

Chapter 14

Ecophysiological Responses of Plants Under Metal/Metalloid Toxicity



Roseline Xalxo, Vibhuti Chandrakar, Meetul Kumar, and S. Keshavkant

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R. Xalxo · V. Chandrakar

School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, India

M. Kumar

Directorate of International Cooperation, Defence Research and Development Organization, New Delhi, India

S. Keshavkant (✉)

School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, India

National Center for Natural Resources, Pt. Ravishankar Shukla University, Raipur, India

Abstract Heavy metal/metalloid (HM) contamination issues are becoming progressively more widespread worldwide, which are witnessed in various locations like foundries, mining industries, smelters, vehicular emissions, coal burning power plants, and agricultural sectors. Heavy metal/metalloid occurs naturally in the Earth's crust, but man-made sources and various industrial activities have led to severe environmental contamination globally. In the present era, accumulation of HMs in agricultural land is of prime concern as it possesses negative consequences on safety of food and its marketability, productivity, and health of soil organisms. Plants cultivated in HM-contaminated sites reveal reduced photosynthesis, mineral nutrients, biomass production, vegetative growth, altered metabolic activities, and higher accumulation of metal(s). Heavy metal/metalloid toxicity causes a redox imbalance and induces the enhancement in the generation of reactive oxygen species (ROS). However, when ROS reach above threshold level, an imbalance in intracellular content of it incurs, thus resulting in oxidative condition. Plants respond to such intensified concentration of HM in the natural environment by invigorating various defense responses such as sequestration of HM into vacuoles, metal chelation, control over uptake of metals ions through their carriers, and amplification of enzymatic and non-enzymatic antioxidative defense mechanisms. These responses inferred by flora are the outcomes of complex signaling cascades functioning in the plant cells so as to mediate the extracellular stimuli into an intracellular response. This section of the book reveals outlook associated with HM phytotoxicity ranging from metal uptake and its transportation, distribution, homeostasis, toxicity at cellular level, and signaling pathway. Our main intent is to highlight predominantly on the mechanisms of HM-instigated oxidative stress-related responses of plants and to unveil signal transduction mechanism(s) under HM stress.

Keywords Environmental pollution · Metal/metalloid transporters · Phytotoxicity · Reactive oxygen species · Sequestration

14.1 Introduction

Plants, during their vegetative life cycle, encounter various biotic and abiotic stresses. Abiotic stresses include drought, temperature (both low and high), salinity, lack of essential nutrients, air pollution, heavy metals (HMs), etc. Heavy metals are recognized as most dangerous pollutants as they possess high toxicity to both flora and fauna and due to their considerable buildup into the environments as a consequence of natural and man-made processes (Yadav 2010; Wang et al. 2014). Among 90 naturally occurring elements, 53 of them are designated as HMs, based on their specific density. Thus, metals having density less than 5 g cm^{-3} and those having density exceeding 5 g cm^{-3} are termed as light metals and HMs, respectively (Xalxo and Keshavkant 2018). On the basis of HM solubility, 17 of them are probably

available to all living beings under physiological conditions and are vital for living cells/organisms and different ecosystems (Schutzendubel and Polle 2002). Various metals, like cobalt-Co, copper-Cu, iron-Fe, molybdenum-Mo, manganese-Mn, nickel-Ni, and zinc-Zn are essential micronutrients, hence are mandatory for the plant's normal growth and development. These elements which act as essential micronutrients participate in redox response, electron transport, and other vital functions of plants. However, other elements, for instance, arsenic-As, cadmium-Cd, chromium-Cr, lead-Pb, mercury-Hg, etc., are potentially lethal for plants, hence reducing their growth and development (Nazar et al. 2012). The accumulation of various HMs and metalloids in soils and waters leads to severe problem globally, as these HMs and metalloids cannot be wrecked down readily either chemically or biologically into nontoxic forms, therefore persisting in various ecosystems for several years (Xalxo and Keshavkant 2018). Environmental contamination with HMs has exceeded beyond the acceptable limit these days and is injurious to all living organisms (Tak et al. 2013). As declared by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), USA, the highest permissible concentrations of some of the HMs in water are 0.002, 0.015, 0.01, 0.01, 0.05, and 0.05 mg L⁻¹ for Hg, Pb, Ar, Cr, Cd, and Ag respectively (Chaturvedi et al. 2015). The soil standards, as customized by the Indian standards for HMs, are Cd = 3–6, Cu = 135–270, Ni = 75–150, Pb = 250–500, and Zn = 300–600 mg kg⁻¹ (Nagajyoti et al. 2010). It is acknowledged that metals at a low concentration might have advantageous consequences that are referred as hormesis (Zengin and Munzuroglu 2006). However, all HMs have the capability to display detrimental consequences at higher level and are proficient to enter into the food web (Chandrakar et al. 2017). The toxic effect of each metal depends on various factors such as its availability to flora and fauna, type of metal, its oxidation state, pH, concentration of metal, the path and the time of exposure, and so on (Mani and Kumar 2014). United States Environmental Protection Agency (UEPA) has listed all these HMs as chief contaminants. The ramifications due to HMs in soil are very complex, and consequences caused by these rely upon various chemical processes. Among various processes, adsorption-desorption and complexation-dissociation reactions normally influence metal activity in soil, while oxidation-reduction process alters the valencies of metal (Swartjes et al. 2007). Excessive concentrations of HMs in plants cause alterations at physiological, biochemical, and molecular levels (Chandrakar et al. 2017; Xalxo and Keshavkant 2018). Heavy metal-induced alterations include decline in the rate of photosynthesis, transpiration, respiration, nitrogen metabolism, and mineral nutrition along with alterations in redox status, concentration of signal molecules, enzymatic and non-enzymatic antioxidants activity, membrane permeability, phytochelatin (PC) content, protein, phenolics content, etc., consequently leading to plant death (Tripathi et al. 2007; Hayat et al. 2012; Qadir et al. 2014; Kumar et al. 2015; Chandrakar et al. 2017; Xalxo et al. 2017). Phytotoxic consequences of HMs on various plant species were revealed in depth by various researchers (Malecka et al. 2001, 2008, 2009; Rucinska and Gwozdz 2005; Krzelowska et al. 2010; Pawlak-Sprada et al. 2011; Chandrakar et al. 2016; Rucinska-Sobkowiak 2016; Drzewiecka et al. 2018; Xalxo and

Keshavkant 2019). This chapter reviews general aspects of HM/metalloid toxicity ranging from metal uptake from soil, their transportation from ground to above aerial parts, and homeostasis to cellular stress responses. The prime objective of this section is to highlight principally on the mechanisms of HM-instigated oxidative injury-associated responses and to show the working of various signal transduction pathways under HM/metalloid toxicity. This particular subject area needs intense exploration to provide vital information to understand cellular responses against HM toxicity.

14.2 Sources of Heavy Metal Pollution in the Environments

There are various sources of HM release in the environments such as natural and anthropogenic routes. Naturally occurring HMs are available in insoluble forms like precipitated or complex forms that are not easily accessible by plants for uptake. Natural processes that cause overaccumulation of HMs in the environments include comets, erosion, volcanic eruptions, and mineral weathering. Various agricultural and horticultural sources include livestock manures, sewage sludge, inorganic fertilizers, and agricultural chemicals like fungicides, pesticides, etc. Inorganic fertilizers, fungicides, and phosphate fertilizers contain variable concentrations of HMs, like Pb, As, Cd, Cr, Ni, Zn, etc., depending on their sources. Heavy metals that are originated from man-made sources are soluble and are present in mobile reactive forms and are readily available to plants. Various anthropogenic sources comprise of battery manufacture, alloy manufacture, atmospheric deposition, manufacturing of explosive, inappropriate piling of solid wastes from industries, tanning of leather preservatives in dyes and woods, etc. (Dixit et al. 2015). The geochemical cycle of HM leads to overaccumulation of it in the environment, which may perhaps cause lethal effects to both flora and fauna, when present above permitted levels (D'amore et al. 2005). The pathways through which HMs generally enter into the natural ecosystems include crumbling of parent materials, amendments in the geochemical cycle by anthropogenic activities, shifting of excavation to different localities, and release of elevated amount of metal waste by manufacturing companies. Mining and processing of ores are chief sources of HM contamination in the ecosystems, and the revival of ecosystems from HM contamination caused by various activities may possibly take several decades.

