Mechanisms of Heavy Metal Toxicity in Plants

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Abstract

Pollution of the environment with the toxic heavy metals has become one of the major causes for worry for human health in both emerging and advanced countries. Metal contamination issues are becoming more and more common in India and elsewhere, with many documented cases of metal toxicity in mining industries, foundries, smelters, coal-burning power plants, and agriculture. As land application becomes one of the foremost waste utilization and disposal practices, soil is increasingly being seen as a major source of metal(loid)s reaching food chain, largely through plant uptake and animal transfer. Heavy metal buildup in soils is of concern in agricultural production due to the adverse effects on food safety and marketability, crop growth due to phytotoxicity, and environmental health of soil organisms. Metal toxicity has high impact and relevance to plants, and consequently, it affects the ecosystem, where the plants form an integral component. A few metals, including copper, manganese, iron, cobalt, zinc, and chromium, are, however, essential to plant metabolism in trace quantities. It is only when metals are present in bioavailable forms and at excessive levels; they have the potential to turn out to be toxic to plants through formation of complex compounds within the cell. Plants growing in metal-contaminated sites exhibit altered metabolism, growth reduction, lower biomass production, and metal accumulation. Various physiological and biochemical processes in plants are affected by metal toxicities. The present-day investigations into toxicity and tolerance in metal-stressed plants are prompted by the growing metal pollution in the environment. This article details the range of heavy metals, toxicity for plants, and mechanisms of plants to cope with metal toxicity.

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5.1 Introduction

Currently, the available literature focuses mainly on different features of heavy metal buildup both in the ecosystem and in the food chain. Reports are also available on the occurrence of high concentration of these metals in crop plants, and on consumption of the produce or the plants, animals and human beings are also affected (Gopal et al. 2003). Being sessile organisms, plants are continuously exposed during their life cycle to adverse environmental conditions that detrimentally affect growth, development, or productivity. The occurrence of toxic compounds, such as heavy metals (HMs), is one important factor that can cause damage to plants by altering most important plant physiological and metabolic processes. Heavy metals are important environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons. The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech Water Treatment and Air Purification 2004). "Heavy metals" in a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3, or five times or more, are greater than water (Hawkes 1997). However, chemical properties of the heavy metals are the most influencing factors compared to their density. Heavy metals include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), zinc (Zn), chromium (Cr), iron (Fe), arsenic (As), silver (Ag), and the platinum group elements (Nagajyoti et al. 2010).

Heavy metals are mostly found in dispersed form in rock formations. Industrialization and urbanization have increased the anthropogenic contribution of heavy metals in the environment. Unlike organic pollutants, heavy metals once introduced into the surroundings cannot be biodegraded. They persist indefinitely and cause pollution of air, water, and soils. Heavy metals have the largest availability in soil and aquatic ecosystems and to a relatively smaller proportion in the atmosphere as particulate or vapors. Heavy metal toxicity in plants varies with plant species, specific metal, concentration, chemical form and soil composition, and pH, as many heavy metals are considered to be essential for plant growth. Some of these heavy metals like Cu and Zn either serve as cofactor and activators of enzyme reactions, e.g., informing enzymes/substrate metal complex (Mildvan 1970) or exert a catalytic property such as prosthetic group in metalloproteins. These essential trace metal nutrients take part in redox reactions, electron transfer, and structural functions in nucleic acid metabolism. Some of the heavy metals such as Cd, Hg, and As are strongly poisonous to metal-sensitive enzymes, resulting in growth inhibition and death of organisms.

5.2 Nature and Classification of Heavy Metals

No organic life can develop and persist without the involvement of metal ions. Current research has revealed that life is as much inorganic as organic. Commonly, the term trace element is rather loosely used in current literature to designate the elements which occur in small concentrations in natural biological systems. The growing public concern over the deteriorating quality of the environment has led to a widespread usage when referring to trace elements. Thus, for all practical purposes, other terms such as "trace metals," "trace inorganics," "heavy metals," "microelements," and "micronutrients" have been treated as synonyms with the term trace elements (Nagajyoti et al. 2010). The elementary constituents of plant, animal, and human life may be classified as major and trace elements, the latter group comprising both essential and nonessential elements (including toxic elements).

Some of the heavy metals such as Fe, Cu, and Zn are essential for plants and animals (Wintz et al. 2002). The availability of heavy metals in medium varies, and metals such as Cu, Zn, Fe, Mn, Mo, Ni, and Co are essential micronutrients

(Reeves and Baker 2000), whose uptake in excess to the plant requirements result in toxic effects (Monni et al. 2000; Blaylock and Huang 2000). They are also called as trace elements due to their presence in trace (10 $mgkg^{-1}$, or mgL^{-1}) or in ultra-trace (1 μgkg^{-1}, or μgL^{-1}) quantities in the environmental matrices. The essential heavy metals (Cu, Zn, Fe, Mn, and Mo) play biochemical and physiological functions in plants and animals. Two major functions of essential heavy metals are the following: (a) participation in redox reaction (Fe, Cu Cr, and Co) and (b) direct participation, being an integral part of several enzymes (Zn, Cd, Ni, Al, etc.).

An alternative classification of metals based on their coordination chemistry categorizes heavy metals as class B metals that come under nonessential trace elements, which are highly toxic elements such as Hg, Ag, Pb, and Ni (Nieboer and Richardson 1980). Some of these heavy metals are bioaccumulative, and they neither break down in the environment nor are easily metabolized. Such metals accumulate in the ecological food chain through uptake at primary producer level and then through consumption at consumer levels. Plants are stationary, and roots of a plant are the primary contact site for heavy metal ions in soil. In aquatic systems, whole plant body is exposed to these ions. Heavy metals are also absorbed directly to the leaves due to particles deposited on the foliar surfaces.

