain et al., 2012). The cell defense system of plant consists of both nonenzymatic system; glutathione (GSH), ascorbic acid (AA), α -tocopherol, β -carotene, and enzymatic system; superoxide dismutases, catalases, peroxidases, GSH reductases, and NADP⁺-reducing enzymes (Hossain et al., 2012).

Plants generate ROS naturally in various organelles such as chloroplasts, mitochondria, and peroxisomes (Shahid et al., 2014). These radicals are highly reactive, unstable and possess free electrons in their last shell. Hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), superoxide anion (O₂⁻), hydroxyl (HO•), alkoxyl (RO•), peroxy (RO₂•) radicals, and organic hydroperoxide (ROOH) are ROS, and their generation increase in the plant tissue due to HM toxicity (Shahid et al., 2017). HMs reduced the plant growth by affecting the photosynthetic activity and photosynthetic pigments (Sheoran and Singh, 1993). Metals also cause water stress by affecting stomatal and transpiration activity, relative water content in leaf due to decrease in size and quantity of xylem tissue, chloroplast, and cell elongation. These metals enter the food chain via edible plant parts so it is necessary to eliminate the HMs from the ecosystem in order to regulate a healthy environment (Adrees et al., 2015).

12.4 Mechanisms of heavy metals uptake and transport in plants

The absorption of HMs in plant tissue is mediated at the extracellular and intracellular levels through the mixed reaction of physiological, biochemical, and molecular mechanisms in polluted soils (Hossain et al., 2012).

Internally plants distribute metals in several distinct ways. These HMs are either localized in root and stem or accumulated in different parts in the nontoxic forms for further distributions and use. In plants, HMs are uptakes by the cortical cells of the root by the competitive uptake with vital elements and follow the symplastic and apoplastic pathways (Salt et al., 1995). The movement of metal to the root surface mainly depends on these following factors: mass flow, that is, metal ions absorbed by the root surface, through the diffusion mechanism as well as by the root interception cause by root growth (Anjum et al., 2012). The uptake of metal may be through the apical region of the root or through the entire surface depending upon the respective metals. In addition, metal uptake also depends upon the root capacity and growth.

There are two ways for the entrance of HMs to plant cell, these are apoplastic (extracellular) and symplastic (intracellular) routes. Apoplastic way of the root is easily permeable to solutes, where metals are absorbed in the root cell walls due to their negatively charged sites (Lasat, 2002) and translocate into the root tissue, whereas the suberin lamellae prevent the solutes from the apoplast to xylem (Taiz and Zeiger, 2002). Metal ions transport through the protein for their further translocation from endodermis to xylem of root (Pilon-Smits, 2005).

Some HMs are chelated through the OAs (Krämer et al., 1996). But it is unclear for some metals that how they chelate and which transport protein is responsible for their transportation into the root xylem (Pilon-Smits, 2005), because of their charged metal ions easily cannot across the lipophilic structure of cell walls. Thus cells must mediate ion transport by transporter protein (Lasat, 2002). Various transporters such as CPx-ATPases, the Nramps, and cation diffusion facilitator family (Williams et al., 2000), and ZIP family in plants are worked for metal uptake and homeostasis for the metal tolerance (Guerinot, 2000; Hall, 2002). Moreover, HM ions such as Cd enter the plant through the transporters for cations such as Fe²⁺ (Thomine et al., 2000).

Membrane transporter possesses an extracellular domain that binds the specific metals and a transmembrane domain that remain in extracellular and intracellular membrane mediums that transfer metal from outside to inside cell (Lasat, 2002). These transporters possess specific transport capacity (V_{max}) and affinity for ion (K_m) (Axelsen and Palmgren, 2001). Transportation of HMs from root to shoot occurs via the xylem by specific membrane transport mechanism. For instance, Ni loading in xylem tissue may be derived by the complexation of Nickel to free histidine (Salt et al., 1995; Krämer et al., 1996). Translocation of metals in xylem tissue is mainly occurred through the transpiration-driven mass flow (Salt et al., 1995). The cell wall of xylem has high cation-exchange capacity (CEC), consequently noncationic metal chelate complexers should be moved effortlessly in xylem (Senden and Wolterbeek, 1990). Cadmium translocated into the xylem tissue through the chelation in the form of cadmium citrate, while PCs and other thiol-containing ligands do not directly involve in Cd transportations (Salt et al., 1995).