14.3 Heavy Metal/Metalloid Transporters

In plants, the transportation of HM ions from ground to upper aerial parts is regulated by specific transporters and metal pumps; also these transporters and metal pumps maintain the metal homeostasis within the cells (Pilon et al. 2009). These transporters are the members of diverse metal transporter families and are usually

made up of membrane proteins that transfer metal ions to epidermal cells from the mesophyll or transfer metal ions from the roots to aboveground parts. At subcellular level, these transporter proteins regulate the uptake and transfer of metal ions across the plasma membranes, across vacuoles through tonoplasts, and other various endomembrane organelles (Maestri et al. 2010). Overexpression of these transporter proteins increases tolerance against HM and does not show injurious effect on homeostasis at physiological condition (Curie et al. 2000; Verret et al. 2004). Few important HM transporters include HM ATPases (HMAs): CPx-type ATPases, ABC (ATP-binding cassette) transporters, NRAMP (natural resistance associated macrophage proteins), CDF (cation diffusion facilitator family), and ZIP (ZRT-IRT-like protein) family. The process through which these HM transporters operate comprises of a sequence of signaling events, for instance, chain reaction that includes addition of phosphate group, hormones, mitogen-activated protein kinases (MAPK), and calcium-calmodulin systems. These signaling events finally lead to balancing of mineral nutrients within the plant cell that is vital for plant survival under unfavorable circumstances.

14.3.1 Heavy Metal ATPases (HMAs): CPx-Type ATPases

The function of HM transporters protein is to transport the metal ions across various cellular compartments, cytoplasm, and vascular tissues especially xylem. This particular HM transporter belongs to a varied class of superfamily that is found in all three kingdoms. The function of CPx-type ATPases is to propel an array of cations across the membranes. CPx-type ATPases include various ATPases, like H⁺-ATPases, Na⁺/K⁺-ATPases, and Ca²⁺-ATPases of fungi and plants, animals, and several other organisms, respectively (Axelsen and Palmgren 2001). Heavy metal/metalloid ATPases are made up of eight transmembrane domains, where the large-sized cytoplasmic loop is sandwiched amid transmembrane 6 and 7 (Mills et al. 2003). The P-type ATPase transporters have been divided into five chief families, i.e., type I, type II, type III, type IV, and type V. Based on their transport specificity, these major families (type I–V) are again categorized into two or more subfamilies (Axelsen and Palmgren 2001). Among various subfamilies, P_{1B} subfamily is assigned for transportation of HMs in plants. Solioz and Vulpe (1996) revealed that P_{1B} possess conserved intramembranous cysteine-prolinecysteine/histidine/serine sequences and hence are reported as the CPx-ATPases. Overexpression of AtHMA4 gene has been shown to ameliorate growth and development of roots in *Arabidopsis thaliana* even in the presence of various noxious levels of Zn, Cd, and Co (Verret et al. 2004). Mills et al. (2005) revealed that AtHMA4 deficit mutant plant leads to augmented sensitivity toward Zn and Cd toxicity, which confirmed the earlier findings. Later, Hanikenne et al. (2008) found that the AhHMA4 (homologue of AtHMA4) from the hyperaccumulating species *Arabidopsis halleri* bestowed tolerance against HMs like Zn and Cd. AhHMA4 is also engaged in translocation of Zn from root-to-shoot, its hyperaccumulation, and maintains Zn-Fe homeostasis.

Parameswaran et al. (2007) performed proteomic analysis of microsomal fractions and found another homologue of AtHMA4, generally expressed in epidermal leaf cells of *Noccaea caerulescens* (Schneider et al. 2013). In an experiment using transgenic approach, OsHMA3 gene, localized in tonoplast that principally encodes a P_{1B}-ATPase transporter, was identified in hyperaccumulating *Oryza sativa* L. that manages transport of Cd from ground part to above shoot portion (Miyadate et al. 2011). Upon Zn and Cd exposure, overexpression of HMA4 and HMA3 gene showed tolerance in *Arabidopsis thaliana* against these stresses. Moreover, overexpression of HMA5 was seen in roots and other plant parts of *Arabidopsis thaliana* upon exposure of Cu (Xu et al. 2010).

14.3.2 ATP-Binding Cassette Transporters (ABC Transporters)

The ABC transporters are exceptionally massive and varied family of membranous proteins present in organisms of all the three monarchy. ATP-binding cassette transporters are engaged in a wide array of transport functions (Theodoulou 2000; Martinoia et al. 2002). These are distinguished by the presence of couple of fundamental structural organizations in two copies, i.e., (1) an extremely hydrophobic transmembrane domain, each one of them consisting four or six transmembrane spans, and (2) a marginal (cytosolic) ATP-binding domain or nucleoside binding fold (Theodoulou 2000). Majority of these proteins serve to propel HM across the membrane and transfer a broad variety of substrates comprising of sugars, ions, lipids, pigments, peptides, antibiotics, and xenobiotics and are also engaged in vacuolar deposition of non-essential HMs within the cells. Till date, couple of subclasses of ABC transporters has been recognized in plants; these are (1) multidrug resistance-associated proteins (MRPs) and (2) the multidrug resistance proteins (MDRs). Bovet et al. (2003) revealed that gene expression of four MRPs was upregulated in roots of *Arabidopsis thaliana* upon application of Cd; however expression of AtMRP3 gene was comparatively more prominent. Furthermore, AtMRP3 was also stimulated upon Cu exposure, but under Zn toxicity, there was slight induction of AtMRP3. During transcriptome-based screening, Bovet et al. (2005) observed that ABC transporters, like AtPDR8, AtABCG36, and PEN3, localized in plasma membranes, were highly upregulated under Cd toxicity in *Arabidopsis thaliana*. Further studies showed that similar ABC transporters were also upregulated upon Pb toxicity. The *Arabidopsis* plants overexpressing PDR8 were more resistant to Cd and Pb toxicity, while RNAi and T-DNA mutant plants were sensitive to these (Kim et al. 2007). A novel ABC transporter IDI7, which is regulated by Fe, has been recognized in the tonoplast of *Hordeum vulgare* L. roots (Yamaguchi et al. 2002). This IDI7 has characteristics similar to ABC transporter and is stimulated under Fe deficit conditions.

14.3.3 *Natural Resistance-Associated Macrophage Proteins (Nramps)*

The Nramps are annotated as an extremely conserved family of membrane proteins. These proteins are engaged in metal ion transport in living organisms including flora and fauna. In plants, this group of protein family was initially recognized in *Oryza sativa* L. where three Nramps, i.e., OsNramp-1, OsNramp-2, and OsNramp-3, were revealed (Belouchi et al. 1995, 1997). Alike, various genes are discovered in a variety of plants such as *Arabidopsis thaliana* in which six Nramps were distinguished (Maeser et al. 2001). The plant Nramps seems to group into two subfamilies, which include first group AtNramps 1 and 6 and second group Nramps 2 to 5 (Maeser et al. 2001). In plants, Nramps are conserved and possess 12 transmembrane domains having transport motif sandwiched between transmembrane-8 and transmembrane-9 (Curie et al. 2000). In *Arabidopsis thaliana* roots, gene expression of AtNramps1, 3, and 4 was overexpressed under Fe deficit conditions (Thomine et al. 2000), whereas AtNramp3 was associated with uptake of Fe and Cd (Thomine et al. 2000). AtNramp3 gene was upregulated under Fe deficiency and was expressed in the xylem and phloem, localized into the vacuolar membranes, and functions in mobilizing metal pools from the vacuoles (Thomine et al. 2003). In *Solanum lycopersicum*, LeNramp1 was exclusively expressed under Fe deficit condition in roots, situated in the vascular parenchyma (Bereczky et al. 2003). Interestingly, it was noted that Nramp transcript level in *Hordeum vulgare* L. was downregulated under Cd toxicity and sufficient supply of nitrogen (N), though Nramp transcript level was notably upregulated by Cd but under N deficiency (Finkemeier et al. 2003). Consequently, above findings demonstrate that Nramp carry out various physiological functions, and few of the Nramps are concerned with Fe and Cd uptake and homeostasis.