5.3 Mechanism of Heavy Metal Toxicity in Plants

Oxygen-free radicals are produced when molecular oxygen accepts electrons from other molecules, and many intracellular reactions reduce oxygen to superoxide (O_2^-) or hydrogen peroxide (H_2O_2). Even though these molecules are not very reactive, they can form hydroxyl radicals (−OH), which are possibly responsible for most of the oxidative damage in biological systems (Halliwell and Cutteridge 1990). The one-electron reduction of molecular oxygen to the superoxide radical is thermodynamically unfavorable (Illan et al. 1976), but can nevertheless take place by interaction with another paramagnetic center. Transition metals such as iron and copper (M) have frequently unpaired electrons and are, therefore, very good catalysts of oxygen reduction, following the reaction:

$$M^n + O_2 \rightarrow M^{n+1} + O_2^-$$

In aqueous solutions at neutral pH, O_2^- can generate H_2O_2, which can subsequently decompose to produce OH by the Haber–Weiss reaction, copper or iron (M) being again involved as follows:

$$M^{n+1} + O_2^- \rightarrow M^n + O_2$$
$$M^n + H_2O_2 \rightarrow M^{n+1} + OH^- + OH$$

These reactions are summarized as

$$O_2 + H_2O_2 \rightarrow O_2 + OH^- + OH$$

When iron is the transition metal in the Haber–Weiss reaction, it is called the Fenton reaction. Hydroxyl radicals (OH) produced by the Haber–Weiss reaction can oxidize biological molecules, leading to major cellular damages and ultimately to cell death. Hydroxyl radicals can be produced in close proximity to DNA, adding or removing H atoms to DNA bases or the DNA backbone, respectively (Pryor 1988). Their action could be responsible for 10^4–10^5 DNA base modifications per cell per day (Ames et al. 1991). Fe^{2+} ions either complexed to a phosphate residue or coordinated with ring nitrogens or free in solution are involved in these hydroxyl radical-mediated DNA alterations (Luo et al. 1994). Metal ions, through Haber–Weiss reaction, also play an important role in the oxidative modifications of free amino acids and proteins, and this aspect has been reviewed by Stadtman (1993). Histidine, arginine, lysine, proline, methionine, and cysteine residues are the most common sites of oxidation in proteins, and their major oxidation products have been identified. Frequently, only one amino acid residue in a given protein is modified by oxidation. These modifications

correspond to site-specific processes, amino acid residues at metal-binding sites being specific targets. A major consequence of oxygen-free radical damage to proteins is to target them for degradation by proteases (Roseman and Levine 1987). Release of Fe^{2+} from [4Fe-4S] clusters of some dehydratases such as aconitases is also another important aspect of protein oxidation (Goldstein and Czapski 1986). Finally, oxygen and transition metals (in particular iron) are implicated in lipid peroxidation, and biological membranes rich in polyunsaturated fatty acids are extremely susceptible to these reactions. Ascorbic acid is known to quench lipid peroxy and alkoxy radicals in the aqueous phase. However, ascorbate can also function as a prooxidant by reducing Fe^{3+} or Cu^{2+}, allowing the metal-catalyzed transition of lipid hydroperoxides to radical species (Scholz et al. 1990).

5.3.1 Genotoxicity

Metal binding to the cell nucleus causes promutagenic damage including DNA base modifications, inter- and intramolecular cross-linkage of DNA and proteins, DNA strand breaks, rearrangements, and depurination. Chemical reactions driving this damage, and the resulting mutations, are characteristics of an oxidative DNA attack (Kasprzak 1995). Metal-mediated production of reactive oxygen species in the DNA vicinity generates principally the promutagenic adduct 8–oxoG (7, 8-dihydro-8-oxoguanine) that could miss pair with adenine in the absence of DNA repair, resulting in C to T transversion mutations (Cunningham 1997). Although oxidative damage explains most of the mechanisms involved in metal-mediated carcinogenicity and acute toxicity, other pathways also have to be considered. DNA methylation aberration has been shown to play an important role in tumorigenesis (Zingg and Jones 1997). Cell treatment with carcinogenic nickel can cause chromatin condensation through competition with magnesium ions. This condensed chromatin is hypermethylated, leading to silencing of putative anti-oncogenic gene expression, thus driving treated cells to a carcinogenic state (Lee et al. 1995). Most of the cellular and molecular aspects of metal toxicity in plants are unknown, even though deleterious effects on crop production have long been recognized. Preliminary observations on putative metal genotoxic effects in plant are scarce. At the organ level, this symptom is common to numerous metals. Nickel accumulation in maize root apex reduces meristem mitotic activity, and this could be due to the lack of integrity of root meristems (L'Huillier et al. 1996). Concentration and time-dependent cadmium, copper, and nickel clastogenic effects were observed in *Helianthus annuus* (Chakravarty and Srivastava 1992). Taken together, these observations suggest that genotoxic effects could be in part responsible for metal phytotoxicity, deserving more work to elucidate the underlying mechanisms.

