

## CHAPTER FOUR

# THE EFFECTS OF HEAVY METAL POLLUTION ON PLANTS AND THEIR TOLERANCE MECHANISMS

MANJU SHRI AND S. DUBEY

Heavy metal pollution of water and soil resulting in toxicity has become a major issue for crop development and yield. This has been exacerbated by cumulative population growth and increasing food requirements. As with other organisms, plants have acquired a range of mechanisms for the detoxification of heavy metals. These include: signal transduction; adherence of metal compounds to the cell wall and root secretions; metal efflux from the plasma membrane; sequestration within vacuoles by metal binding ligands, such as phytochelatins and metallothioneins; and antioxidant enzymes and heat shock proteins. Substantial progress has been made in recent years to develop transgenic plants for the removal of heavy metals from the environment. This chapter presents an updated understanding of the uptake, accumulation, and detoxification of heavy metals in plants.

### **Introduction**

Over the course of the last few years, the intensification of human activity—rapid industrial development and contemporary agricultural approaches—has resulted in widespread heavy metal pollution, which induces toxicity in living entities (Eapen and D'souza, 2005; Kavamura and Esposito, 2010; Miransari, 2011). Soils have been contaminated with heavy metals through the application of pesticides, fertilizers, and sewage sludge, as well as the poor disposal of heavy metal wastes from smelting and mine tailings (Yang et al., 2005). Though heavy metals are naturally found elements, difficulties emerge when they are discharged in excess into the surroundings due to human activity. Categorized according to their density ( $>5 \text{ g/cm}^3$ ), the 53 d-block elements have been characterized as

“heavy metals” (Jarup, 2003). Macro and micronutrients play essential roles in fundamental plant processes, such as sugar metabolism, nitrogen fixation, chlorophyll biosynthesis, photosynthesis, DNA synthesis, protein modification, and redox reactions. For instance, Zn is a cofactor for approx. 300 enzymes and 200 transcription factors related to the maintenance of membrane integrity, auxin metabolism, and reproduction (Ricachenevsky et al., 2013).

Nevertheless, at higher concentrations, heavy metals make plants show symptoms of toxicity. As such, their uptake is highly regulated by plant cells (Fidalgo et al., 2013). Some non-essential heavy metals, such as Cd, As, Cr, Pb, Al, and Hg, are toxic even at low concentrations and may inhibit the physiological metabolism of plants (Garzón et al., 2011; Chong-qing et al., 2013). Heavy metals have many detrimental effects directly impacting growth, photosynthesis, chlorosis, the plant water balance, nutrient absorption, senescence, and even triggering plant death.

In addition to their toxic effects on plants, heavy metals also pose a threat to human health due to their persistence in nature. For instance, Pb is a highly toxic heavy metal with a soil retention time of 150-5,000 years and is reported to maintain high concentrations for as long as 150 years (Yang et al., 2005). Plants growing in heavy metal-contaminated sites generally accumulate higher amounts of heavy metals and thus contamination of the food chain occurs. Contaminated food chains are a primary route for the entry of heavy metals into animal and human tissues, causing a number of diseases ranging from dermatitis to various cancers (McLaughlin et al., 1999). This problem can become even worse if sufficient measures are not taken at the right time. Therefore, research in this area is driven by the goal of decreasing the entry of heavy metals into crop plants, thereby reducing the risk of contamination of animals and human beings.

Due to this toxicity, heavy metal contamination in the environment remains a matter of great concern. Several studies reporting the potential of phytoremediation have highlighted the molecular and genetic basis for detoxification of heavy metals in genetically modified plants on heavy metal polluted sites (Yan et al. 2020). This chapter presents current knowledge on heavy metal metabolism, including their uptake, accumulation, and detoxification in plants.

## Plant responses to heavy metal stress

As plants are sessile in nature, they cannot move away from environmental stresses. Heavy metal exposure generates wide-ranging physiological and biochemical alterations and plants employ various strategies to survive heavy metal toxicity. Plants counter heavy metal stress through mechanisms that can be characterized as follows: (i) perceiving external stress stimuli; (ii) signal transduction; and (iii) eliciting suitable actions to combat the adverse effects of stress by controlling the physiological, biochemical, and molecular status of cells. It is challenging to identify variations in signal transduction after plants have been subjected to heavy metal stress at the level of the entire plant. However, studying early impacts, for instance oxidative stress, transcriptomic and proteomic alterations, and metabolite accumulation, can help us examine signal transduction changes when plants are exposed to stress. For example, overexpression of genes related to dehydration stress in barley after exposure to Cd and Hg was reported by Tamás et al. (2010). In the same way, during heavy metal exposure, oxidative stress and glutathione reduction can be used as early signals of sensing and signal transduction in alfalfa roots, as reported by Hernandez et al. (2012). Zhang et al. (2002) studied how seed germination and seedling growth in wheat was suppressed when exposed to high arsenic concentrations. Likewise, a decline in plumule and radicle length of *Helianthus annuus L.* plantlets after As exposure was reported by Imran et al. (2013). Furthermore, it has been found that arsenic causes a decline in the photosynthetic pigment, impairs the chloroplast membrane, and reduces enzyme activity by binding with the sulfhydryl group of proteins; it has also been reported that it modifies the nutrient balance and protein metabolism (Ahsan et al., 2010). It has been suggested that heavy metals cause toxicity in plants through four mechanisms. These consist of: (i) resemblance with nutrient cations, which creates competition for entry at the root surface; (ii) binding of heavy metals to the sulfhydryl group (SH), leading to the disruption of the structure and function of proteins, which renders them inactive; (iii) dislocation of vital cations from precise binding sites leading to reduced activity; and (iv) overproduction of reactive oxygen species (ROS), which subsequently impair important biomolecules (Sharma and Dietz, 2009). To examine the effect of this stressor, plant roots, the first point of contact with heavy metals, have been extensively used. Plants growing in heavy metal polluted areas show reduced growth, performance, and yield (Keunen et al., 2011; Anjum et al., 2014). Root development relies on cell division and elongation and inhibited root growth due to a reduction in the