14.3.4 *Cation Diffusion Facilitator (CDF) Family*

Cation diffusion facilitator transporter gene was originally differentiated in *Arabidopsis* and was named as ZAT. Upregulation of this gene in genetically modified plants results in resistance against Zn stress (van der Zaal et al. 1999). It was anticipated that ZAT gene is engaged in the vesicular/vacuolar sequestration of Zn and therefore is concerned with Zn homeostasis and tolerance. A ZAT gene, ZTP1, has been recognized in *Thlaspi caerulescens* and was seen to be upregulated principally in the leaf tissue, followed by root tissue (Assuncao et al. 2001). Degree of expression was higher in plants grown on calamine (which are enriched in Zn, Cd, and Pb) soil as compared with non-metalliferous soil. Majority of plants expressing these genes were found to be the Zn-tolerant, signifying the role of ZTP1/ZAT-like transporters in intracellular sequestration and tolerance against Zn stress (van der Zaal et al. 1999; Assuncao et al. 2001). Persans et al. (2001) have

distinguished CDF transporter (TgMTP1) which is accountable for metal ion accumulation within the shoot vacuoles in *Thlaspi goesingense*. Another CDF transporter, ShMTP1, has also been recognized in the *Stylosanthes hamata* (tropical legume) that can be grown in acid or high Mn^{2+} containing soils (Delhaize et al. 2003). The ShMTP1 provide tolerance against Mn^{2+} toxicity by sequestering metal ions and functions antiporter of proton/ Mn^{2+} in yeast and *Arabidopsis thaliana* (Delhaize et al. 2003). An experiment conducted in *Arabidopsis thaliana* revealed the presence of eight genes showing homology with the members of CDF family (Maeser et al. 2001).

14.3.5 The ZIP Family

The ZIP proteins are basically affianced in the uptake of Fe and Zn and their accumulations (Guerinot 2000). Nevertheless, they may possibly be engaged in response against Cd toxicity (Xu et al. 2012) and Ni tolerance (Nishida et al. 2011). Approximately, 85 ZIP family members are recognized from various prokaryotes and eukaryotes organisms, out of which 15 of them belongs to *Arabidopsis* (Maeser et al. 2001). This protein contains eight transmembrane domains, where the extracellular region contains the amino- and carboxyl-terminal ends (Guerinot 2000). Regarding single member of this family, *Arabidopsis* consists of three iron-regulated transporter (IRT) genes that regulate the upward movement of metal ions in plants from soil. The upregulation of several of IRT genes like IRT1, IRT2, and IRT3 was stimulated by increased concentration of Zn, revealing their functions in regulating Zn-Fe homeostasis in *Arabidopsis thaliana*. It is interesting to note that upregulation of IRT genes does not explicitly lead to HM tolerance. For instance, IRT1 overexpression in *Arabidopsis thaliana* led to over accumulation of Fe, Mn, Co, and Zn that caused negative effects to plant (Barberon et al. 2011). Interestingly, IRT3 overexpression in *Arabidopsis thaliana* has diverse consequences on accumulation of Fe and Zn; Zn accrual increases in the shoot, whereas Fe accretion increases in the roots of transgenic lines (Lin et al. 2009).

14.4 Potential Threats of Heavy Metals/Metalloids

14.4.1 Morphological Amendments

Presence of HM in agricultural soil and groundwater higher than the permissible level causes perturbations in the metabolism of plant cells resulting in various morphological modifications like wilting, curling, leaf necrosis, reductions in leaf number and area thus diminished photosynthesis rate and biomass accumulation, dissipation of mineral contents, reduced elongation rate, stunt growth, and reduced

yield (Chandrakar et al. 2017; Xalxo and Keshavkant 2018). Mostofa and Fujita (2013) demonstrated that exposure of *Oryza sativa* L. seedlings to Cu caused severe oxidative stress which appeared in terms of chlorosis (loss of normal green coloration of leaves), necrosis (death of cell or tissues), and leaf rolling. Upon individual and combined application of Cu and/or Cd, there was a noteworthy decline in diameter of root, its breadth, and width of leaf midrib in *Sorghum bicolor* (Kasim 2006). Plants when exposed to lethal dose of Cd showed visible symptoms of injury like chlorosis, reductions in growth and development, and root tip browning which ultimately led to cell death (Guo et al. 2008). Other symptoms of Cd toxicity include noteworthy decrease in the length and diameter of the internodes, leaf area, and thickness. Heavy metals have greater affinity for sulfhydryl groups of enzymes, and binding of HM ions with these groups causes distortion of membranes; therefore loss of turgor and rigidity are accountable for plant wilting under HM stress conditions. The presence of HMs in the soil restricts the water uptake and essential minerals, thus creating dehydration inside the cells, ultimately leading to leaf curling (Panda et al. 2009). Insufficiency of vital nutrients in plants also causes additional symptoms of HM toxicity, i.e., necrosis, which predominately affects the green leaves under which leaves turn brown or black in color. Farnese et al. (2017) reported that accumulation of As results in necrosis of outer margins of leaf, indicator of cell demise in particular area, which finally leads to death of the leaf. After performing root bioassay of *Brassica napus* L. seedlings, which was exposed to As, Farooq et al. (2017) affirmed that decrease in root length resulted in lethal effect of the stresses on cell elongation. Pandey and Sharma (2002) and Rahman et al. (2005) also reported necrosis and chlorosis in *Brassica oleracea* L. and *Hordeum vulgare* L., respectively, under Ni toxicity. Steiner et al. (2012) while working on *Jatropha curcas* plants found visible symptoms of Al toxicity like presence of minute necrotic spots and chlorosis on the margins of juvenile leaves and in the margins and center of adult leaves, respectively. Recently, study conducted by Ardisana et al. (2018) revealed chlorosis and necrosis in *Solanum lycopersicum* under Pb stress, indicating altered mineral nutrient absorption and rate of photosynthesis.

14.4.2 Physiological Effects

14.4.2.1 Growth and Productivity

Root Plasmolysis

Heavy metals being a non-essential constituent and extremely fatal to flora significantly affect growth and crop production. From the soil, HMs are taken up and then transported by various transporters localized in the plasma membranes of roots (Ovecka and Takac 2014). When plants are exposed to HM toxicity, usually, roots are the foremost organ that encounters HMs, where metal ions result in discoloration

and plasmolysis after entering into the cells (Shaibur and Kawai 2011). As plasmolysis is an active process and well-known characteristic feature of viable cells, it is used as a marker to observe the cell viability under various environmental stresses (Lang et al. 2014). Plants when exposed to HM toxicity show augmented lignifications in root cells and microtubule dissolution, which later results into rapid amplification in cell width, thus increasing diameter of root (Lou et al. 2015). Aluminium toxicity inhibits extension of the main axis and lateral root because of constrained cell division, and as a result root system appeared under developed and fragile, and apices were inflamed and injured (Panda et al. 2009).

Biomass

Plants when exposed to HM toxicity have popularly been reported to cause a noteworthy diminution in the biomass. This possibly may be because of the fact that root tissues are the foremost organ that comes in contact with HMs inside the soil (Chandrakar et al. 2018). As HMs have high affinity for sulfhydryl groups of numerous proteins and enzymes that are located in the radicular membranes, it causes interruption in root functions and cellular death (Shaibur and Kawai 2011). The reductions in rate of germination, root elongation, and biomass accumulation are consequences of HM contamination (Schutzendubel et al. 2001). Various researchers revealed that the prime influences of HM toxicity are the inhibition in rate of germination, biomass accumulation, root extension, and normal growth (Munzuroglu and Geckil 2002; Suzuki 2005; Chandrakar et al. 2016; Xalxo and Keshavkant 2019). In plants, reduction in germination rate and growth responses may result because of negative consequences of HMs on cellular functioning where maximum accessible energy is used for the production of stress linked vital compounds like phytochelatins (PCs), antioxidants, etc. Distinguished scientists have reported rigorous decrease in biomass production, which eventually cause decrease in crop productivity under HM toxicity (Ahmad et al. 2011; Asgher et al. 2014; Chandrakar et al. 2017; Xalxo and Keshavkant 2018). Most probable reason shown for reduction in biomass accumulation was increased permeability of cell membrane, thereby unavoidably increasing leakage of cellular constituents/fundamental nutrients which were primary components for the generation of energy and plant development (Farooq et al. 2015). Additionally, under HM stress, the water loss is increased, while water uptake is reduced by the roots which also results in diminished biomass accumulation (Gomes et al. 2013; Sidhu et al. 2018). Hence, it is obvious that when plants are exposed to HMs beyond their permissible limits, they negatively affect plant growth and development (Chandrakar et al. 2016; Xalxo and Keshavkant 2018).