5.3.2 Heavy Metals on Antioxidant Activity

Heavy metal toxicity is reported to increase the activity of enzymes such as glucose-6-phosphate dehydrogenase and peroxidase in the leaf of plants grown in polluted soil (Van Assche and Clijsters 1987). Metal ions play an important role in the antioxidant network, as these are essential cofactors of most antioxidant enzymes. For example, all isoforms of superoxide dismutase (SOD) contain bound heavy metal ions. Cu and Zn constitute the cofactor of the Cu/Zn-SOD associated with chloroplast; glyoxysomes contain Mn-SOD. Fe-SOD has been found in the chloroplast of some plants. Metals are involved in the direct or indirect generation of free radicals (FR) and reactive oxygen species (ROS) in the following ways: (1) direct transfer of electron in single-electron reduction, (2) disturbance of metabolic pathways resulting in an increase in the rate of FR and ROS formation, (3) inactivation and downregulation of the enzymes of the antioxidative defense system, and (4) depletion of low molecular weight antioxidants (Aust et al. 1985). The ROS produced in leaf cells are removed by complex enzyme catalase (CAT),

ascorbate peroxidases (APX), glutathione peroxidases (GPX), superoxide dismutase (SOD), and glutathione reductases (GR) of antioxidant systems. Heavy metals induced oxidative damage in senescing oat leaf cells (Luna et al. 1994), primary leaves of mung bean (Weckex and Clijsters 1997), and in wheat leaves (Panda and Patra 2000). Proline is known to play a role in the detoxification of active oxygen in *Brassica juncea* and *Cajanus cajan* under heavy metal stress (Alia Prasad and Pardha Saradhi 1995). Accumulation of proline has been observed in various plant species subjected to heavy metal stress (Shah and Dubey 1998). It is reported that antioxidant enzyme activities increase under Zn stress in *Brassica juncea* (Prasad and Hagmeyer 1999). Verma and Dubey (2003) have reported that with increase in the level of Pb treatment, antioxidant enzymes such as guaiacol peroxidase, SOD, APX, and GR activities increased compared to control in rice plants. It has been further suggested that SOD and GR play a pivotal role in combining oxidative stress in rice plants and Pb toxicity. Thus, it is evident from the several research reports that judicious use and presence of heavy metals have toxic effects on plants, animals, and other living organisms and affect the same after certain limits. Therefore, it is well needed to intensify the research programs for better understanding of heavy metal toxicity on plants and allied areas to maintain the ecological harmony of the globe.

5.4 Mechanisms of Plants to Cope with Metal Toxicity

Like all other organisms, plants have evolved different mechanisms to maintain physiological concentrations of essential metal ions and to minimize exposure to nonessential heavy metals. Plant tolerance to a particular heavy metal is governed by an interrelated network of physiological and molecular mechanisms, and understanding of these mechanisms and their genetic basis is an important aspect to developing plants as agents of phytoremediation. Plants have both constitutive and adaptive mechanisms to withstand excess heavy metals (Meharg 1994). Physiological, biochemical, and molecular approaches continue to be employed to identify the underlying mechanisms of heavy metal accumulation, tolerance, and adaptive mechanisms to cope with heavy metal stress. Some mechanisms are ubiquitous because they are also required for general metal homeostasis, and they minimize the damage caused by high concentrations of heavy metals in plants by detoxification, thereby conferring tolerance to heavy metal stress. Other mechanisms target individual metal ions (indeed some plants have more than one mechanism to prevent the accumulation of specific metals), and these processes may involve the exclusion of particular metals from the intracellular environment or the sequestration of toxic ions within compartments to isolate them from sensitive cellular components. As a first line of defense, many plants exposed to toxic concentrations of metal ions attempt to prevent or reduce uptake into root cells by restricting metal ions to the apoplast, binding them to the cell wall or to cellular exudates or by inhibiting long-distance transport (Manara 2012). If this fails, metals already in the cell are addressed using a range of storage and detoxification strategies, including immobilization, synthesis of specific heavy metal transporters, chelation, trafficking, and sequestration of heavy metals by particular ligands (phytochelatins and metallothioneins). When these options are exhausted, plants activate oxidative stress defense mechanisms contrasting the effects of ROS and MG (such as upregulation of antioxidant and glyoxalase system), and the synthesis of stress-related proteins and signaling molecules, such as heat shock proteins, hormones, the biosynthesis of Pro, polyamines, and signaling molecule such as salicylic acid and nitric oxide (Hossain et al. 2012).

5.5 Effects of Toxic Heavy Metals on Plants

Plants, like all living organisms, are often sensitive both to the deficiency and to the excess availability of some heavy metal ions as essential

micronutrient, while the same at higher concentrations and even more ions such as cadmium (Cd), mercury (Hg), arsenic (As) are strongly poisonous to the metabolic activities (Foy 1978). Contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence and their acute and chronic toxic effect on plants grown from such soils (Reeves and Baker 2000).

5.5.1 Zinc Toxicity in Plants

Zinc (Zn) is an essential micronutrient that affects several metabolic processes of plants and has a long biological half-life. Concentrations of Zn found in contaminated soils frequently exceed to those required as nutrients and may cause phytotoxicity. Zn concentrations in the range of 150–300 mg/kg have been measured in polluted soils (Warne et al. 2008). High levels of Zn in soil inhibit many plant metabolic functions, result in retarded growth, and cause senescence. The phytotoxicity of Zn and Cd is indicated by decrease in growth and development, metabolism, and an induction of oxidative damage in various plant species such as *Phaseolus vulgaris* (Cakmak and Marshner 1993) and *Brassica juncea* (Prasad and Hagmeyer 1999). Cd and Zn have reported to cause alternation in catalytic efficiency of enzymes in *Phaseolus vulgaris* (Somasekharaiah et al. 1992) and pea plants (Romero-Puertas et al. 2004). Zinc toxicity in plants limited the growth of both root and shoot (Ebbs and Kochian 1997; Fontes and Cox 1998). Zinc toxicity also causes chlorosis in the younger leaves, which can extend to older leaves after prolonged exposure to high soil Zn levels (Ebbs and Kochian 1997). The chlorosis may arise partly from an induced iron (Fe) deficiency as hydrated Zn^{2+} and Fe^{2+} ions have similar radii (Marschner 1986). Excess Zn can also give rise to manganese (Mn) and copper (Cu) deficiencies in plant shoots. Such deficiencies have been attributed to a hindered transfer of these micronutrients from root to shoot. This hindrance is based on the fact that the Fe and Mn concentrations in plants grown in Zn-rich media are greater in the root than in the shoot (Ebbs and Kochian 1997). Another typical effect of Zn toxicity is the appearance of a purplish-red color in leaves, which is ascribed to phosphorus (P) deficiency (Lee et al. 1996).