(Table 12.1). The movement of metals in the xylem strands from the root cell to stem is mainly occurred through the transpiration, that is, to create a negative pressure to move water and solutes upward (Taiz and Zeiger, 2002), and in the leaf, metals are transported through the membrane transport proteins. Metals are transported by precise membrane transporter proteins, and when they are taken up by the symplast, compartmentalized in specific organelles, they could not be much harmful to essential cellular processes. Mainly these HMs are accumulated into the vacuole or cell wall (Burken, 2003; Cobbett and Goldsbrough, 2002), whereas in tissue HMs are gathered in the epidermal layer or hairs.

12.5 Mechanism of heavy metals detoxification/tolerance in plants

Plants acquire several cellular mechanisms, including extracellular and intracellular, to avoid the metal toxicity. On the primary stage, root cells check the entry of metals by adopting avoidance strategy, but when somehow these metals enter the root cells, detoxified by several mechanisms such as cell wall binding, OAs, chelation, and sequestration, these intracellular detoxification mechanisms are called tolerance strategy of plants against the metal toxicity. Antioxidative defense systems as well as the formation of stress-related proteins in plants are also the part of tolerance mechanism in metal toxic plants. So the plant utilizes two types of strategies against the metal toxicity, avoidance (restriction to metal uptake) and tolerance (intracellular detoxification) (Dalvi and Bhalerao, 2013). These metals are sequestered in the leaf cell, bounded by the chelators.

Chelators implicated in metal sequestration comprise the tripeptide glu-cys-gly (GSH) and its oligomer, the PCs and MTs show active role in sequestration, tolerance, and in regulation of vital metals (Goldsbrough, 2000).

12.6 Avoidance mechanisms

Avoidance strategies limit the uptake of HMs and check the entry of metal ions into plant cells through the root tissues, these mechanisms are the first line of extracellular defense system against the metal toxicity. This extracellular defense system involves several strategies such as immobilization by mycorrhiza, complexation through root exudates, and alteration of rhizosphere pH, secretion of metal-binding OAs, or development of redox barrier (Fig. 12.2).

Ectomycorrhizas and *Arbuscular mycorrhiza* are the mycorrhizal association, which grow on HM-polluted soil. *Mycorrhizas acquired* exhibits effective exclusion barriers such as absorption, adsorption, or chelation mechanisms to check the influx of HMs in to host plant (Dalvi and Bhalerao, 2013) (Fig. 12.2). Besides this, root also releases amino acids or OAs, water, inorganic ions, carbohydrates, etc. as well as excretes bicarbonates, protons, carbon dioxide, and secretes mucilage, siderophores, allelopathic compounds, etc. (Fig. 12.2), which are communally called root exudates and play an important role to survive the plant in polluted areas by forming stable ligand complexes and make the metals less toxic (Dalvi and Bhalerao, 2013). Cellular exclusion is an imperative adaptation strategy against the metals toxicity. Huge amount of metals in roots are mainly present in apoplastic space (Hossain et al., 2012).

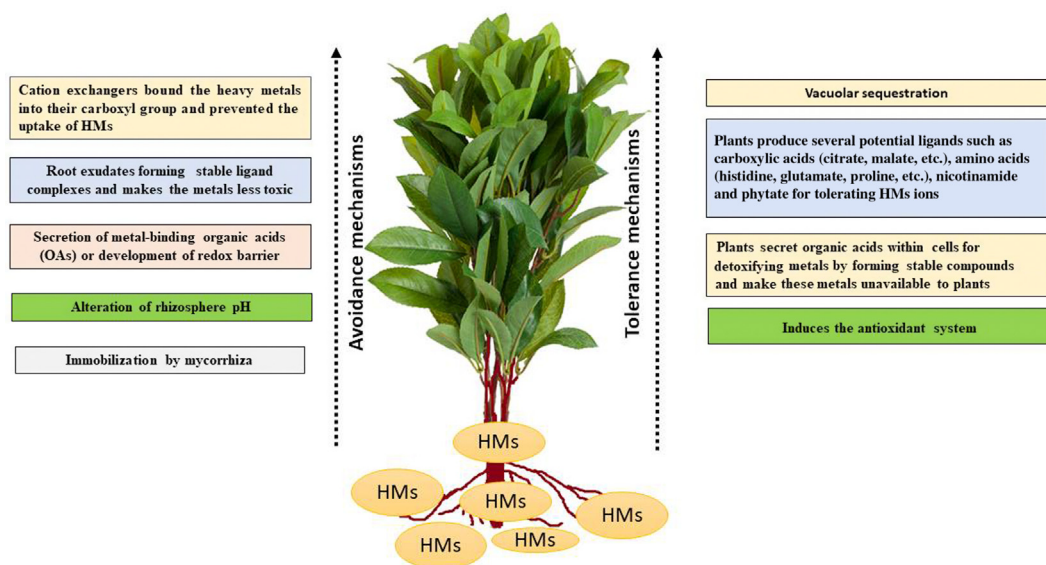


FIGURE 12.2 Mechanism of HM detoxification/tolerance in plants. *HM*, Heavy metals.