Cell Division, Elongation, and Expansion

Plants when exposed to HM lead to inhibited cell division in the root meristematic zone, thus resulting in diminished growth. Reduction in mitotic activity thus decreased the rate of cellular division in the root apical meristem consequently leading to inhibited expansion and elongation of the novel cells (Suzuki 2005; Farooq et al. 2015). Heavy metal toxicity also reduces root cellular turgor leading to inhibition in cell enlargement. Exposure to HMs adversely influences the cell division machinery that is responsible for the cell cycle regulation in plants. Heavy metals also suppress the formation of proteins like cyclins and Cdk that are involved in cell cycle check points (Sharma et al. 2012). Inhibition of the enzyme activity involved in DNA repair system may be the outcome of binding of HMs to thiol groups which are accountable for DNA damage (Patra et al. 2004). Chromosomal aberrations with lower mitotic index, altered cell divisions (mitotic and meiotic), disruption of nuclear and plasma membranes, distortion in thylakoid structure, augmentation in amount of nucleoli, starch granules and plastoglobuli, severe plasmolysis, deficiency/absence of chromatin materials, and amplification in vacuoles count and size are few of the ultra-morphological alterations revealed in various plant parts under various HM toxicities (Patra et al. 2004; Aravind and Prasad 2005).

14.4.2.2 Photosynthetic System

Chlorophyll Synthesis

Among various vital biological processes, photosynthesis is one of the most studied phenomena during different biotic and abiotic stresses. Different plant species vary in patterns of metal accretion which affects biosyntheses of pigments, necessary for photosynthesis (Shakya et al. 2008; Vesely et al. 2011; Xalxo and Keshavkant 2018). Heavy metal may possibly associate with the photosynthetic machineries and may lead to a broad array of detrimental consequences together with photooxidative damage. One of the major negative effects of HM stress is inhibition of photosynthesis because HM invariably affects the function of photosynthetic system by hampering the machinery of both light and dark reactions. It is revealed that plant exposed to HM slows down the photosynthetic rate, transpiration rate, stomatal conductivity, plant growth and development, and ultimately loss in yield (Shakya et al. 2008). Babu et al. (2010) reported that the negative consequences of metal ions on growth, activities of leaf enzymes, and photosynthesis along with activities of both photosystem, i.e., PS-I and PS-II, were found to be dose dependent and were further amplified with an increase in exposure time. Heavy metal ions also influence the electron transport system and modify the energy transfer at multiple sites. Isolated chloroplasts were examined to reveal any alteration on the electron transport system due to HM ions, and it was found that PS-II was more vulnerable to HM toxicity as compared to PS-I. The activity of PS-I can be diminished on higher concentration

of HM exposure (Ivanov et al. 2006). Reduction in the concentration of various enzymes like ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity and/or its biosynthetic enzymes δ -aminolevulinic acid dehydratase and protochlorophyllide reductase may be the reason for reduction in the chlorophyll content (Farooq et al. 2016). Gene expression analysis of δ -aminolevulinic acid dehydratase showed that transcript level of this gene was downregulated under various abiotic stresses (Agnihotri and Seth 2016). Heavy metals affect chlorophylls and also carotenoids in plants because phytotoxic outcome of HM appears to be linked with excess generation of ROS which stimulate the production of carotenoids (Ghnaya et al. 2009; Kumar et al. 2012). Carotenoids, a low molecular weight compound owing to their antioxidant property, function by quenching higher concentration of ROS (Gill and Tuteja 2010). Among various established mechanisms in plants, to avoid photooxidative damage, one of the fundamental photoprotective mechanisms is the liberation of excessive excitation energy (Janik et al. 2008). Various accessory pigments like zeaxanthin (Zx), antheraxanthin (Ax), and the de-epoxidized components of xanthophyll cycle are engaged in the avoidance mechanism for dissipation of energy in PS-II (Toth et al. 2007). The *Pinus sylvestris* grown in areas contaminated with heavy metals such as Al, Pb, and Cu revealed reduction in the xanthophyll content (Matysiak 2001). Janik et al. (2008) reported that reduction in the second step of violaxanthin de-epoxidation leads to accumulation and augmentation in the *cis*-isomers fraction of violaxanthin in leaves of *Secale cereal* upon Cd exposure (Janik et al. 2008). On the other hand, when *Lemna minor* plants were exposed to Cd stress, amplification in the level of all the pigments of xanthophyll cycle was observed as an antioxidative response (Appenroth et al. 1996). It is well acknowledged that xanthophylls play important role in quenching of ROS-induced reactions. Xanthophylls also avoid excess production of free radicals, thus limiting the oxidative damage caused by excess ROS. Both adaptation and survival of plants under HM toxicity depend on their competence to resist the oxidative injury via antioxidant systems.

Chloroplast Membrane

Inside the cell, apart from mitochondria, peroxisomes, and other organelles, chloroplasts are one of the chief sites for the formation of free radicals and are pretty susceptible to HM-induced injury. Predominantly, this free radical modifies the function of chloroplast membrane and elements of the photosynthetic electron transport chain, thereby damaging the light reaction of photosynthesis (Ventrella et al. 2011). Li et al. (2006) and Schneider et al. (2013) revealed that *Pteris vittata* and *Leucaena leucocephala* when exposed to As toxicity resulted in structural injury to the chloroplast internal membranes. Toxic level of Cr has also shown to damage chloroplast ultrastructure, thus disturbing the photosynthetic process (Peralta et al. 2001). Plants, when exposed to Cd stress, lead to amendment in membrane function by inducing lipid peroxidation reaction and disturbances in chloroplast metabolism by reducing chlorophyll biosynthesis and decreasing the activities

of enzymes involved in CO₂ fixation (Fodor et al. 1995). Degradation of chloroplasts and alteration in their internal membranes due to HMs has shown detrimental consequences on pace of photosynthesis which is responsible for decreased level of photosynthetic pigment and rate of carbon assimilation (Farnese et al. 2017). Plants when exposed to HM stress also lead to structural disorganization of chloroplast membranes and perturbations in functioning of integral photosynthetic activity, consequently leading to alterations (breakage and swelling) of thylakoid membranes and reduced synthesis of photosynthetic pigment. Reduction in pigment biosynthesis may possibly be due to the absence of appropriate regulatory mechanism for its synthesis at lethal concentrations of HMs (Sharma et al. 2012; Chandrakar et al. 2016). Heavy metal-induced over produced ROS-mediated lipid peroxidation reaction may also be accountable for destruction of chloroplast membranes (Mascher et al. 2002; Chandrakar et al. 2017; Xalxo and Keshavkant 2018).

CO₂ Fixation

The net fixation of CO₂ is a fundamental process that dictates the total amount of carbon that recycles into the biosphere. Plants on exposure to HM stress show reduction in the rate of CO₂ fixation, thus reducing photosynthetic rate (Demirevska-Kepova et al. 2004; Gusman et al. 2013). Heavy metal-induced overproduction of free radical results in distortion of chloroplasts membranes, thus interfering with CO₂ assimilation and photosynthetic activity (Stoeva and Bineva 2003; Demirevska-Kepova et al. 2004; Gusman et al. 2013). Demirevska-Kepova et al. (2004) observed reduction in biomass accumulation and inhibition in enzyme activity, thus affecting CO₂ fixation. Stoeva et al. (2005) revealed that subjection of As-stress to *Zea mays* L. decreased the rate of CO₂ fixation with a noteworthy diminution in the functioning of PS-II. Application of As also leads to significant decrease in the chlorophyll fluorescence ratio, rate of CO₂ fixation, PS-II functioning, and consequently diminished photosynthetic efficiency (Stoeva and Bineva 2003; Chandrakar et al. 2016). Plant growth reduction due to HM contamination may possibly be attributed due to inhibition of enzymes of Calvin cycle, thus diminished rate of CO₂ fixation, and also by restrictive transport of carbohydrates from source to sink regions. Because of reduced pace of CO₂ fixation, recovering of NADP⁺ get stopped (final electron acceptor), leading to decreased photosynthetic electron transport. This overall process leads to decrease in the rate of electron transport and excess generation of excitation energy. As a result, the excess excitation energy thus produced, stimulate the alternative pathways of electron transport like, water-water cycle and photorespiration, leading to excess leakage of electrons out of electron transport chain (Gusman et al. 2013).