5.5.2 Cadmium Toxicity in Plants

Cadmium is a nonessential element that adversely affects plant growth and development. It is considered as one of the significant pollutants due to its high toxicity and more solubility in water. The monitoring limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil (Salt et al. 1995). Concentration of cadmium varies extensively among plant species and varieties within species. Cadmium absorption and translocation varied with cultivars of amaranthus (Varalakshmi and Ganeshamurthy 2009) and radish (Varalakshmi and Ganeshamurthy 2013). The variation in Cd uptake among amaranthus cultivars may be due to genotypic variations in cultivars to absorb or translocate specific toxic metals. Cadmium can alter the uptake of mineral nutrients by plants through its effects on availability of nutrients from the soil. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, leaf rolls, growth inhibition, browning of root tips, and finally death (Mohanpuria et al. 2007; Guo et al. 2008). The inhibition of root Fe(III) reductase induced by Cd led to Fe(II) deficiency, and it seriously affected photosynthesis (Alcantara et al. 1994). In general, cadmium has been shown to interfere with the uptake, transport, and use of several elements, viz., Ca, Mg, P, and K and water by plants (Das et al. 1997). Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots (Hernandez et al. 1996). Appreciable inhibition of the nitrate reductase activity was also found in plants of *Silene cucubalus* (Mathys

1975). Nitrogen fixation and primary ammonia assimilation decreased in nodules of soybean plants during Cd treatments (Balestrasse et al. 2003). Metal toxicity can affect the plasma membrane permeability, causing a reduction in water content; in particular, Cd has been reported to interact with the water balance (Costa and Morel 1994). Cadmium treatments have been shown to reduce ATPase activity of the plasma membrane fraction of wheat and sunflower roots (Fodor et al. 1995). Cadmium produces alterations in the functionality of membranes by inducing lipid peroxidation (Fodor et al. 1995) and disturbances in chloroplast metabolism by inhibiting chlorophyll biosynthesis and reducing the activity of enzymes involved in CO_2 fixation (De Filippis and Ziegler 1993).

5.5.3 Copper Toxicity in Plants

Copper (Cu) is considered as a micronutrient for plants (Thomas et al. 1998) and plays an important role in CO_2 assimilation and ATP synthesis. Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain (Demirevska-Kepova et al. 2004). But enhanced industrial and mining activities have contributed to the increasing occurrence of Cu in ecosystems. Mining activities generate a large amount of waste rocks and tailings, which get deposited at the surface. Excess of Cu in soil plays a cytotoxic role, induces stress, and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis et al. 2001). Interveinal chlorosis is a common initial symptom of copper toxicity. The chlorosis often takes the form of cream or white spots or lesions. With increasing exposure, leaf tips and margins can become necrotic. In acute Cu toxicity, leaves may become wilted before eventually becoming necrotic (Foy et al. 1995). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman and Oliver 1991). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al. 2001). Copper toxicity affected the growth of *Alyssum montanum* (Ouzounidou 1994) and Cd of cucumber (Moreno-Caselles et al. 2000) and *Brassica juncea* (Singh and Tewari 2003). Copper and Cd in combination have affected adversely the germination, seedling length, and number of lateral roots in *Solanum melongena* (Neelima and Reddy 2002). Citrus seedlings exposed to excess Cu produce few new roots and have a thickened tap root (Zhu and Alva 1993). Thickening of root apices was also evident in *Pinus* seedlings (Arduini et al. 1995). In *Betula papyrifera* (paper birch) and *Lonicera tatarica* (honeysuckle) seedlings high, Cu concentrations have been shown to inhibit the production of root hairs (Patterson and Olson 1983).

5.5.4 Mercury Toxicity in Plants

Mercury poisoning has become a problem of current interest as a result of environmental pollution on a global scale. Natural emissions of mercury form two-thirds of the input; manmade releases form about one-third. Significant amounts of mercury may be added to agricultural land with sludge, fertilizers, lime, fungicides, and manures. The most important sources of contaminating agricultural soil have been the use of organic mercurials as a seed coat dressing to prevent fungal diseases in seeds (Patra and Sharma 2000). The large input of mercury (Hg) into the arable lands has resulted in the widespread occurrence of mercury contamination in the entire food chain. Mercury is a unique metal due to its existence in different forms, e.g., HgS, Hg^{2+}, Hg^{0}, and methyl-Hg. However, in agricultural soil, ionic form (Hg^{2+}) is predominant (Han et al. 2006). Mercury released to the soil mainly remains in solid phase through adsorption onto sulfides, clay particles, and organic matters. Increasing evidence has shown that Hg^{2+} can readily accumulate in higher and aquatic plants (Kamal et al. 2004; Israr et al. 2006). High level of Hg^{2+} is strongly phytotoxic to plant cells. Toxic level of Hg^{2+} can induce visible injuries and physiological disorders in plants (Zhou et al. 2007). For

example, Hg^{2+} can bind to water channel proteins, thus inducing leaf stomata to close and physical obstruction of water flow in plants (Zhang and Tyerman 1999). High level of Hg^{2+} interferes the mitochondrial activity and induces oxidative stress by triggering the generation of ROS. This leads to the disruption of biomembrane lipids and cellular metabolism in plants (Cargnelutti et al. 2006). Mercury affects both light and dark reactions of photosynthesis. Replacement of the central atom of chlorophyll, magnesium, by mercury in vivo, prevents photosynthetic light harvesting in the affected chlorophyll molecules, resulting in a breakdown of photosynthesis (Patra and Sharma 2000).