mitotic process has been found in numerous plants upon heavy metal exposure (Thounaojam et al., 2012). A higher toxic effect on cell division during Cr (VI) stress in comparison to Cr (III) treatment was reported by Liu et al. (1992), while Sundaramoorthy et al. (2010) reported that Cr (VI) triggered a delay in the cell cycle that tended to decrease root growth due to the suppression of cell division. Pena et al. (2012) examined how Cd toxicity and ROS production alters the G1/S transition and progression through the S phase by inhibited cyclin dependent kinase (CDK) expression. It has been observed that higher concentrations of Cu influence both elongation and meristem zones by modifying auxin supply through the PINFORMED1 (PIN1) protein (Yuan et al., 2013). Likewise, Petö et al. (2011) confirmed that superfluous Cu hinders root length and modifies root structure by causing changes in auxin concentration, which disturbs nitric oxide function. The plant cytoskeleton is also a target of heavy metal stress, as shown by a reduction in root growth with an increase in root diameter (Zobel et al., 2007). As such, much research has proposed that heavy metals produce a reduction in root growth that disturbs water and nutrient uptake, thus disturbing their movement to the above-ground plant parts, adversely influencing shoot development, and lessening biomass accumulation. Roots apply numerous approaches to address these issues. These include the production and deposition of callose to reduce or avoid heavy metal toxicity by constraining the entry of heavy metals and intensifying the elasticity of the root framework. To improve plant tolerance to HM stress, roots also permit their uptake and transport to above ground plant parts for vacuole compartmentalization (Fahr et al., 2013). Plasma membranes function as an extremely restrictive entry check point for undesirable elements into cells. Kenderešová et al. (2012) examined the greater resistance of *Arabidopsis halleri* and *Arabidopsis arenosa* to metal toxicity in comparison to *Arabidopsis thaliana* on account of their lower membrane depolarization, signifying that fluctuations in the membrane's electric potential would be a good means of checking the effects of heavy metal toxicity. After entering the cell, heavy metals modify metabolic activities, inducing decreased growth and a decline in biomass cumulation (Nagajyoti et al., 2010). Tinted stem and root length, as well as chlorosis in younger leaves, may result due to metal stress and this can spread to mature leaves after extended exposure (Srivastava et al., 2012). Heavy metals are extremely toxic and affect the cellular and molecular mechanism of plants, causing modifications in their physiological and biochemical processes. They can negatively impact seed growth, photosynthesis, gas exchange, and respiration; denature enzymes; inactivate important molecules by blocking their functional groups; and

alter hormone proportions, nutrient absorption, and protein and DNA synthesis (Singh et al., 2013). Their toxic effects have been investigated in relation to several photosynthetic indicators, such as photosynthetic rate (Pn) and intracellular CO<sub>2</sub> concentration (Ci) in tomato plantlets under Cd exposure (Dong et al., 2005). Substantial decay in chlorophyll levels, followed by a decrease in the photochemical proficiency of photosystem II (PSII), was confirmed by Maleva et al. (2012) with Mn, Cu, Cd, Zn, and Ni stress in *Elodea Densa*. Li et al. (2012) investigated the reduced chlorophyll and carotenoids levels and the quantum yield of PSII in *Thalassia hemprichii* under Cu, Zn, Pb, and Cd exposure, suggesting that photosynthesis is highly affected by metal toxicity. In addition, heavy metals reduce CO<sub>2</sub> absorption by either lessening the activity of RUBP carboxylase, or by binding with the thiol group of RUBISCO. For example, Monnet et al. (2001) reported that Zn impeded the activity of RUBISCO in *Phaseolus vulgaris* by exchanging Zn<sup>+2</sup> for Mg<sup>+2</sup>. Decreased RUBISCO activity was also observed by Muthuchelian et al. (2001) during Cd stress in *Erythrina variegata*. These scientists also found diminished CO<sub>2</sub> fixation, which may be due to a reduction in ATP (Husaini and Rai, 1991), as Cd ions decrease the proton source for reduction reactions (Ferretti et al., 1993). Likewise, it was found that Cu decreases RUBISCO activity in *Chenopodium rubrum* (Schafer et al., 1992) by reacting with the essential cysteine residue of the enzyme (Siborova, 1988). This reduction in gas exchange, pigments, photosynthetic level, quantum yield of PSII, stomatal conductivity, and CO<sub>2</sub> absorption is possibly due to structural alterations prompted by metal toxicity. The impacts emerging as a result of such structural changes have been stated by numerous research studies (Sánchez-Pardo et al., 2012). Furthermore, heavy metals also exhibit destructive consequences on other physiological practices, such as nitrogen metabolism, thus interfering with plant growth. Increased protease activity has been reported to be a consequence of heavy metal toxicity (Chaffei et al., 2003), minimizing the function of enzymes associated with nitrate and ammonia assimilation. It has been shown that nitrogen assimilation is affected by Cd stress in terms of nitrate uptake and transportation, nitrate reductase, and GS activity inhibition (Lea and Mifflin, 2004). Heavy metal-facilitated modification of hormonal proportions is related to their cytotoxicity in plants (Wilkinson et al., 2012). As an example, remarkable alterations have been detected in auxin intensity—indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA)—and in the expression of about 69 microRNAs in *Brassica juncea* under As stress (Srivastava et al., 2013). Conversely, *B. juncea* growth was improved with additional delivery of

IAA during As exposure, suggesting an approach to counterbalance the impact of As stress by regulation of the hormone level.

## Heavy metal uptake and transport

Hyperaccumulator plants have a phenomenal capability to take up heavy metals from the soil (Yang et al., 2002). However, the uptake of heavy metals by hyperaccumulators is influenced by numerous factors, such as water content, pH, and organic constituents. Likewise, metals need an appropriate transportation mode to enter the plant. A number of investigators have studied how pH influences proton excretion by roots, which acidify the rhizosphere, hence improving metal disintegration, as well as the development of metal-accumulator plants (Kuriakose and Prasad, 2008). Organic materials discharged from the roots affect the growth of hyperaccumulator plants. Krishnamurti et al. (1997) found that Cd solubility was affected by organic acids released via Cd complex formation. Heavy metal mobilization and enhanced absorption results due to the release of pH and organic substances from the rhizosphere of the hyperaccumulating plant (Peng et al., 2005). Elevated heavy metal uptake has also been connected to boosted root propagation (Whiting et al., 2000) and has been attributed to the upregulation of certain genes. To pinpoint the genes involved in overexpression, numerous experiments have been undertaken using the hyperaccumulator species *Arabidopsis halleri* and *Thlaspi caerulescens* and congener non-hyperaccumulating species. Studies on *T. caerulescens* and *A. halleri* have revealed that increased Zn uptake is due to overexpression of genes belonging to the ZIP (zinc/iron-regulated transporter-like proteins) family encoding plasma membrane-located transporters (Assunção et al., 2001). These are ZTN1 and ZTN2 in *T. caerulescens* and ZIP6 and ZIP9 in *A. halleri*. The decreased uptake of Cd at increasing Zn concentrations has been noted in both genera. This clearly demonstrates that expression of the ZIP genes is Zn regulated (Assunção et al., 2010) and Cd influx is mainly due to Zn transporters having a strong preference for Zn over Cd (Weber et al., 2006). Evidence exists that As, being a chemical analog of phosphate, enters the plant cell via phosphate transporters (Kanoun-Boulé et al., 2009). Similarly, a study of the As hyperaccumulator *Pteris vittata* and non-hyperaccumulator *Pteris tremula* showed that the plasma membranes in root cells of *P. vittata* had a higher density of phosphate/arsenate transporters than *P. tremula* (Caille et al., 2005), which is possibly due to constitutive gene overexpression. In addition, a study into the Se hyperaccumulators *Astragalus bisulcatus* (Fabaceae) and *Stanleya pinnata* (Brassicaceae)

revealed that there was a higher Se/S ratio in the shoots of the Se hyperaccumulator species in comparison to the non-hyperaccumulator sister species. This observation is also supported by the fact that enhanced Se uptake used sulfate transporters (Galeas et al., 2007).