Stomatal Conductance

Heavy metals are well known to destroy the structures of plasma membranes. Any damage to the outer membranes results in disparity in the uptake and transportation of essential nutrients and ions in the plant cells which ultimately lead to reduction in the stomatal conductance (Paivoke and Simola 2001). Inhibition of key enzymes like δ -aminolevulinic acid dehydratase and increased activity of chlorophyllase (pigment degrading enzyme) leads to the increase/decrease in the concentration of pigment and stomatal conductance under HM stress (Sharma and Dubey 2005; Xalxo and Keshavkant 2019). Subjection of plants to HMs/metalloids leads to deposition of these in the cuticles which envelop the guard cells and its auxiliary cells that result in decreased stomatal opening (Rucinska-Sobkowiak 2016). Lead toxicity has been shown to decrease the stomatal aperture, which later leads to closing of stomata that result in slower diffusion of water vapors due to the greater diffusion gradient of it (Bondada and Oosterhuis 2000). Presence of Pb limits the compounds that are responsible for maintenance of cell wall plasticity and turgor pressure thus resulting in lowering of cellular water potential and thereby inducing stomatal closure. Closed stomata may also be an outcome of inhibited energy system or altered K^+ fluxes through membranes or Pb-induced ABA accumulation or all of these (Sharma and Dubey 2005). Furthermore, As interrupts with microtubules assembly during cell division and thus normal cell cycle regulation get hampered. Eventually, the regular pattern of cell division ceases, and there is a formation of abnormal stomata, thus leading to reduction in growth of plant (Gupta and Bhatnagar 2015). Heavy metal-induced detrimental effects include decrease in rates of photosynthesis, transpiration, and stomatal conductance which hampers the water and ion uptake (Milivojevic et al. 2006). Anjum et al. (2016) observed closing of stomata due to the Cd or As contamination which leads to deprivation of CO_2 ; thus it arrests photosynthetic carbon assimilation and also reduces rate of transpiration. In *Arachis hypogaea*, Shi and Cai (2008) revealed reduction in net photosynthetic rate in association with decreased stomatal conductance and photosynthetic pigment under Cd stress. Studies have reported that Ni stress inhibits rate of photosynthesis due to the decreased stomatal conductance (Seregin and Kozhevnikova 2006; Ahmed and Hader 2010). Heavy metal-induced decline in photosynthesis rate suggests that restriction in the stomatal conductance leads to decreased internal CO_2 level and as a result decrease in rate of photosynthesis (Gusman et al. 2013).

14.4.2.3 Nutrient and Water Uptake

Micro- and Macronutrients

Mineral nutrients are the major components of the plant structural component, thereby providing mechanical support to plants. These micronutrients also play a vital role in plant growth and development. However, HM toxicity may interfere

with uptake and transport of essential nutrients, thus disturbing the mineral composition. Both micro- and macronutrients aid in biosyntheses of macromolecules, like proteins, carbohydrates and nucleic acids, growth substances, and chlorophylls, and most importantly help in stress tolerance (Paivoke and Simola 2001). Reactive oxygen species is accountable for oxidative damages to various biomolecules like proteins, lipids, and nucleic acids, which in turn are responsible for different physiological amendments such as diminished growth, nutrient deficiency, decreased nutrient transport, genotoxicity, and retarded photosynthesis (Nagajyoti et al. 2010). Roots are the first tissues that interact with HMs and accumulate them inside the cells and disturb the nutrient uptake (Stoeva et al. 2005). Selectivity and permeability of root cell membranes also get modified due to HM contamination, thereby resulting in reduced uptake of nutrients (Gusman et al. 2013). Adequate concentration of nutrients helps plants in accumulation of biomass. Excess accrual of HM competes with nutrient ions for their binding with transport protein and thus interferes with the uptake of nutrients (Gusman et al. 2013). Significant reduction in uptake of nutrients and transfer of ions from ground to aboveground parts were revealed in As stressed *Triticum aestivum* L., and reduced nutrient uptake was associated with decreased photosynthetic rate (Liu et al. 2008; Gusman et al. 2013). Plants obtain N from soil, in the form of nitrate or ammonium, but incidence of HM disturbs the N assimilation; thus genes that are accountable for transportation of nitrate and ammonium in plants are downregulated (Norton et al. 2008).

Water Relations

Apart from providing mechanical support to the plant and fixing it in the soil, the prime purpose of roots is the water absorption and transport of inorganic nutrients from soil. Generally, accumulation of HM is found higher in roots than other plant parts as it is the foremost organ to come in contact with HM ions (Seregin and Ivanov 2001; Chen et al. 2004; Małecka et al. 2008). Excessive availability of HM hampers the proficiency of the water flow by decreasing the rate of transpiration (Barcelo and Poschenrieder 1990). In roots, transport of water by the symplast and apoplast pathways also gets disturbed by HM exposure resulting into reduced water uptake (Steudle 2000, 2001). Heavy metal also inhibits the aquaporin activity and/or alters the expression of these proteins, thus resulting in impairment of water transport through the membranes (Przedpelska-Wasowicz and Wierzbicka 2011). Subjection of HM leads to accumulation of callose in cell walls that is accountable for the reduced movement of water. Samardakiewicz et al. (2012) revealed that subjection of Pb to *Lemna minor* roots resulted in accretion of callose within the plasmodesmata. On the other hand, deposition of metals on cell walls leads to thickening of these, which increases the apoplast resistance to water flow. It has previously revealed that Pb was responsible for thickening in the cell wall (Krzesłowska et al. 2009) and increased lignin content in *Pisum sativum* L. (Paivoke 1983) and *Glycine max* L. (Pawlak-Sprada et al. 2011), callose in *Lemna minor* roots (Samardakiewicz et al. 2012), and callose, pectins, cellulose, and sudanophilic lipid compounds in the

protonemata of *Funaria hygrometrica* (Krzesłowska et al. 2009). Obstruction in leaf development due to the water deficit may be due to hampered transport of water to above parts of plants particularly to leaf. A quick reduction in osmotic potential, root vacuolization, and reorganization of tissues may be regarded as the strategy to enhance water retention in plants (Rucinska-Sobkowiak et al. 2013). It is proposed that modifications in the aquaporin permeability are linked with the alteration of aquaporin proteins by phosphorylation/dephosphorylation, the processes which may be controlled by abscisic acid (ABA). Plant exposure to HM may possibly stimulate augmentation in signaling compounds such as calcium ions, polyamines, and various plant hormones like ethylene, auxin, jasmonic acid (JA), salicylic acid (SA), and ABA.

14.4.3 Phytotoxicity of Heavy Metals/Metalloids at Cellular and Molecular Levels

14.4.3.1 Lipid Peroxidation

Lipid peroxidation is an indicator of oxidative injury, induced by various abiotic and biotic stresses including HM, and has been acknowledged in varied plant species (Cho and Park 2000; Cargnelutti et al. 2006; Ahsan et al. 2007; Wang et al. 2008; Cavusoglu et al. 2009; Pandey et al. 2009; Maheshwari and Dubey 2009; Chandrakar et al. 2017; Xalxo and Keshavkant 2018). Plants subjected to HM toxicity displayed an augmentation in lipid peroxidation reaction as a consequence of excess ROS generation (Chandrakar et al. 2017; Xalxo and Keshavkant 2018), and also acceleration in it might be done by the lipoxygenase with the formation of hydroperoxides (Huang et al. 2012). Heavy metal-induced production of free radical leads to deterioration of cellular components involving polyunsaturated fatty acid (PUFA) residues of phospholipids, which are tremendously susceptible to oxidation (Abd-Allah et al. 2015). The PUFAs like linoleic acid (18:2) and linolenic acid (18:3) are predominantly vulnerable to attack of singlet oxygen (1O_2) and hydroxyl radical ($\cdot OH$), resulting in the formation of lipid hydroperoxides (Ahmad et al. 2010). Lipid peroxidation is regarded as most detrimental process that occurs in plants during HM toxicity. Several aldehydic products such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), along with hydroxyl and keto fatty acids, are generated as consequences of PUFA peroxidation under HM stress (Chandrakar and Keshavkant 2018). Treatments of Cd, As, and Pb notably increased the accumulation of lipid peroxidized products in *Pisum sativum* L. (Metwally et al. 2005), *Glycine max* L. (Chandrakar et al. 2017), and *Trigonella foenum graecum* L. (Xalxo and Keshavkant 2018), respectively. In plant cells, some of the PUFA oxidation products function as secondary messengers either directly or after enzymatic modifications (Mittler 2002; Miller et al. 2010).