5.5.5 Chromium Toxicity in Plants

Chromium is found in all phases of the environment including air, water, and soil. Chromium compounds are highly toxic to plants and are injurious to their growth and development. Although some crops are not affected by low Cr ($3.8–10^{-4}$ μM) concentrations (Huffman and Allaway 1973a, b), Cr is toxic to higher plants at 100 μg kg^{-1} dry weight (Davies et al. 2002). Since seed germination is the first physiological process affected by Cr, the ability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal (Peralta et al. 2001). Seed germination of the weed *Echinochloa colona* was reduced to 25 % with 200 μM Cr (Rout et al. 2000). High levels (500 ppm) of hexavalent Cr in soil reduced germination up to 48 % in the bush bean *Phaseolus vulgaris* (Parr and Taylor 1982). Peralta et al. (2001) found that 40 ppm of Cr (VI) reduced by 23 % the ability of seeds of Lucerne (*Medicago sativa* cv. Malone) to germinate and grow in the contaminated medium. Reductions of 32–57 % in sugarcane bud germination were observed with 20 and 80 ppm Cr, respectively (Jain et al. 2000). The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on the subsequent transport of sugars to the embryo axes (Zeid 2001). Protease activity, on the other hand, increases with the Cr treatment, which could also contribute to the reduction in germination of Cr-treated seeds (Zeid 2001). Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Tang et al. 2001). Prasad et al. (2001) reported that the order of metal toxicity to new root primordia in *Salix viminalis* is Cd > Cr > Pb, whereas root length was more affected by Cr than by other heavy metals studied. Chromium stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation, and enzyme activities (Nagajyoti et al. 2010). In higher plants and trees, the effect of Cr on photosynthesis is well documented (Van Assche and Clijsters 1983). However, it is not well understood to what extent Cr-induced inhibition of photosynthesis is due to disorganization of chloroplasts' ultrastructure (Vazques et al. 1987), inhibition of electron transport, or the influence of Cr on the enzymes of the Calvin cycle. Chromate is used as a Hill reagent by isolated chloroplast. The more pronounced effect of Cr (VI) on PS I than on PS II activity in isolated chloroplasts has been reported by Bishnoi et al. (1993a, b) in peas. Chromium stress can induce three possible types of metabolic modification in plants: (i) alteration in the production of pigments, which are involved in the life sustenance of plants (e.g., chlorophyll, anthocyanin) (Boonyapookana et al. 2002); (ii) increased production of metabolites (e.g., glutathione, ascorbic acid) as a direct response to Cr stress, which may cause damage to the plants (Shanker et al. 2003a, b); and (iii) alterations in the metabolic pool to channelize the production of new biochemically related metabolites, which may confer resistance or tolerance to Cr stress (e.g., phytochelatins, histidine) (Schmfger 2001).

5.5.6 Lead Toxicity in Plants

Lead (Pb) is one of the major heavy metals of the antiquity and has gained considerable importance as a potent environmental pollutant. Among heavy metals, lead is a potential pollutant

that readily accumulates in soils and sediments. Although lead is not an essential element for plants, it gets easily absorbed and accumulated in different plant parts. Leafy vegetables accumulated maximum levels of Pb, followed by root vegetables (carrot and radish), tomato, and French bean (Varalakshmi and Ganeshamurthy 2012). It exerts adverse effect on morphology, growth, and photosynthetic processes of plants. Excess lead causes a number of toxicity symptoms in plants, e.g., stunted growth, chlorosis, and blackening of root system. Lead is known to inhibit seed germination of *Spartina alterniflora* (Morzck and Funicclli 1982) and *Pinus halepensis* (Nakos 1979). Inhibition of germination may result from the interference of lead with important enzymes. Mukherji and Maitra (1976) observed 60 μM lead acetate inhibited protease and amylase by about 50 % in rice endosperm. Early seedling growth was also inhibited by lead in soybean (Huang et al. 1974), rice (Mukherji and Maitra 1976), maize (Miller et al. 1975), barley (Stiborova et al. 1987), tomato, eggplant (Khan and Khan 1983), and certain legumes (Sudhakar et al. 1992).