### **Root-to-shoot translocation of heavy metals**

In contrast to non-hyperaccumulator plants, hyperaccumulators do not retain heavy metals absorbed through the roots but translocate them from root to shoot efficiently via the xylem. There are various proteins that help in this translocation: heavy metal-transporting ATPases (CPx-type, P1B-type); cation diffusion facilitator (CDF) proteins; natural resistance-associated macrophage proteins (Nramp); MATE (multidrug and toxin efflux) proteins; and zinc–iron permease (ZIP) proteins. Different proteins have roles in the uptake of various heavy metals. The CPx-type ATPases are involved in transporting toxic metals like Cd, Cu, Zn, and Pb using ATP across cell membranes (Williams et al., 2000). The P1B-type ATPases have a comparable role transporting heavy metals, but they regulate metal homeostasis as well as tolerance (Axelsen and Palmgren, 1998). Studies have shown the upregulation of these heavy metal ATPases in hyperaccumulator plants in comparison to non-accumulator plants, suggesting their importance for hyperaccumulation (Papoyan and Kochian, 2004). Along with these HMAs, another class of proteins, Nramp, has also been shown to be involved in transporting heavy metal ions. In rice, three Nramps—OsNramp1, OsNramp2, and OsNramp3—have been found to be expressed in different tissues and transport distinct, but related ions (Belouchi et al., 1997). Cation diffusion facilitators (CDF) have been found to be involved in the transportation of Zn, Co, and Cd, as well as regulating the efflux of cations out from the cytoplasmic compartment (Mäser et al., 2001). ZNT1, a member of the ZIP family, has been found to be expressed at high levels in the roots and shoots of *T. caerulescens* (Pence et al., 2000). In the root tissues of Arabidopsis the another ZN transporter ZAT1 was found to be expressed at higher level. (Van der Zaal et al., 1999). Members of the MATE family has also been found to be involved in the transport of heavy metals: FDR3, a MATE protein, was found to be expressed in the roots tissues of *T. caerulescens* and *A. halleri* (Talke et al., 2006). The above mentioned studies all provide strong evidence that multiple transporter proteins are involved in the translocation of heavy metals.

## **Different strategies used by plants to detoxify heavy metals**

### **1. Heavy metals and signal transduction in plants**

To deal with heavy metal stress, different strategies are used by plants, including compartmentalization and metal export among others. When a plant senses increased heavy metal concentrations in its vicinity, a complicated signal transduction network is activated. Stress signaling molecules and stress-related proteins are synthesized. As a result, specific genes are transcriptionally activated that are specific to metal stress, hence the response to heavy metals is epigenetically controlled (Cicatelli et al., 2013). This mechanism requires the coordination of complex biochemical and physiological processes. Generally, the signaling pathway consists of a sequential strategy: perception of a heavy metal concentration; activation of signaling molecules like lipids; and the modulation of endocytic pathways (Galvan-Ampudia and Testerink, 2011). Along with plant developmental processes, stress responses are also mediated by other signaling molecules like jasmonates (lipid derived signals) and annexins, etc. (Jami et al., 2010). Different heavy metals induce different responses with different signaling molecule cascades, but a general signal transduction pathway involves the following: ROS; signaling pathway; calcium-calmodulin system; phosphorylation cascade; mitogen-activated protein (MAP) kinase; and certain hormones (DalCorso et al. 2010).

### **2. Phosphorylation cascades**

In many cellular processes, phosphorylation is a major event (Chen et al. 2013). Under stress, different proteins of the thylakoid membranes undergo phosphorylation or dephosphorylation in response to biotic or abiotic stresses (Tikkanen and Aro, 2012). In most cases, phosphorylation occurs on threonine and serine residues, but it can also occur on tyrosine residues resulting in many developmental and stress responses (Sasabe et al., 2011). Alteration of cytokinesis involves the activation of MAPKKK and proteins like mitotic kinesin. This activation is done by cyclin dependent kinases (Sasabe et al., 2011).

### **3. MAP kinase**

Downstream signaling events after sensing ROS in plant cells include calmodulin (the Ca-binding protein) and the activation of phospholipid signaling and G-proteins, ultimately leading to the accretion of



phosphatidic acid and/or activation of MAPK pathways (De Pinto et al. 2012). In this signaling mechanism, the pathway has three kinases that are activated sequentially: MAPK kinase kinase (MAPKK); MAPK kinase (MAPKK or MKK); and MAP kinases (MAPK or MPK) (Opdenakker et al., 2012). These kinases then phosphorylate different cellular compartments. This signaling pathway has been shown to be initiated as a result of different stresses (DalCorso et al., 2010). In reaction to a type of stress, e.g. cold in plants, the MAP kinase cascade is activated. In turn, this activates other signaling pathways, resulting in an adaptative response to the stress (Yang et al., 2010). Thanks to this pathway, as the concentration of stress causing agents increases, the plant's growth, cell division, and differentiation decrease (Smekalova et al., 2013). A study showed that MPK3 and MPK6 are activated in *A. thaliana* in response to short term exposure to CdCl<sub>2</sub> at low concentrations (1 μM), following the accumulation of ROS (Liu et al., 2010). In another study, a rice MAPK cascade called *Oryza sativa* MAPKK (OSMCK4 and OSMCK3) was analyzed. When exposed to arsenite, rice plants showed elevated transcription levels of OSMCK3 in leaves and roots after 30 minutes, while levels of OSMCK4 were raised after 3 hours (Rao et al., 2011). This cascade plays diverse roles in signaling and transferring information from sensors to responders and controlling processes like proliferation, differentiation, and death. Different biotic and abiotic stresses produce different responses; these stresses include temperature extremes, heavy metals, salinity, high/low osmolarity, and drought etc. (Pitzschke and Hirt, 2010).

#### 4. Calcium-calmodulin system

This system uses proteins as secondary messengers for plant responses to the presence of heavy metals. Under stress conditions, the concentration of calcium greatly increases in the cell, stimulating calmodulin proteins. This system regulates a variety of mechanisms including the regulation of certain genes, transport of ions across the membranes, metabolic processes, and tolerance of different metals (Dal Corso et al., 2010). This system is primarily activated in response to stress and has a significant role in cold tolerance and acclimatization in coordination with other signaling pathways (Yang et al., 2010). It has been found that some heavy metals, such as Cd and Cu, disturb intracellular levels of Ca, impairing the calmodulin signaling pathway, which regulates Cd tolerance mechanisms. It was found that transgenic tobacco plants overexpressing calmodulin were effectively able to resist Ni and Pb toxicity (Lindberg et al. 2012). A regulated member of this family, CRLK1 (calcium-calmodulin receptor

like kinase 1), enhances cold tolerance in plants even under freezing conditions (Yang et al., 2010a).