14.4.3.2 Oxidative Damage to DNA

Subjection to abiotic stress like HM can harm DNA which can affect the strong steadiness of the plant genome (Chandrakar et al. 2017). The excessively accumulated ROS are the key mediators of damage to cellular organization and significant biomolecules (Tuteja et al. 2009; Chandrakar et al. 2017). Binding of metals to the cellular nuclei results in modifications in DNA nucleotides, inter- and intramolecular cross-linking of DNA and proteins, and DNA fragmentation and reorganization, which can further induce mutation (Nagajyoti et al. 2010). For example, when ROS is produced by oxidative stress nearby DNA, it forms a mutagenic adduct 8-OxoG (7,8-dihydro-8-oxoguanine) that may possibly establish bonding with adenine. If unchecked by the repair machinery, this may lead to transverse mutation from cytosine to thiamine (Cunningham 1997). Common genotoxic effects reported due to the As toxicity involve disruption of mitotic spindle fibers. In the seeds of *Hordeum vulgare* L., the degree of anomalies found in the chromosomes was related to As aggregation (Bandyopadhyay and Maity 1995). Chromosomal behavioral analysis of Cd exposed *Pisum sativum* L. root tips revealed increased frequency of chromosomal abnormalities like fragmentation, early and late separation of chromosomes, single and double bridges, and chromosome agglutination (Siddique et al. 2009).

14.4.3.3 Oxidative Damage to Proteins

Proteins on direct reaction with excess ROS undergo oxidative modifications. Interactions with MDA and HNE (lipid peroxidation products) also result in oxidative stress (Xalxo and Keshavkant 2018). Both MDA and HNE produced as a result of lipid peroxidation undergo non-enzymatic condensation with free amino groups of proteins or nucleic acids, which leads to browning. Glycosylamine formed as a result rearranges to produce the Amadori product (1-amino-deoxyketose), which consequently degrades into intermediate products (MDA and HNE). These intermediates thus obtained mix with other amino groups which results in the formation of glycosylation end products. Oxidized protein is universally present and thus can be used as a marker to diagnose HM-imposed oxidative stress. Carbonylation is another irreversible and most frequently transpiring oxidative protein modulation under HM stress. It may result due to oxidation of amino acid side chains (leading to formation of arginine and proline to α -glutamyl semialdehyde, threonine to amino ketobutyrate, and lysine to amino adipic semialdehyde). Reaction between proteins and lipid peroxidized products, for instance, HNE and MDA, may also initiate carboxylation reaction. Scientists have revealed that the content of protein carbonylation is found more in the mitochondria in comparison to the chloroplasts and peroxisomes which signifies that the mitochondria are more vulnerable to oxidative injury (Ahmad et al. 2010; Gill and Tuteja 2010). Heavy metal-induced oxidative amendments of proteins lead to various detrimental consequences on the cellular physiology of plants. The oxidative damage caused by excess ROS can be mild to brutal on cell functions

depending upon various factors such as fraction of biological molecules that are modified and for how much time the alteration persisted in the cell.

14.5 Production of Reactive Oxygen Species and Antioxidant Defense Systems: Markers in Ecophysiological Studies

Overaccumulation of ROS is considered as the first biochemical modification when plants are exposed to HM-contaminated sites (Gill and Tuteja 2010; Chandrakar et al. 2017; Yadu et al. 2017; Xalxo and Keshavkant 2018). The ROS comprises of $^1\text{O}_2$, superoxide radical ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroperoxyl radical (HO_2), $\cdot\text{OH}$, hydroperoxide (ROOH), peroxy radical (ROO^{\cdot}), and alkoxy radical (RO^{\cdot}). These free radicals are extremely reactive and noxious and cause injury to various biomolecules like proteins, lipids, carbohydrates, and DNA which eventually leads to death of the cells (Gill and Tuteja 2010). Excess accrual of ROS, as a consequence of diverse environmental stresses including metal toxicity, affects crop yield and food security globally (Xalxo and Keshavkant 2018). Reactive oxygen species are generally shown to damage nucleic acids, oxidize proteins, and cause lipid peroxidation, thus hampering many cellular functions (Chandrakar et al. 2017; Xalxo and Keshavkant 2018). A severe imbalance occurs between production and elimination of ROS, and this disproportion later results in remarkable physiological challenges to the plants that is cumulatively termed as “oxidative syndrome” (Kovacik et al. 2010; Morina et al. 2010; Chandrakar et al. 2017; Yadu et al. 2017; Chandra et al. 2018). Heavy metals such as As, Cu, Cd, Cr, Fe, Hg, Pb, and Zn have the capability to stimulate the excess generation of ROS inside the cells (Duquesnoy et al. 2010; Vanhoudt et al. 2010; Korpe and Aras 2011; Márquez-García et al. 2011). The mechanism of ROS generation is dissimilar for redox-active and redox-inactive metals (Pourrut et al. 2008; Opendakker et al. 2012). Redox-active metals like Cu and Fe act as catalyst in the Haber-Weiss/Fenton reactions, wherein H_2O_2 splits into $\cdot\text{OH}$ at a neutral pH (Sahi and Sharma 2005; Valko et al. 2006). On the other hand, redox-inactive metals like Cd, Pb, Hg, As, Zn, and Ni hamper enzymatic activities as they have strong affinity for $-\text{SH}$ groups of the proteins (Pourrut et al. 2011; Chandrakar et al. 2016). As redox-inactive metals have electron-sharing affinities, they form covalent bonds with sulfhydryl groups of proteins. Heavy metals, particularly Pb, bind to functional groups (COOH) of protein molecules and inactivate enzymes (Sharma and Dubey 2005; Gupta et al. 2010). Additionally, HM induces displacement of vital cations from specific binding sites of enzymes, thus disturbing the intracellular ROS balance, resulting in excess accumulation of ROS. Heavy metals are also proficient in depleting reduced glutathione (GSH) within plant cells (Bhaduri and Fulekar 2012). Depletion of major antioxidants due to the accumulation of HMs inside the cells also disrupts the ROS balance. Heavy metals also directly bind to GSH and its derivatives whose presence is obligatory to scavenge excess ROS generated in the plants, hence leading to ROS accrual in cell

(Lee et al. 2003). In addition, plasma membrane-integrated NADPH oxidase is engaged in HM-induced oxidative injury (Pourrut et al. 2008, 2013; Potocky et al. 2012). Reactive oxygen species generation via NADPH oxidase was revealed by Rodriguez-Serrano et al. (2006), Hao et al. (2006), and Pourrut et al. (2008) in *Pisum sativum* L., *Triticum durum* L., and *Vicia faba* L. treated with Cd, Ni, and Pb, respectively.