Lead also inhibited root and stem elongation and leaf expansion in *Allium* species (Gruenhage and Jager 1985), barley (Juwarkar and Shende 1986), and *Raphanus sativus*. The degree to which root elongation is inhibited depends upon the concentration of lead and ionic composition and pH of the medium (Goldbold and Hutterman 1986). Concentration-dependent inhibition of root growth has been observed in *Sesamum indicum* (Kumar et al. 1992). A high lead level in soil induces abnormal morphology in many plant species. For example, lead causes irregular radial thickening in pea roots and cell walls of the endodermis and lignification of cortical parenchyma (Paivoke 1983). Lead also induces proliferation effects on the repair process of vascular plants (Kaji et al. 1995). Lead administrated to potted sugar beet plants at rates of 100–200 ppm caused chlorosis and growth reduction (Hewilt 1953). In contrast, there was no visual symptom of lead toxicity in alfalfa plants exposed to 100 mg/mL (Porter and Cheridan 1981). Low amounts of lead (0.005 ppm) caused significant reduction in growth of lettuce and carrot roots (Baker 1972). Inhibitory effects of Pb^{2+} on growth and biomass production may possibly derive from effects on metabolic plant processes (Van Assche and Clijsters 1990). The primary cause of cell growth inhibition arises from a lead-induced simulation of indol-3 acetic acid (IAA) oxidation. Lead is also known to affect photosynthesis by the inhibiting activity of carboxylating enzymes (Stiborova et al. 1987). High level of Pb also causes inhibition of enzyme activities (Sinha et al. 1988a, b), water imbalance, changes in hormonal status, alterations in membrane structure and permeability and disturbs mineral nutrition (Sharma and Dubey 2005) and also induces oxidative stress by increasing the production of ROS in plants (Reddy et al. 2005). Pb inhibits the activity of enzymes at cellular level by reacting with their sulfhydryl groups (Nagajyoti et al. 2010).

5.5.7 Arsenic Toxicity in Plants

The element arsenic (As) is an environmental toxin that is found naturally in all soils. The metalloid enters into farming systems through a variety of means that include natural geochemical processes, the past and present use of As-based pesticides, mining operations, irrigation with As-contaminated groundwater, and fertilization with municipal solid wastes. Arsenic is a nonessential element and generally toxic to plants. Roots are usually the first tissue to be exposed to arsenic, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, arsenic can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production (Garg and Singla 2011). At sufficiently high concentrations, arsenic interferes with critical metabolic processes, which can lead to death. Most plants possess mechanisms to retain much of their As burden in the root. However, a genotype-dependent proportion of the As is

translocated to the shoot and other tissues of the plant. Arsenate (As) is an analog of phosphate (P) and competes for the same uptake carriers in the root plasmalemma of plants (Meharg and Macnair 1992). The As tolerance in grasses results from suppression of a high-affinity P/As uptake system (Meharg and Macnair 1992). This suppression reduces As influx to a level at which plant can easily detoxify it, presumably by constitutive mechanisms (Meharg 1994). The As tolerance is achieved by a single gene encoding for the suppressed P/As transport (Meharg and Macnair 1992). Despite this clear understanding of the process controlling decrease in As uptake, tolerant grasses still assimilate As, albeit at much lower rate compared with non-tolerant. Nevertheless, assimilation over the life history of plants growing on contaminated soil can result in a very high As concentration, e.g., 3470 mg/g As in *Agrostis tenuis* and 560 mg/g As in *Holcus lanatus* (Porter and Peterson 1975).

5.5.8 Cobalt Effects on Plants

Cobalt, a transition element, is an essential component of several enzymes and coenzymes. It has been shown to affect growth and metabolism of plants, in different degrees, depending on the concentration and status of cobalt in rhizosphere and soil. Cobalt interacts with other elements to form complexes. The cytotoxic and phytotoxic activities of cobalt and its compounds depend on the physicochemical properties of these complexes, including their electronic structure, ion parameters (charge–size relations), and coordination. Thus, the competitive absorption and mutual activation of associated metals influence the action of cobalt on various phytochemical reactions. Cobalt (Co) naturally occurs in the earth's crust as cobaltite [CoAsS], erythrite [Co_3 $(AsO_4)_2$], and smaltite [$CoAs_2$]. Plants can accumulate small amount of Co from the soil. The uptake and distribution of cobalt in plants are species dependent and controlled by different mechanisms (Li et al. 2004; Bakkaus et al. 2005). Very little information is available regarding the phytotoxic effect of excess cobalt. Phytotoxicity study of cobalt in barley (*Hordeum vulgare* L.), oilseed rape (*Brassica napus* L.), and tomato (*Lycopersicon esculentum* L.) has recently shown the adverse effect on shoot growth and biomass (Li et al. 2009). In addition to biomass, excess of cobalt restricted the concentration of Fe, chlorophyll, protein, and catalase activity in leaves of cauliflower. Further, high level of Co also affected the translocation of P, S, Mn, Zn, and Cu from roots to tops in cauliflower. In contrast to excess Cu or Cr, Co significantly decreased water potential and transpiration rate (Nagajyoti et al. 2010), while diffusive resistance and relative water content increased in leaves of cauliflower upon exposure to excess Co (Chatterjee and Chatterjee 2000).

5.5.9 Nickel Toxicity in Plants

Among different environmental heavy metal pollutants, Ni has gained considerable attention in recent years, because of its rapidly increasing concentrations in soil, air, and water in different parts of the world. Nickel is a transition metal and found in natural soils at trace concentrations except in ultramafic or serpentine soils. However, Ni^{2+} concentration is increasing in certain areas by human activities such as mining works, emission of smelters, burning of coal and oil, sewage, phosphate fertilizers, and pesticides (Gimeno-Garcia et al. 1996). Ni^{2+} concentration in polluted soil may range from 20- to 30-fold (200–26,000 mg/kg) higher than the overall range (10–1000 mg/kg) found in natural soil (Izosimova 2005). Although Ni is metabolically important in plants, it is toxic to most plant species when present at excessive amounts in soil and in nutrient solution. High Ni concentrations in growth media severely retards seed germinability of many crops. This effect of Ni is a direct one on the activities of amylases, proteases, and ribonucleases, thereby affecting the digestion and mobilization of food reserves in germinating seeds. At vegetative stages, high Ni concentrations retard shoot and root growth, affect branching development, deform various plant parts, produce abnormal flower shape,