## 5. The role of hormones in heavy metal stress

During heavy metal signaling, particular hormones have a very important role in the regulation of plant development, growth, and reproduction, as well as key functions in the regulation of defense mechanisms against biotic and abiotic stresses (Pieterse et al., 2012). This regulation is in response to concentrations of different metals (DalCorso et al., 2010). Recently, plant hormones such as indole-3-acetic acid (IAA), also known as auxins, brassinosteroids (BR), and abscisic acid (ABA), have been found to play a vital role in stress management (Vázquez et al., 2013). Auxins seem to play an important role in stress regulation as shown by exogenous ABA inducing overproduction of phytochelatins (PC) under Cd, Zn, and Cu stress in *Prosopis juliflora* (Usha et al., 2009). Steroid hormones, also known as brassinosteroids (BR), are critical to developmental and detoxification processes—they promote the formation of PCs, which chelate heavy metal ions accumulated in plant cells (Choudhary et al., 2010). In another study, the effects of exogenous application of BRs on raddish and mustard plants under copper stress were studied. It was found that BR promoted shoot and root growth by overcoming copper toxicity—BRs were found to be responsible for antioxidant activity and increased PC formation (Choudhary et al., 2011a). When combined, secondary messengers, like hormones and MAPK, ensure effective transcription and signaling under stress conditions (Smekalova et al., 2013). Ethylene is a hormone normally produced in response to age-related stresses (Khan et al., 2014). Jasmonic acid and salicylic acid are also produced. The hormone signaling pathway has positive and negative regulators that are crucial to hormonal crosstalk in stress and defense mechanisms. Auxins, cytokinins, ABA, and brassinosteroids, etc. adapt to changes and outcomes in antagonistic and synergistic connections that play important roles in abiotic stress tolerances (Ha et al., 2012). In a recent study, various concentrations of EBL (24-epibrassinolide), a brassinosteroid obtained from *Brassica juncea*, were applied to seeds of the same species 8 hours prior to sowing. These seeds were then exposed to Ni stress and showed improved growth with lower uptake of Ni ions (Kanwar et al., 2013). The use of an EBL foliar spray also improved the antioxidant system by promoting the formation of catalase, superoxide dismutase, proline, and peroxidase in bean plants with Cd toxicity (Rady, 2011). Choudhary et al. (2011b) studied the influence of EBL on other hormones and found that, under Cr stress, EBL increased the formation of IAA to promote seedling

growth in raddish plants. It was also seen to enhance the production of ABA, increasing Cr tolerance (Choudhary et al., 2011b).

## **6. Formation of metal complexes**

Another important strategy for heavy metal detoxification is heavy metal chelation by high affinity ligands. In plants, two types of metal binding peptides are produced—phytochelatins (PC) and metallothioneins (MT). Besides these, many other small molecules are also used in metal chelation inside the cytosol.

## **7. Phytochelatins**

Phytochelatins (PC) are a family of polypeptides that are rich in cysteine. PCs are synthesized from GSH (glutathione) and are found in plants and fungi. The enzyme, PC synthase, is activated when metal ions bind to it, converting GSH to PC. Heavy metals ions, such as Hg, Cu, Ni, Au, Zn, and Cd, induce the biosynthesis of PCs; Cd is known to be the strongest PC inducer. The PC-Cd complex is formed by the attachment of Cd to PC through the thiolic group (-SH) of the cysteine residue. These complexes are sequestered into the vacuole by ABC proteins, as mentioned above. Plants are unable to metabolize or neutralize Cd. Rather, they limit Cd circulation in the cytosol or transport it away through the xylem and phloem. PCs also contribute to homeostasis of Cu and Zn by providing transitory storage for the ions. It has been found that both heavy metal resistant and heavy metal susceptible plants produce PCs. The process of detoxification is not limited to metal chelation: after the chelated metal complex is transported into the vacuole, it is stabilized there by the formation of complexes with organic acids or sulfides. As part of this complex mechanism, PCs also transport metal ions.

## **8. Metallothioneins**

Like PCs, metallothioneins (MT) are a major family of cysteine rich, metal binding low molecular weight peptides found in many organisms. Although the structure of plant MTs is different to those found in other organisms, they interact with heavy metals through the thiolic group of their cysteine residues. It has been suggested that they are involved in homeostasis and the sequestering of important heavy metal ions (Coldsbrough, 2010). They also provide protection against intracellular oxidative damage. Based on the arrangement of cysteine residues, MTs can be divided into three classes. Different isoforms of MTs exist and their capability to bind and confiscate heavy metals also varies. Hormones,

cytotoxic agents, and heavy metal ions, such as Cu, Au, Zn, Hg, Ni, Co, and Cd, are MT inducers. A study showed three *B. rapa* MT genes (BrMT1-BrMT3) to be differentially regulated in various heavy metal stresses and the expression of all 3 genes was variable when the seedlings were treated with Fe. Upon Cu exposure, BrMT1 expression was increased compared to BrMT2, whereas BrMT3 expression remained unchanged (Ahn et al., 2012). The tonoplast ABC transporter protein was also up-regulated in transgenic OsMT1 plants, which sequestered Cd metal ions inside the vacuole, thus assisting Cd detoxification (Yang and Chu, 2011). Although animal and fungal MTs have a clear role in heavy metal detoxification, the precise relationship between plant MTs and heavy metals remains unclear.

### **9. Amino acids, organic acids, and phosphate derivatives**

Organic acids, including malate, citrate, and oxalate, have the ability to bind metals and are therefore deployed in heavy metal tolerance mechanisms. Organic acids confer metal tolerance in two ways—external exclusion and internal tolerance. In the former, organic acids are secreted from plant roots and make a stable metal-ligand complex with metal, thus hindering metal ions from entering and accumulating in sensitive sites in the roots (Sharma and Dietz, 2009). Citrate is synthesized in plants by the citrate synthase enzyme, which has higher affinity for metals compared to malate and oxalate. It has been found that citrate plays a principal role in Fe chelation, although other heavy metal ions, like Zn, Ni, Cd, and Co, also have high affinity for citrate. The amount of citrate produced depends greatly on Ni exposure (Hassan and Aarts, 2011).

Amino acids and derivatives chelate metals thus conferring plant resistance against toxic metals. Histidine is considered the most important free amino acid in heavy metal metabolism. The presence of putative carboxyl, amino, and imidazole groups makes histidine a versatile metal chelator (Krämer, 2010). It has been found that histidine plays a major role in tolerance to nickel. In Ni hyperaccumulator species *Alyssum* and *N. goesingense*, the concentration of histidine in the xylem exudate is higher compared to closely related nonaccumulator species (McNear et al., 2010). Nicotianamine (NA) is a non-proteogenic and low molecular weight amino acid that is found in root and leaf cells and also in the phloem (Hassan and Aarts, 2011). It is synthesized as a result of a condensation reaction of three S-adenosyl-Lmethionine molecules catalyzed by NA synthase (Talke et al., 2006). NA chelates Cu, Fe, and Zn by complex formation, before storing them in the vacuole. In *A. thaliana* and other

plants, NA is involved in the influx and efflux of Cu, Zn, and Fe by transporting metals from one cell to another (Klatte et al., 2009). The membrane YSL transporter family transports the metal-NA complexes; such complexes are substrates for these transporters (Gendre et al., 2007). Phytate is a principal form of stored phosphorus in plant cells. The molecule consists of six phosphate groups, which allow the chelation of various cations, including Ca, Mg, Fe, Mn, and K (Kumar et al., 2010).