The cell wall and plasma membrane could be a potential site for the HM tolerance due to their accumulation properties such as Italian ryegrass (*Lolium multiflorum*), which accumulates about 60% of copper in the root cell wall and plasma membrane (Iwasaki et al., 1990).

Plant CEC depends upon the exchange sites of roots cell wall (Horst and Marschner, 1978). Sensitive wheat cultivars have less cell wall CEC than tolerant cultivars (Masion and Bertsch, 1997), which shows the tolerant varieties have high CEC to prevent the entry of HMs (Hossain et al., 2012). Reichman (2002) had described about utilization strategy of plant to produce metal tolerance by active efflux that decreases the intracellular concentration to subtoxic levels.

12.7 Metal binding to cell wall

Extracellular carbohydrates present in cell walls check the uptake of HMs into the cytosol. Cell wall pectins contain polygalacturonic acid, which are cation exchangers and bound the HMs into their carboxyl group and prevent the uptake of HMs (Fig. 12.2). Several studies reported that metal tolerance influenced by the metal uptake, which is generally modulated by the chemical properties of the cell wall. On the other side the plant cell wall minimally influenced the HM tolerance because of lacking of sites of metal absorption. Therefore the complete mechanism of the plant cell wall against HMs toxicity is still not well understood and revealed (Dalvi and Bhalerao, 2013).

Plants are capable of lessening the negative impacts of toxic HMs by controlling the metal allocation and localization within the cells. Besides hyperaccumulator, much higher

amount of metals present in plants root in comparison to shoot to minimize the transportation and accretion of HMs to the cells of plant shoot (Hossain et al., 2012). So the tolerant genotypes have a lesser amount of HMs in the shoot than the sensitive genotypes. Therefore tolerant cowpea (*Vigna unguiculata*) genotypes have found homogenized allocation of Mn in the leaf thoroughly, while the nontolerant genotypes agglomerate Mn in the specific area of leaf in the form of dark brown spots of manganese oxide precipitates (Hossain et al., 2012).

12.8 Tolerance mechanisms

HM tolerance mechanisms include accumulation, restoration, and immobilization of HMs as metals get bonded with the amino acids, proteins, or peptides and makes a complex. However, it is known as the “plant’s second line of defense,” which chiefly accelerates intracellular detoxification of metals in the plants. Furthermore, Tong et al. (2004) described that on toxicity of HMs, plants primarily bind or modify the metal ions to minimize the metal transport across the plasma membrane and which metal ions entered the plant cells were detoxified by their inactivation or converting them into less toxic forms (Dalvi and Bhalerao, 2013). Once an HM got entry in cells, plants acquire various strategies to cope with it, such as transporting of HMs out of the cells, sequestration of ions into the vacuole or other cell organelles where sensitive metabolic process occurs (Clemens, 2001). So the central vacuole is a suitable storage sites for toxic HMs accumulation, and the two vacuolar proton pumps, a vacuolar proton-ATPase and a vacuolar proton pyrophosphatase, facilitate the vacuolar up take of solutes, which are catalyzed by the channels or transporters. When these metals are sequestered in vacuoles they bind by chelators that are polypeptides. The two significant metal-binding polypeptides are found in plants such as MTs and PCs. MTs have significant properties, thereby regarded as gene-encoded, cysteine-rich polypeptides with low molecular weight (Robinson et al., 1993). Numerous MT genes (MT1, MT2, MT3, and MT4) now have also been found involved in different higher plants, including *Arabidopsis* (Goldsbrough, 2000). PCs are also class III MTs, which may be cysteine rich having general structure (3 -Glu Cys) n -Gly with $n = 2-11$. PCs are produced from GSH by a specific transpeptidase named 3-glutamyl cysteine dipeptidyl transpeptidase, a phytochelatin synthase (PCS) (Vatamaniuk et al., 2004), required for posttranslational activation by the HMs (Klapheck et al., 1995). The best activator for the enzyme PCS is cadmium whereas moderately activate in the presence of silver, lead, zinc, etc. (Cobbett, 2000; Pickering et al., 2000). PCs sequestered the metal-PC complexes in the cell vacuole via the tonoplast membrane through ABC transporter (Schat et al., 2002), further they stabilized by the acid-labile sulfide (Cobbett and Goldsbrough, 2002). These PCs play an imperative role in the exclusion of cadmium and arsenic, while playing an insignificant role in the alleviation of HMs such as Cu, Zn, Ni, and SeO_3 (Cobbett, 2000). Hyperaccumulators possess extra mechanisms to detoxify metal for instance Ni hyperaccumulator—*Thlaspi goesingense* has high tolerance of Ni as it makes complex with the histidine, which further causes metal inactivation (Krämer et al., 1996). At cytoplasmic level, PCs and MTs have significant role in the metal tolerance, by forming complexes with metals, and store these complexes into the vacuole without any negativity (Hall, 2002).