To defend itself against these ROS, plant cells and its components (chloroplasts, mitochondria, and peroxisomes) utilize antioxidant security systems. Prior investigations have confirmed that cellular antioxidant system is significant for defense against several stresses (Tuteja 2007; Singh et al. 2008; Chandrakar et al. 2017). The antioxidant defense machinery comprises of enzymatic and non-enzymatic factors. The superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) are termed as enzymatic antioxidants, while GSH, ascorbate (AsA), carotenoids, and tocopherols (lipid soluble) are placed under non-enzymatic group (Chandrakar et al. 2017; Yadu et al. 2017; Xalxo and Keshavkant 2018). These antioxidants prevent additional buildup of ROS intracellularly, thus maintaining redox homeostasis in the cell (Gill and Tuteja 2010). Many examples of amendment in the antioxidant machinery imposed by HMs in various plant species are present in the literature (Miller et al. 2010; Ahmad et al. 2011; Chandrakar et al. 2017; Xalxo and Keshavkant 2018). Antioxidative restoration to metal toxicity depends upon the metal type, quantity, and plant species. In plants, major role of ROS homeostasis maintenance is played by AsA and GSH antioxidants. Elevation in AsA and reduction in GSH amount have been studied in *Oryza sativa* L. seedlings after Ni stress (Maheshwari and Dubey 2009). Exposure of *Glycine max* L. seedlings to Ni leads to loss in AsA amount (Prasad et al. 2005). Treatment of *Phaseolus vulgaris* L. seedlings to Cd, Pb, Hg, and Cu, separately, resulted in an amplification in AsA and tocopherol levels (Zengin and Munzuroglu 2006); similarly Cd treatment also exhibited a concentration reliant increase in the GSH levels of germinating *Vicia faba* L. seeds (Szollosi et al. 2009). The level of antioxidant enzymes escalated in the leaves of *Pisum sativum* L. and *Trigonella foenum graecum* L. plants in retaliation to Cd and Pb, respectively (Dixit et al. 2001; Xalxo and Keshavkant 2018). The SOD and CAT also enhanced in the plant cells after Hg exposure (Patra et al. 2004); also, gene expression of these enzymes was upregulated by Cr and Pb in *Zea mays* L. and *Trigonella foenum graecum* L., respectively (Labra et al. 2006; Xalxo and Keshavkant 2018). The antioxidant enzyme responses to HM stress depend on the tissues, for instance, application of Cd-induced GST in *Pisum sativum* L. roots, but no induction of GST was observed in leaves (Dixit et al. 2001). Similarly, GST was upregulated by Cu in germinating *Oryza sativa* L. embryos, but downregulation was observed when whole seed was taken into consideration (Ahsan et al. 2007; Zhang et al. 2009).

14.6 Signal Transduction Under Heavy Metal/Metalloid Toxicity

To conserve the normal quantity of essential metal ions, plants have developed various methods to reduce their contact with HMs, which are non-essential for them. Few mechanisms required for homeostasis are common in all, but some of the processes target individual metal ions for their elimination from intracellular environment. When these two systems fail to overcome stress responses, plants put up a stress defense system including several proteins and signal transducing molecules like heat shock proteins, hormones, ROS, and most significantly the specific metal-responsive genes (Maksymiec 2007). However, diverse signal transduction pathways get initiated in response to HMs, most common of these includes the calcium calmodulin pathway, ROS signaling, hormones, and the MAPK phosphorylation pathway. Researchers have reported that HM toxicity in plants causes alterations in calcium (Ca) concentration, MAPK cascade, and transcriptional modifications of the genes involved in stress defense (Shao et al. 2008, 2009; Thapa et al. 2012).

14.6.1 Calcium Calmodulin System

Calcium, a vital macronutrient, enters into plants via root systems and is transferred to the other parts of the plant through xylem (Tuteja et al. 2009; Ahmad et al. 2012). Calcium ion not only acts as a major component in all signaling systems but also plays a role during normal development and several biotic and abiotic stresses including HM (Tuteja and Mahajan 2007; Himschoot et al. 2015). In plants, exposure of HM alters the concentration of cytoplasmic Ca^{2+} due to the influx of Ca^{2+} from outer environment of the cell, or due to the discharge of the stored intracellular Ca^{2+} inside the cell (Steinhorst and Kudla 2014). Plants consist of a range of Ca^{2+} sensors like calcineurin B-like proteins (CBLs), calmodulins (CaMs), calmodulin-like proteins, and Ca^{2+} -dependent protein kinases (CDPKs). These biological sensors sense and decipher alterations in the levels of cytosolic Ca^{2+} and transmit it as signal for further response (Luan et al. 2002; Sanders et al. 2002; Dodd et al. 2010; Steinhorst and Kudla 2013). The Ca^{2+} -dependent protein kinases are sensory proteins that bind to Ca^{2+} directly prior to phosphorylation of substrates which are involved in signal pathway during various stresses (Hamel et al. 2006; Schulz et al. 2013). Various experiments have been performed to explore the role of CBLs in plants vs. abiotic stresses. The identification and classification of CBLs have been done in several plant species including *Cicer arietinum* L. (Tripathi et al. 2009), *Glycine max* L. (Li et al. 2012), *Vitis vinifera* (Tripathi et al. 2009), and *Solanum lycopersicum* (de la Torre et al. 2013) to enhance their endurance toward abiotic stresses. In plants, higher intracellular accumulation of Ca^{2+} was observed under Cd stress, thus implementing adaptive techniques that help to mitigate the toxicity of

HM (Yang and Poovaiah 2003). The Ca-calmodulin system was seen to be intricately involved in tolerance mechanisms of *Nicotiana tabacum*, in response to Ni and Pb toxicity. High amount of toleration toward Ni and sensitivity to Pb were observed in transgenic *Nicotiana tabacum* plant expressing NtCBP4 (*Nicotiana tabacum* calmodulin-binding protein). Another Ca^{2+} sensor, i.e., CDPKs, senses and relays the downstream signaling pathway cascade (Asano et al. 2012). It aids in the regulation of *Oryza sativa* L. growth via continuous expression of CDPK gene (Manimaran et al. 2015). Reports have also proposed the possible mitigation of HM stress by the induction of exogenous Ca^{2+} which assists via influencing various vital processes of plants. Moreover, addition of Ca^{2+} also affects the action of defensive antioxidant enzymes (APX, GR, and SOD) (Ahmad et al. 2015). Thus, the study of varied expression patterns of the Ca^{2+} -binding proteins in plants against HM stress will be advantageous in further analysis of the functions and mechanisms behind Ca^{2+} -dependent signaling in plants.

14.6.2 Plant Hormones

Phytohormones are universally present and play principal roles in growth and development during plant life cycle (Divi and Krishna 2009). Auxin is known to be the best hormone for plant growth regulation; ABA positively influences bud dormancy and closing of stomata; cytokinin helps in delaying aging; gibberellin induces seed germination; and brassinosteroids regulate growth and development of plants (Sun et al. 2005; Vert et al. 2005; Jaillais and Chory 2010). Phytohormones are low molecular weight mediators between various signaling pathways, and they also influence plant retaliation to abiotic stresses (Bostock 2005). Significantly, ethylene (ET), SA, and JA are closely linked with the plant defense mechanisms vs. acting stress. The EIN2, EIN3, JAZ proteins, and AP2/ERF TF gene family are strongly influenced by ET and JA (Mauch-Mani and Mauch 2005; Fujita et al. 2006; Kazan 2015). An investigation by Singh and Shah (2014) showed that subjection to JA increases antioxidant responses, hence leading to Cd stress endurance in *Oryza sativa* L. Thus, clearly suggesting that modulation in extent of plant hormones alters the plant response to HM stress. Increase in ET biosynthesis was reported by Rodriguez-Serrano et al. (2009) in *Pisum sativum* L., Masood et al. (2012) in *Brassica oleraceae* L., and Chmielowska-Bak et al. (2013) in *Glycine max* L. under Cd stress. Experiments done by Popova et al. (2009) in Cd stressed *Pisum sativum* L. revealed augmentation in the SA level which helped to defend leaves from lipid peroxidation. The results suggested that elevated concentration of intracellular SA accumulated after Cd treatment may act directly as an antioxidant or indirectly activate other antioxidant responses. Comparative transcriptome analysis of *Oryza sativa* L. seedlings treated with As(V) and As(III) showed that As modified the signaling pathway and defense mechanism of the plant, revealing that As(III) profoundly affects hormonal signaling processes such as ABA metabolism (Chakrabarty

et al. 2009). Additionally, the detailed study of signal cascade involving hormones under HM stress can help in growth of plants with increased stress bearing.