## **10. Oxidative stress defense and damaged proteins repair mechanisms**

If the intracellular concentration of metal ions overwhelms the plant's defense mechanisms and strategies, it begins to suffer oxidative stress due to the inhibition of metal dependent antioxidant enzyme and the production of methylglyoxal (MG) and reactive oxygen species (ROS) (Hossain and Fujita, 2010). This includes the induction of enzymes such as catalase (CAT) and superoxide dismutase (SOD), and the production of non-enzymatic free radical scavengers. The literature shows many cases of such induction. For example, APX and CAT production were found to be induced in response to excess Fe exposure in the leaves of *Nicotiana plumbaginifolia*. Similarly, CAT3 was induced in *B. juncea* plants in response to Cd exposure (Minglin et al., 2005). Cd has been shown to cause oxidation of CAT in pea plants, reducing plant activity. The plant responds by upregulating the transcription of the CAT gene (Romero-Puertas et al., 2007). SOD activity was shown to increase in response to prolonged activity of metals and wheat leaves were shown to respond to excess Cd by increasing SOD levels (Lin et al., 2007).

## **11. Glutathione (GSH)**

Production of ROS also encourages the activation of the ascorbic acid glutathione scavenging system. GSH is a low molecular weight, non-enzymatic antioxidant; it is a major redox buffer and antioxidant found abundantly in all plant cell compartments (Yadav, 2010). GSH plays a role in the control of H<sub>2</sub>O<sub>2</sub> levels and up-regulation of GSH is of crucial importance because it induces defensive strategies against ROS and MG through various pathways, including the activation and expression of enzymes associated with GSH and argininosuccinate (AsA) (Hossain et al., 2012). GSH plays a key role in responding to various environmental stresses, metal tolerance, and metal chelation because it acts as an ROS scavenger and a substrate for PC biosynthesis (Krämer, 2010). GSH shields proteins against denaturation triggered by oxidation under stress conditions. It is also indirectly involved in protecting membranes by

maintaining a reduced state of zeaxanthin and  $\alpha$ -tocopherol (Hossain and Fujita, 2011). GSH (Glu-Cys-Gly) is the major intracellular antioxidant inside cells and is a precursor to PCs; it also forms complexes with heavy metals, such as Cd (Wójcik and Tukiendorf, 2011). Nevertheless, the role of GSH as an antioxidant depends on its intracellular concentration and varies substantially under Cd toxicity. It has been observed in relation to *B. juncea* and *B. campestris* that an increase in Cd concentration increases the GSH concentration in response (Anjum et al., 2008). In *Phaseolus vulgaris* and *Pisum sativum*, Cd treatment induces ascorbate peroxidase (APX) (Romero-Puertas et al., 2007). An elevation in GSH concentration in the leaves, roots, and stems under various metal stresses (Hg, Cd, and Pb) was reported by Huang et al. (2010). In *Holcus lanatus*, an As tolerant species, GSH levels increased significantly, induced by As, compared to species that were As-sensitive (Verbruggen et al., 2009). Recent studies have demonstrated that nitric oxide (NO) influences GSH synthesis, as revealed in a study with *Medicago truncatula* in which increased NO in the roots increased GSH and glutathione (GS) gene expression (Xu et al., 2011). S-nitrosoglutathione (GSNO) is formed during the interaction of GSH with NO, possibly interconnecting the reactive nitrogen and ROS based signaling pathways (Xiong et al., 2010).

## 12. Glutathione S-transferases (GST)

GSTs belong to a superfamily of multifunctional phase II metabolic isoenzymes, which are best known for their ability to detoxify xenobiotics. GSTs catalyze the conjugation of a reduced form of GSH with various compounds to form derivatives that can be sequestered in the vacuole or secreted from the cell. In addition, they also defend against oxidants and abiotic induced oxidative stress (Hossain et al., 2012b). Increased GST activity was seen in barley (*Hordeum vulgare*) when subjected to Cu, Hg, Co, Cd, Pb, and Zn (Valentovičová et al., 2009). Another study on rice seedlings showed increased activity of GST in response to Cd stress (50  $\mu$ M Cd, 7 days) (Hu et al., 2009). GST activity was shown to increase substantially in the callus of onions in response to Cd stress (1 mM CdCl<sub>2</sub>) (Rohman et al., 2010). Time and dose affects GST levels in plants and thus it is likely that, in the case of severe toxicity and when basal antioxidant mechanisms are used up and depleted, more effective responses like GST are activated, (Hossain et al., 2010). A study by Dixit et al. (2011) observed that tobacco plants with upregulated GST gene expression show less lipid peroxidation due to reduced Cd accumulation than wild type plants, indicating better Cd tolerance.

### 13. Glutathione peroxidases (GPX)

GPXs belong to family of enzymes with peroxidase activity. They are an important part of the antioxidant network of plants and found in different cellular organelles. Their primary activity is the catalyzation of the reduction process of free  $\text{H}_2\text{O}_2$  to water, ROOHs (organic hydroperoxidases), and lipid hydroperoxidases to alcohol in the presence of GSH and other reducing agents (Foyer and Noctor, 2011). Mammalian glutathione peroxidases possess higher affinity to lipid peroxidases than  $\text{H}_2\text{O}_2$  and are homologous to most of the plant's GPX genes. *A. thaliana* exposed to Cd stress (11 and 10  $\mu\text{M}$  for 7 days) showed an increase in GPX activity, indicating protection by GPX against lipid peroxidases (Semane et al., 2007). Additionally, it was found that Cu, Hg, and Ni induced increased GPX activity, whereas Co, Zn, and Pb exposure had no substantial effect on activity (Valentovičová et al., 2009). It was reported that GPX activity is induced under As stress in other plants (Gupta et al., 2009).

### 14. Dehydroascorbate reductase (DHAR)

DHAR is an important enzyme required in the AsA-GSH (ascorbate-glutathione) reaction in higher plants. Ascorbic acid is oxidized through spontaneous disproportion to form dehydroascorbic acid (DHA). DHAR then reduces DHA to AsA using GSH (Yang et al., 2009). It was observed that the enzyme activity of DHA reductase increased in two barley genotypes when exposed to Cd stress for 1-25 days (Chen et al., 2010). In the same way, DHA reductase activity was amplified in wheat leaves and roots when exposed to different concentrations of Cd (Paradiso et al., 2008). Under Ni stress, its activity was shown to increase in rice roots and shoots, showing that Ni maintains elevated levels of AsA by activating the AsA regenerating system (Maheshwari and Dubey, 2009). An increase in DHAR activity was seen in two tomato cultivars, "Josefina" and "Kosaco", when subjected to boron stress, in addition to a reduction in AsA content, indicating that enzyme activity increases to reduce oxidative stress (Cervilla et al., 2007). When exposed to Cd stress transgenic, *A. thaliana* plants, in which genes for CAT and GST were overexpressed, saw a considerable increase in DHA reductase activity was seen; however, it was reduced in non-transgenic plants (Zhao et al., 2009).