decrease biomass production, induce leaf spotting, disturb mitotic root tips, and produce Fe deficiency that leads to chlorosis and foliar necrosis. Additionally, excess Ni also affects nutrient absorption by roots, impairs plant metabolism, inhibits photosynthesis and transpiration, and causes ultrastructural modifications. Ultimately, all of these altered processes produce reduced yields of agricultural crops when such crops encounter excessive Ni exposures. Excess of Ni^{2+} in soil causes various physiological alterations and diverse toxicity symptoms such as chlorosis and necrosis in different plant species (Pandey and Sharma 2002; Rahman et al. 2005; Yadav 2010), including rice (Das et al. 1997). Plants grown in high Ni^{2+}-containing soil showed impairment of nutrient balance and resulted in disorder of cell membrane functions. Thus, Ni^{2+} affected the lipid composition and H-ATPase activity of the plasma membrane as reported in *Oryza sativa* shoots (Ros et al. 1992). Exposure of wheat to high level of Ni^{2+} enhanced MDA concentration (Pandolfini et al. 1992). Moreover, Gonnelli et al. (2001) reported an increase in MDA concentration of Ni^{2+}-sensitive plants compared to a Ni^{2+}-tolerant saline. Such changes might disturb membrane functionality and ion balance in the cytoplasm, particularly of K^{+}, the most mobile ion across plant cell membrane. High uptake of Ni^{2+} induced a decline in water content of dicot and monocot plant species. The decrease in water uptake is used as an indicator of the progression of Ni^{2+} toxicity in plants (Pandey and Sharma 2002; Gajewska et al. 2006).

5.5.10 Manganese Toxicity in Plants

Manganese (Mn) is an essential element for plant growth. It can, however, be detrimental when available in excess in the growth medium. Excess Mn in the growth medium can interfere with the absorption, translocation, and utilization of other mineral elements such as Ca, Mg, Fe, and P (El-Jaoual and Cox 1998). High Mn concentration in plant tissues can alter activities of enzymes and hormones, so that essential Mn-requiring processes become less active or nonfunctional (Epstein 1961; Horst 1988a, b). Accumulation of excessive manganese (Mn) in leaves causes a reduction of photosynthetic rate (Kitao et al. 1997a, b). Symptoms of Mn toxicity as well as the concentration of Mn that causes toxicity vary widely among plant species and varieties within species. Necrotic brown spotting on leaves, petioles, and stems is a common symptom of Mn toxicity (Wu 1994). This spotting starts on the lower leaves and progresses with time toward the upper leaves (Horiguchi 1988). With time, the speckles can increase in both number and size resulting in necrotic lesions, leaf browning, and death (Elamin and Wilcox 1986a, b). General leaf bronzing and shortening of internodes have been documented in *Cucumis sativus* (Crawford et al. 1989). Another common symptom is known as "crinkle leaf," and it occurs in the youngest leaf, stem, and petiole tissue. It is also associated with chlorosis and browning of these tissues (Wu 1994). Roots exhibiting Mn toxicity are commonly brown in color and sometimes crack (Foy et al. 1995). Chlorosis in younger leaves by Mn toxicity is thought to be caused through Mn-induced Fe deficiency (Horst 1988a, b). Excess Mn is reported to inhibit synthesis of chlorophyll by blocking an Fe-concerning process (Clarimont et al. 1986). Manganese toxicity in some species starts with chlorosis of older leaves moving toward the younger leaves with time (Bachman and Miller 1995). This symptom starts at the leaf margins progressing to the interveinal areas, and if the toxicity is acute, the symptom progresses to marginal and interveinal necrosis of leaves (Bachman and Miller 1995). In the only research on Mn toxicity of Australian native trees, *Corymbia gummifera* (red bloodwood) displayed small, chlorotic leaves that were often distorted in shape and death of terminal buds (Winterhalder 1963).

5.5.11 Iron Toxicity in Plants

Iron as an essential nutrient for all plants has many important biological roles in the processes

as diverse as photosynthesis, chloroplast development, and chlorophyll biosynthesis. Iron is a major constituent of the cell redox systems such as heme proteins including cytochromes, catalase, peroxidase, and leghemoglobin and iron sulfur proteins including ferredoxin, aconitase, and superoxide dismutase (SOD). It functions to accept and donate electrons and plays important roles in the electron-transport chains of photosynthesis and respiration. But iron is toxic when it accumulates to high levels. Although most mineral soils are rich in iron, the expression of iron toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the microbial reduction of insoluble Fe^{3+} to soluble Fe^{2+} (Becker and Asch 2005). Iron toxicity is believed to be involved in several extremely complicated physiological diseases of rice under flooded conditions. These include "bronzing" in Sri Lanka and elsewhere, "Alkagare type" disorder in Japan, and "Akiochi" in Korea (Aizer et al. 1975). The Fe^{2+} excess causes free radical production that impairs cellular structure irreversibly and damages membranes, DNA, and proteins (Arora et al. 2002; de Dorlodot et al. 2005). Iron toxicity in tobacco, canola, soybean, and *Hydrilla verticillata* is accompanied with reduction of plant photosynthesis and yield and the increase in oxidative stress and ascorbate peroxidase activity (Sinha et al. 1997). Excess Fe also apparently contributes to "freckle leaf" of sugarcane in Hawaii, along with excess AI, Mn, and Zn (Clements et al. 1974). In tobacco, excess Fe produces brittle, tender, dark-brown to purple leaves which have poor burning qualities and flavor (Foy 1978). In navy bean, Fe toxicity associated with Zn deficiency produced black spots on the foliage (Foy 1978).