14.6.3 Role of Reactive Oxygen Species

The recognition of stress signal and initiation switching of signal transduction pathways are the adaptive reactions that decides the existence of plants under stressed environment. Production and aggregation of ROS, particularly $O_2^{\cdot-}$, H_2O_2 , $\cdot OH$ and 1O_2 , is one of the stress stimuli. Due to HM stress, ROS is formed in huge amount which is termed as “oxidative burst,” an alarming signal to trigger the gene expression of transcription factors (TFs) and various other protective genes (Sharma et al. 2012). The excessive formation of ROS disturbs the balance of the normal cellular functioning, resulting in oxidative harm to biomolecules (Karuppanapanadian et al. 2011; Kapoor et al. 2015). These free radicals play a dual role that is based on time, site, and their quantities. Firstly, ROS at low concentration acts as a signal messenger and helps in eliciting response against several stresses, while at elevated concentration, it poses threat to the plant cell. Reactive oxygen species receptors include histidine kinases, redox-sensitive TFs, ROS-sensitive phosphatases, and redox-regulated ion channels (Steffens 2014). Various scientists have revealed that ROS generation due to the HMs is confirmed by the accruals of lipid peroxidation products like MDA and HNE (Sharma et al. 2012; Rai et al. 2015; Chandrakar et al. 2018; Xalxo and Keshavkant 2018). Studies have confirmed that HM like Cd causes lipid peroxidation and PCD in varied plant due to the agitation in the metabolism of cell organelles like chloroplasts and mitochondria (Cho and Seo 2005; Metwally et al. 2005; Bi et al. 2009). It has been revealed that subjection to Hg toxicity induces Ca^{2+} accrual, decreases free radical generation, and activates MAPKs, which contributes to the plant resistance to Hg stress (Chen et al. 2014). As stated by various researchers, As contamination negatively affects regular growth and development of plants and instigates oxidative injury (Shri et al. 2009; Islam et al. 2015; Chandrakar et al. 2017). During stress condition, enzymatic and non-enzymatic antioxidant systems undergo several modifications (Dwivedi et al. 2010; Kanwar and Poonam 2015; Chandrakar et al. 2017). As a defense strategy, few mechanisms tackle toxicity in the plants. The primary detoxification mechanism comprises increased antioxidant activity which preserves homeostasis intracellularly by scavenging excess accumulated ROS, and secondary mechanism includes HM sequestration into the vacuoles by various peptides (Kumar et al. 2015). During stress condition, antioxidant system is triggered to lessen the negative consequences due to excess ROS (Rai et al. 2011; Chandrakar et al. 2017; Yadu et al. 2017; Xalxo and Keshavkant 2018). Shri et al. (2009), Chandrakar et al. (2017), Yadu et al. (2017), and Xalxo and Keshavkant (2018) reported amplified activities of CAT, SOD, POD, APX, and GR in *Oryza sativa* L., *Glycine max* L., *Cajanus cajan* L., and *Trigonella foenum-graecum* L. seedlings, respectively, exposed to various HMs.

14.6.4 Mitogen-Activated Protein Kinase Cascade

Mitogen-activated protein kinases are serine/threonine kinases and are signaling molecules conserved throughout evolution in the plants (Jonak et al. 2002). It is composed of MAP kinase kinase kinases, MAP kinase kinases, and MAP kinases (MAPKs/MPKs) (Nakagami et al. 2005). These molecules aid during stressed environments and are significant in the establishment of tolerance while introducing the adaptive downstream signaling. Phosphorylation occurs in signal cascade of several TFs such as DREB, bZIP, NAC, and WRKY and thus affects normal functioning like differentiation, growth, and developmental and stress responses of cells (Lin and Aarts 2012). Transcriptional initiation of these MAPKs in *Oryza sativa* L. confirms its regulatory function in plant maturation, safety, and stress signaling (Kim et al. 2003). Genomic study of different plants explained that components of this signaling pathway are determined by multigene families. Approximately, 20 MAPKs, 10 MAP kinase kinases, and 60–80 MAP kinase kinase kinases are a part of the *Arabidopsis* genome (Chen et al. 2012b), and similar quantities of these have also been reported in other species like *Oryza sativa* L. and *Zea mays* L. (Rao et al. 2010). The purpose of MAPKs has been deliberated under variety of stresses. The MAPKs under physical factors like cold, heat, drought, and salinity are differentially regulated in *Oryza sativa* L., proposing the participation of this gene family in stress signaling (Kumar et al. 2008; Chen et al. 2012a; Zhang et al. 2012). Jonak et al. (2004) reported four isoforms of MAPK in *Medicago sativa* seedlings when subjected to Cu or Cd stress. Similar results were also obtained by Yeh et al. (2007) in *Oryza sativa* L. after Cd-induced toxicity. In conclusion, all of these pathways together aid in the regulation of TFs that regulates genes necessary for plant adjustment during stress.

14.7 Assisting Plant to Survive: Future Directives and Perspectives

Accumulation of excessive HM in the environment has become a major threat due to its adverse effects in all the living organisms. Negative effects of HM toxicity on flora and fauna are a matter of serious concern globally. Even at low concentrations, HMs are very reactive and their concentrations gets build up in the food web, leading to detrimental effects to all the living organisms. Various detrimental consequences of elevated or lethal concentrations of HM ions to sensitive plants include reduced growth, crop productivity, and yields. Developing the mechanisms to help plants for the management of HM toxicity may be useful in creating new ideas for the remediation of HM. Presently, researchers are more worried in developing novel technologies of least expensive and eco-friendly land retrieval techniques. Currently, there is a couple of biotechnological approaches to limit the negative consequences of HM contami-

nations in the natural environments. First approach involves increasing the HM uptake capacity of plants for the amplification of their efficacy in phytoremediation of HM from contaminated sites. Another strategy includes the enhancing plant tolerance against HMs, which increases plant growth and crop yield. Genetic manipulation of transporter gene responsible for localization of HM is reassuring step as the encoded proteins can effectively regulate the entry, circulation, and accrual of metal ions in the plant tissues. On the other hand, the modifications of membrane transporters to decrease the uptake and transfer of particular HM ions may result in disparity in maintaining the redox state of essential metal ions. Therefore, molecular and cellular adaptation seems mandatory to improve their tolerance against HM toxicity, which hampers the HM entry into the food chain. It is confirmed that the overaccumulations of HMs are also responsible for imbalance in the homeostasis of antioxidants and slanting the balance that induces the ROS generation and its interference in normal functioning of cellular macromolecules (proteins, lipids, nucleic acids, etc.) and organelles. Additionally, PCs and metallothionins (MTs), responsible for metal sequestration, and their functional diversity and molecular adaptability are attracting the scientist regarding the HM detoxification and regulating the cellular ion balance. Both PCs and MTs interact with antioxidant defense system directly or indirectly and aid plants in transporting and distributing excessive HM ion between different tissues in a time- or tissue-specific manner. Glutathione also plays a major role in HM entry, passage, compartmentation, sequestration, and formation of specific metal-binding ligands such as PCs. The modification in gene expression of PCs is one of the potential mechanisms for amplifying the plant efficiency for phytoremediation. Understanding the consequences of gene expression of the GSH/PC biosynthetic pathways, probably in a tissue-specific manner, on metal tolerance and accumulation may lead to understanding of their usefulness in remediation of HM toxicity. There are evidences that used transgenic plants which overexpressed PCs and conferred HM tolerance. Presently, scientists are using microorganisms for the removal or remediation of HM stress. Strategies developed by microbes for sustained survival in HM-polluted sites include various mechanisms such as bioaccumulation, biomineralization, biosorption, and biotransformation. Signaling pathways like MAPK cascades trigger environmental and developmental signals into adaptive and programmed responses in response to HM stress. Various important physiological and developmental processes like stress and hormonal responses and innate immunity are controlled by MAPK cascade. Extensive investigations are required for finding of suitable substrates of MAPKs and their association with other signaling molecules. Importantly, for detailed understanding of the detrimental effects of HMs on seeds/seedlings, the knowledge about the signaling networks needs to be explored including the interaction of ROS, reactive nitrogen species, and seed/plant hormones with HMs.

14.8 Conclusions

It is evident from various researches that non-judicious use and excessive presence of HM have lethal consequences on flora and fauna. It is evident that there are various substitutes, by political or social actions decreasing the discharge of toxic pollutants into the natural environments, utilization of genetically modified plants or the use of naturally occurring tolerant crops, which may aid to defeat contaminated environmental conditions. Nevertheless, extensive research programs are required for enhanced understanding of deleterious impacts of HM on the living system and its related areas to control the ecological redox of our environment.

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