### 15. Glutathione reductase (GR)

GR belongs to an enzyme family that catalyzes the reduction of GSSG to GSG using NADPH. Heavy metals, in particular Cd, reduce the GSH/GSSG

ratio and activate antioxidant enzymes, such as GR and SOD. The cysthiol group of reduced glutathione (GSH) is oxidized and glutathione reductase (GR) catalyzes the reverse reaction using NADPH, thus acting as a defense mechanism against Cd-generated oxidative stress (Yannarelli et al., 2007). GR helps cells to resist toxicity caused by RO metabolites. It maintains the reduced form of GSH and ascorbate in cells, which sequentially retain the cellular redox state of heavy metal stress (Hossain et al., 2011). In their study, Nouairi et al. (2009) reported significantly increased glutathione reductase activity in *B. napus* at lower concentrations of Cd ions, which dropped when concentrations were raised after 15 days of treatment. In *B. juncea* leaves, GR enzyme activity levels were unaffected by variable concentrations of Cd ions. In a study on two cultivars of mung beans, GR activity was increased in both Cd tolerant and Cd sensitive genotypes in response to Cd stress (Anjum et al., 2011). However, in another study it was observed that GR activity decreases considerably in response to Cd stress on mung bean seedlings (Hossain et al., 2010), indicating that GR activity is greatly influenced by genotypic differences. Another study reported that GR enzymatic activity in *B. napus* roots decreased, while in leaves the glutathione reductase buildup was comparatively high when exposed to Cu (Russo et al., 2008). Upregulation of GR contributes to the maintenance of the GSH/GSSG ratio and higher GSH levels under heavy metal stress, which are, in turn, used by many enzymes that depend on GSH involved in ROS and MG metabolism (Hossain et al., 2012).

## 16. Heat shock proteins (HSP)

Heat shock proteins are signaling molecules released in response to metal induced and other forms of abiotic stress. HSPs are found in all types of cell and are not just expressed in response to elevated temperatures, but in response to other stresses as well (Dubey, 2011). They protect and repair proteins and act as molecular chaperons to ensure correct folding. The induction of HSPs by several heavy metal ions (Al, Cu, Hg, Cd, and Zn) has been reported (Dubey, 2011).

## Conclusion

With the growth in the human population, different human activities have resulted in the increase of toxic compounds in soil that are not naturally present. Heavy metal pollution brings various health problems and risks and can result in accumulation of heavy metals in the food chain of both plants and animals. Plants have developed certain defense mechanisms to



shield themselves and respond to stimuli to minimize the risks. Antioxidant systems, both enzymatic and nonenzymatic, symptoms, and tolerance mechanisms vary from plant to plant. This chapter has explained the different mechanisms used by plants to defend themselves against various toxic heavy metals.

## References

- Ahn, Young Ock, Sun Ha Kim, Jeongyeo Lee, HyeRan Kim, Haeng-Soon Lee, and Sang-Soo Kwak. 2012. "Three *Brassica rapa* metallothionein genes are differentially regulated under various stress conditions". *Molecular biology reports* 39, No. 3: 2059-2067.
- Ahsan, Nagib, Dong-Gi Lee, Kyung-Hee Kim, Iftexhar Alam, Sang-Hoon Lee, Ki-Won Lee, Hyoshin Lee, and Byung-Hyun Lee. 2010. "Analysis of arsenic stress-induced differentially expressed proteins in rice leaves by two-dimensional gel electrophoresis coupled with mass spectrometry". *Chemosphere* 78, No. 3: 224-231.
- Anjum, Naser, A, Sarvajeet, S, Gill, Ritu Gill, Mirza Hasanuzzaman, Armando C Duarte, Eduarda Pereira, Iqbal Ahmad, Renu Tuteja, and Narendra Tuteja. 2014. "Metal/metalloid stress tolerance in plants: role of ascorbate, its redox couple, and associated enzymes". *Protoplasma*, 251, No. 6: 1265-1283.
- Anjum, Naser Aziz, Shahid Umar, Altaf Ahmad, Muhammad Iqbal, and Nafees Ahmad Khan. 2011. "Cadmium causes oxidative stress in moongbean [*Vigna radiata* (L.) Wilczek] by affecting antioxidant enzyme systems and ascorbate-glutathione cycle metabolism". *Russ. J. Plant Physiol.*, 58: 92-99.
- Anjum, Naser Aziz, Shahid Umar, Altaf Ahmad, Muhammad Iqbal, and Nafees Ahmad Khan. 2008. "Ontogenic variation in response of *Brassica campestris* L. to cadmium toxicity". *J. Plant Interact.*, No. 3: 189-198.
- Assunção, AG, Da Costa Martins, P, De Folter, S, Vooijs, R, Schat, H, and Aarts, MGM. 2001. "Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*". *Plant Cell Environ.*, 24, No. 2: 217-226.
- Assunção, Ana GL, Eva Herrero, Ya-Fen Lin, Bruno Huettel, Sangita Talukdar, Cezary Smaczniak, Richard GH Immink et al. 2010. "*Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency". *Proceedings of the National Academy of Sciences*, 107, No. 22: 10296-10301.

- Axelsen, Kristian B, and Michael G Palmgren. 1998. "Evolution of substrate specificities in the P-type ATPase superfamily". *Journal of molecular evolution*, 46, No. 1: 84-101.
- Belouchi, Abdelmajid, Tony Kwan, and Philippe Gros. 1997. "Cloning and characterization of the OsNramp family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions". *Plant Mol. Biol.* 33, No. 6: 1085-1092.
- Caille, N, Zhao, FJ, and McGrath, SP. 2005. *New Phytol.* 165: 755-761.
- Chen, Fei, Fang Wang, Feibo Wu, Weihua Mao, Guoping Zhang, and Meixue Zhou. 2010. "Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance". *Plant Phys. Biochem.* 48, No. 8: 663-672.
- Chen, Yang-Er, Zhong-Yi Zhao, Huai-Yu Zhang, Xian-Yin Zeng, and Shu Yuan. 2013. "The significance of CP29 reversible phosphorylation in thylakoids of higher plants under environmental stresses". *J. Exp. Bot.* 64, No. 5: 1167-1178.
- Chiraz, Chaffei, Gouia Houda, and Ghorbel Mohamed Habib. 2003. "Nitrogen metabolism in tomato plants under cadmium stress". *J. Plant Nutr.* 26, No. 8: 1617-1634.
- Choudhary, Sikander Pal, Mukesh Kanwar, Renu Bhardwaj, BD Gupta, and RK Gupta. 2011b. "Epibrassinolide ameliorates Cr (VI) stress via influencing the levels of indole-3-acetic acid, abscisic acid, polyamines and antioxidant system of radish seedlings". *Chemosphere* 84, No. 5: 592-600.
- Choudhary, Sikander Pal, Renu Bhardwaj, BD Gupta, Prabhu Dutt, RK Gupta, Mukesh Kanwar, and Stefania Biondi. 2011a. "Enhancing effects of 24-epibrassinolide and Putrescine on the antioxidant capacity and free radical scavenging activity of *Raphanus sativus* seedlings under Cu ion stress". *Acta Phys Plant* 33, No. 4: 1319-1333.
- Choudhary, Sikander Pal, Renu Bhardwaj, Bishan Datt Gupta, Prabhu Dutt, Rajinder Kumar Gupta, Stefania Biondi, and Mukesh Kanwar. 2010. "Epibrassinolide induces changes in indole-3-acetic acid, abscisic acid and polyamine concentrations and enhances antioxidant potential of radish seedlings under copper stress". *Phys. Plant.* 140, No. 3: 280-296.
- Cicatelli, Angela, Valeria Todeschini, Guido Lingua, Stefania Biondi, Patrizia Torrigiani, and Stefano Castiglione. 2013. "Epigenetic control of heavy metal stress response in mycorrhizal versus non-mycorrhizal poplar plants". *Environmental Science and Pollution Research* 21, No. 3: 1723-1737.