HM tolerance and detoxification in plants occurs via two mechanisms: external and internal tolerance and detoxification. However, under external detoxification mechanism, it involves excretion of OAs from roots and forms a stable compound with metal ions and affects their mobility and bioavailability to the plants (Fig. 12.2). In the internal tolerance, chelation occurs through OAs into the cytosol or makes metal ions less toxic (Hall, 2002). Plants produce several potential ligands such as carboxylic and amino acids (AAs) for the tolerance of HM ions (Hall, 2002; Balaji et al., 2003; Yang et al., 2005; Sharma and Dietz, 2009; Singh and Chauhan, 2011; Sunitha et al., 2013). These acids within cells detoxify metals by forming stable compounds and make these metals unavailable to plants, and it also has role in nitrogen metabolism under which it acts as metabolic intermediates in the generation of ATP from carbohydrates. Consequently, metabolic anomaly in these mechanism reflected by the changes in the OA concentration. So the increase concentration of OAs at the metals toxicity could be detoxification mechanisms or consecutively irregularities in the metabolism produced OA as an indicator of metal stress (Dalvi and Bhalerao, 2013). Many researchers have been worked on the hyperaccumulator plants during the last few decades.

Hyperaccumulation depends on the plant species, pH, organic matter content, CEC of the soil as well as the types of metals (Sarma, 2011). In hyperaccumulator plants, there is a fast and an efficient movement of metals from root to shoot by the xylem tissue, which could be driven by the transpiration (Salt et al., 1995). Hyperaccumulation of HMs occurs even at low external metal amount. HM uptake is tremendously high in hyperaccumulators in root tissues, because of highly active membrane transporter in the plasma membrane. These transporters tolerate metals stress via the process of intracellular compartmentalization and chelation (Pilon-Smits and Pilon, 2002).

Several chelators such as OAs or nicotianamine also play a dynamic role in the transportation of metal ions through the xylem tissue (Sunitha et al., 2013). On metal exposure, plants synthesize various types of novel proteins in which most of the proteins play a regulatory role for HM influx in the plant that ultimately leads to the metal homeostasis and its exclusion. Heat shock proteins, which are stress related, act as “molecular chaperones” and work in posttranscriptional process. Moreover, it might also play an imperative role in the defacing and restoring of proteins under stressed condition. Increased generation of ROS is the primary indicator of HM-induced stress. At low level, during normal metabolic processes, these ROS constantly produce in the plant. Therefore hydrogen peroxide (H_2O_2) acts as a signaling messenger that modulates defense system. ROS have dual function: at the elevated concentration, they damage the tissue; while at normal level, they induce the antioxidant system. However, a decreased level of oxidative burst with increased resistivity to HMs occurs through the complex ROS destruction mechanism at the molecular and cellular levels.

Peleg and Blumwald (2011) suggested that the increased synthesis of hormones due to HM toxicity shows the adaptation of plants. Hormones such as salicylic acid (SA), jasmonic acid (JA), ethylene, and gibberellic acid (GA) are involved in the plant defense signaling pathways. JA increased the biosynthesis of GSH and ethylene, which play an active role in the defense of HMs toxicity (Bajguz and Hayat, 2009). Besides this, vacuolar sequestration, morphological features of the plant perform an essential role in the sequestration of HMs thereby removes the induced toxicity (Fig. 12.2). Moreover, various

reported studies also discussed about the mechanism of metal mitigation through sequestration and chelation, and also the role of glandular trichomes and epidermal structures (hydropotes) in these processes.

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