5.6 Conclusions

Numerous harmful health effects upon exposure to toxic heavy metals in the environment is a matter of serious concern and a universal issue. Thus, it is evident from the several research reports that the occurrence of heavy metals has toxic effects on plants, animals, and other living organisms and affects the same after certain limits. Therefore, it is well required to intensify the research programs for better understanding of heavy metal toxicity on plants and allied areas to maintain the ecological harmony of the globe. Metal toxicities in plants are often not clearly identifiable entities; instead, they may be the results of complex interactions of the major toxic ions with other essential or nonessential ions and with other environmental factors. Although excesses of different metals may produce some common effects on plants in general, there are many cases of specific differential effects of individual metals on different plant genotypes. Such occurrences must be recognized in approaching any problem of metal toxicity. It now seems likely that the phytotoxic mechanisms of a given metal ion involve different biochemical pathways in different plant species and varieties.

Plant heavy metal tolerance is a multigenic trait and controlled at multiple levels. Once the genetic control mechanisms of metal tolerance are identified, it may be possible to combine metal tolerance with other desirable traits to produce plants that are better adapted to soils polluted with heavy metals. Such problems are often not economically correctable with conventional fertilization and liming practices. Additionally, the selection of near-isogenic lines may provide valuable tools for studying specific biochemical mechanisms of tolerance. Once these mechanisms are better understood, it may be possible to select or breed, even more precisely, those plants having greatest tolerance to a particular metal toxicity. Such biochemical knowledge concerning metal tolerance mechanisms could also lead to the development of improved chemical (fertilization, liming, organic matter, etc.) and physical (tillage, drainage, etc.) management practices for problem soils.

5.7 Future Strategies

A wealth of recent studies demonstrated that plants protect themselves from heavy metal

toxicity, besides other mechanisms, through an elevated level of nonenzymatic and enzymatic components of antioxidant and glyoxalase defense systems. Significant progress has been achieved in regard to heavy metal toxicity, and tolerance and different key components ensuring heavy metal tolerance in plants have been identified; however, many key questions remain unanswered. Additionally, different heavy metals appear to have different mechanisms to stimulate toxicity symptoms, and plants employ different mechanisms as resistant reactions to particular heavy metals. Likewise, the reaction response and tolerance mechanisms are also different when a plant is grown under excess heavy metals in hydroponic or actual field conditions. Therefore, it is very difficult to hypothesize a common resistance mechanism against all heavy metals and metalloids. Importantly, among the various nonenzymatic antioxidant molecules that plants usually utilize against heavy metal stress, glutathione (GSH) is the most vital one. Besides detoxification of reactive oxygen species (ROS) directly or detoxification of ROS and methylglyoxal (MG) through antioxidative and glyoxalase systems, GSH also plays additional roles, including heavy metal (HM) uptake, transport, and sequestration and formation of specific metal-binding ligands such as PCs. The reviewed literature confirms the central role of GSH metabolism in heavy metal tolerance in plants as evidenced by proteomic, genomic, and transgenic studies over a range of plant species. Although significant progress has been made in different aspects of GSH and its related enzymes in heavy metal-induced ROS and MG metabolism and heavy metal chelation in a large number of plant species, most of the studies were conducted under laboratory conditions. Therefore, to get more realistic information, we need to move from the laboratory to actual field conditions by using commercial plant species at various phases of plant growth. The increasing identification and study of the remarkable natural variation in the capacity of plants to accumulate and tolerate heavy metals are and will continue to provide a wealth of information. Therefore, concerted efforts by various research domains will further increase our understanding of the fundamental mechanisms involved in hyperaccumulation processes that naturally occur in metal hyperaccumulating plants. This should allow us to develop plants that are more ideally suited for phytoremediation of heavy metal-contaminated soils. Therefore, molecular and cellular adaptation of plant cells in response to heavy metal stress appears to be necessary to improve plant heavy metal tolerance that ultimately reduces the chance of entering heavy metal into the food chain.

Differential cultivar tolerances to metal toxicity almost certainly involve differences in the structure and function of membranes. But such differences are extremely difficult to measure in living plants. Any fractionation, extraction, or tissue fixation procedure used will introduce the possibility of measuring only artifacts. Electron microscope procedures involving the use of X-ray emission can determine the physical location of metal ions in root sections, provided that membranes have not been disturbed during tissue preparation. Comparative physiological studies need to be undertaken between closely related genotypes (near-isogenic lines, if possible) of the same species which differ widely in tolerance to a given metal toxicity. Investigations are needed on plant anatomical and rhizospheric changes responsible for the variability in absorption, translocation, and uptake of trace elements by seed and fodder of food crops. Mass awareness has to be created about pollutant elements in soil–plant–animal continuum and remedial measures to establish optimum level of elements for good health of animal and humans. A joint multidisciplinary team consisting of soil scientist, nutrition scientist, physiologists, and veterinary and medicine doctors has be constituted to establish definite quantitative association of animal and human health.

There is need for developing systematic database using GPS to monitor health hazards from heavy metal contamination and minor element toxicities in soil, plant, human, and animal chain. Maps of trace element deficiency and toxicity need to be produced to create awareness of

such areas for taking remedial measures by the people, planners, and policy makers. These research efforts require close collaboration between soil scientists, plant breeders, plant physiologists, plant biochemists, and perhaps pathologists. Until recently, such cooperation has been rare in both state and central institutions concerned with agricultural research. A multidisciplinary approach is now essential if world food needs are to be met.

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