- DalCorso, Giovanni, Silvia Farinati, and Antonella Furini. 2010. "Regulatory networks of cadmium stress in plants". *Plant Signaling & Behavior* 5, No. 6: 663-667.
- De Pinto, MC, V Locato, and L De Gara. 2012. "Redox regulation in plant programmed cell death". *Plant, cell & environment* 35, No. 2: 234-244.
- Dubey R. 2011. "Metal toxicity, oxidative stress and antioxidative defense system in plants". In Gupta SD (ed.) *Reactive Oxygen Species and Antioxidants in Higher Plants*. CRC Press, Boca Raton, 177-203.
- Eapen, Susan, and SF D'souza. 2005. "Prospects of genetic engineering of plants for phytoremediation of toxic metal". *Biotechnology advances* 23, No. 2: 97-114.
- Fahr, Mouna, Laurent Laplaze, Najib Bendaou, Valerie Hocher, Mohamed El Mzibri, Didier Bogusz, and Abdelaziz Smouni. 2013. "Effect of lead on root growth". *Front. Plant Sci.* 4: 175.
- Ferretti, M, R Ghisi, L Merlo, F Dalla Vecchia, and C Passera. 1993. "Effect of cadmium on photosynthesis and enzymes of photosynthetic sulfate and nitrate assimilation pathways in maize (*Zea mays* L.)". *Photosynthetica* 29: 49-54.
- Fidalgo, Fernanda, Manuel Azenha, António F Silva, Alexandra de Sousa, Ana Santiago, Pedro Ferraz, and Jorge Teixeira. 2013. "Copper-induced stress in *Solanum nigrum* L. and antioxidant defense system responses". *Food and Energy Security* 2, No. 1: 70-80.
- Foyer, Christine, H, and Graham Noctor. 2011. "Ascorbate and glutathione: the heart of the redox hub". *Plant physiology* 155, No. 1: 2-18.
- Galeas, Miriam, L, Li Hong Zhang, John, L, Freeman, Mellissa Wegner, and Elizabeth AH Pilon-Smits. 2007. "Seasonal fluctuations of selenium and sulphur accumulation in selenium hyperaccumulators and related nonaccumulators". *New Phytologist* 173, No. 3: 517-525.
- Galvan-Ampudia, Carlos S., and Christa Testerink C. 2011. "Salt stress signals shape the plant root". *Current Opinion in Plant Biology* 14, No. 3: 296-302.
- Garzón Garzón, Teresa, Benet Gunsé, Ana Rodrigo Moreno, A. Deri Tomos, Juan Barceló, and Charlotte Poschenrieder. 2011. "Aluminium-induced alteration of ion homeostasis in root tip vacuoles of two maize varieties differing in Al tolerance". *Plant Science* 180, No. 5: 709-715.
- Gendre, Delphine, Pierre Czernic, Geneviève Conéjéro, Katia Pianelli, Jean-François Briat, Michel Lebrun, and Stéphane Mari. 2007. "TcYSL3, a member of the YSL gene family from the hyper-

- accumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter". *The Plant Journal* 49, No. 1: 1-15.
- Goldsbrough, Peter. 2010. "12 Metal tolerance in plants: The role of phytochelatin and methallothioneins". *Phytorem. Contam. Soil Water*, 221.
- Gupta, Meetu, Pallavi Sharma, Neera Bhalla Sarin, and Alok Krishna Sinha. 2009. "Differential response of arsenic stress in two varieties of *Brassica juncea* L.". *Chemosphere* 74, No. 9: 1201-1208.
- Ha, Sukbong, Radomira Vankova, Kazuko Yamaguchi-Shinozaki, Kazuo Shinozaki, and Lam-Son Phan Tran. 2012. "Cytokinins: metabolism and function in plant adaptation to environmental stresses". *Trends in Plant Science* 17, No. 3: 172-179.
- Halušková, L'ubica, Katarína Valentovičová, Jana Huttová, Igor Mistrík, and Ladislav Tamás L. 2009. "Effect of abiotic stresses on glutathione peroxidase and glutathione S-transferase activity in barley root tips". *Plant Physiology and Biochemistry* 47, No. 11-12: 1069-1074.
- Hassan, Zeshan, and Mark GM Aarts. 2011. "Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants". *Environmental and Experimental Botany* 72, No. 1: 53-63.
- Hernández, Luis, E, Cristina Ortega-Villasante, M Belén Montero-Palmero, Carolina Escobar, and Ramón O Carpena. 2012. "Heavy metal perception in a microscale environment: a model system using high doses of pollutants". In *Metal Toxicity in Plants: Perception, Signaling and Remediation*, 23-39.
- Hossain, MA, and M Fujita. 2011. "Regulatory role of components of ascorbate-glutathione (AsA-GSH) pathway in plant tolerance to oxidative stress". In Anjum NA, Umar S, Ahmad A (eds.), *Oxidative Stress in Plants: Causes, Consequences and Tolerance*, 81-147.
- Hossain, Mohammad Anwar, Mirza Hasanuzzaman, and Masayuki Fujita. 2010. "Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress". *Molecular Biology of Plants* 16, No. 3: 259-272.
- Hossain, Mohammad Anwar, Pukclai Piyatida, Jaime A. Teixeira da Silva, and Masayuki Fujita. 2012. "Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation". *Journal of Botany*.
- Huang, Guo-Yong, You-Shao Wang, Cui-Ci Sun, Jun-De Dong, and Zong-Xun Sun. 2010. "The effect of multiple heavy metals on ascorbate, glutathione and related enzymes in two mangrove plant

- seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Ocean Hydrobiol. Studies* 39, No. 1: 11-25.
- Hussain, Y, and RC Rai. 1991. "Studies on nitrogen and phosphorus metabolism and the photosynthetic electron transport system of *Nostoc linckia* under cadmium stress". *J. Plant Physiol.* 138: 429-435.
- Imran, Muhammad Asif, Rass Masood Khan, Zulfiqar Ali, and Tariq Mahmood. 2013. "Toxicity of arsenic (As) on seed germination of sunflower (*Helianthus annuus* L.)". *International Journal of Physical Sciences* 8, No. 17: 840-847.
- Jami, Sravan Kumar, Robert D Hill, and PB Kirti. 2010. "Transcriptional regulation of annexins in Indian Mustard, *Brassica juncea* and detoxification of ROS in transgenic tobacco plants constitutively expressing AnnBj1". *Plant Signaling & Behavior* 5, No. 5: 618-621.
- Järup, Lars. 2003. "Hazards of heavy metal contamination". *British Medical Bulletin* 68, No. 1: 167-182.
- Jing, Dong, Wu, Feibo, and Zhang Guoping. 2005. "Effect of cadmium on growth and photosynthesis of tomato seedlings". *J. of Zhejiang University Science B* 6, No. 10: 974-980.
- Kanoun-Boulé, Myriam, Joaquim AF Vicente, Cristina Nabais, MNV Prasad, and Helena Freitas. 2009. "Ecophysiological tolerance of duckweeds exposed to copper". *Aquatic Toxicology* 91, No. 1: 1-9.
- Kanwar, Mukesh Kumar, Renu Bhardwaj, Sikandar Pal Chowdhary, Priya Arora, Priyanka Sharma, and Subodh Kumar. 2013. "Isolation and characterization of 24-Epibrassinolide from *Brassica juncea* L. and its effects on growth, Ni ion uptake, antioxidant defense of Brassica plants and in vitro cytotoxicity". *Acta Physiologiae Plantarum* 35, No. 4: 1351-1362.
- Kavamura, Vanessa Nessner, and Elisa Esposito. 2010. "Biotechnological strategies applied to the decontamination of soils polluted with heavy metal". *Biotechnology Advances* 28, no. 1: 61-69.
- Kenderešová, Lucia, Andrea Staňová, Ján Pavlovkin, Eva Ďurišová, Miriam Nadubinská, Milada Čiamporová, and Miroslav Ovečka. 2012. "Early Zn<sup>2+</sup>-induced effects on membrane potential account for primary heavy metal susceptibility in tolerant and sensitive *Arabidopsis* species". *Annals of Botany* 110, No. 2: 445-459.
- Keunen, Els, Tony Remans, Sacha Bohler, Jaco Vangronsveld, and Ann Cuypers. 2011. "Metal-induced oxidative stress and plant mitochondria". *International Journal of Molecular Sciences* 12, No. 10: 6894-6918.

- Khan, Mamoon, Wilfried Rozhon, and Brigitte Poppenberger. 2014. "The role of hormones in the aging of plants: a mini-review". *Gerontology* 60, No. 1: 49-55.
- Klatte, Marco, Mara Schuler, Markus Wirtz, Claudia Fink-Straube, Rüdiger Hell, and Petra Bauer. 2009. "The analysis of Arabidopsis nicotianamine synthase mutants reveals functions for nicotianamine in seed iron loading and iron deficiency responses". *Plant Physiology* 150, No. 1: 257-271.
- Krämer, Ute. 2010. "Metal hyperaccumulation in plants". *Annual Review of Plant Biology* 61: 517-534.
- Krishnamurti, GSR, G Cieslinski, PM Huang, and KCJ Van Rees. 199. "Kinetics of cadmium release from soils as influenced by organic acids: implication in cadmium availability". *Journal of Environmental Quality* 26, No. 1: 271-277.
- Kumar, Vikas, Amit K Sinha, Harinder PS Makkar, and Klaus Becker. 2010. "Dietary roles of phytate and phytase in human nutrition: a review". *Food Chemistry* 120, No. 4: 945-959.
- Kuriakose, Saritha V, and MNV Prasad. 2008. "Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes". *Plant growth regulation* 54, No. 2:143-156.
- Lea, Peter J, and Ben J Mifflin. 2004. "Glutamate synthase and the synthesis of glutamate in plants". *Plant Physiology and Biochemistry* 41, No. 6-7: 555-564.
- Li, Lei, Xiaoping Huang, Devajit Borthakur, and Hui Ni. 2012. "Photosynthetic activity and antioxidative response of seagrass *Thalassia hemprichii* to trace metal stress". *Acta Oceanologica Sinica* 31, No. 3: 98-108.
- Lin, Fanyun, Jianhong Xu, Jianrong Shi, Hongwei Li, and Bin Li. 2010. "Molecular cloning and characterization of a novel glyoxalase I gene TaGly I in wheat (*Triticum aestivum* L.)". *Molecular Biology Reports* 37, No. 2: 729-735.
- Lindberg, Sylvia, Md Abdul Kader, and Vladislav Yemelyanov. 2012. "Calcium signalling in plant cells under environmental stress". In *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*, 325-360. Springer, New York, NY.
- Liu, Donghua, Wusheng Jiang, and Maoxie Li. 1992. "Effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa*". *Hereditas* 117, No. 1: 23-29.
- Liu, Xiao-Min, Kyung Eun Kim, Kang-Chang Kim, Xuan Canh Nguyen, Hay Ju Han, Mi Soon Jung, Ho Soo Kim et al. 2010. "Cadmium

- activates Arabidopsis MPK3 and MPK6 via accumulation of reactive oxygen species". *Phytochemistry* 71, No. 5-6: 614-618.
- Maheshwari, Ruchi, and RS Dubey. 2009. "Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings". *Plant Growth Regulation* 59, No. 1: 37-49.
- Maleva, MG, GF Nekrasova, GG Borisova, NV Chukina, and OS Ushakova. 2012. "Effect of heavy metal on photosynthetic apparatus and antioxidant status of elodea". *Russian Journal of Plant Physiology* 59, No. 2: 190-197.
- Mäser, Pascal, Sébastien Thomine, Julian I Schroeder, John M Ward, Kendal Hirschi, Heven Sze, Ina N Talke et al. 2001. "Phylogenetic relationships within cation transporter families of Arabidopsis". *Plant Physiology* 126, No. 4: 1646-1667.
- McLaughlin, MJ, Parker, DR, and Clark, JM. 1999. "Metal and micronutrients-food safety issues". *Field Crops Research* 60, No. 1-2: 143-163.
- McNear Jr, David H, Rufus L Chaney, and Donald L Sparks. 2010. "The hyperaccumulator *Alyssum murale* uses complexation with nitrogen and oxygen donor ligands for Ni transport and storage". *Phytochemistry* 71, No. 2-3: 188-200.
- Minglin, Lang, Zhang Yuxiu, and Chai Tuanyao. 2005. "Identification of genes upregulated in response to Cd exposure in *Brassica juncea* L.". *Gene*. 363: 151-158.
- Miransari, Mohammad. 2011. "Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metal". *Biotechnology Advances* 29, No. 6: 645-653.
- Monnet, Fabien, Nathalie Vaillant, Philippe Vernay, Alain Coudret, Huguette Sallanon, and Adnane Hitmi. 2001. "Relationship between PSII activity, CO<sub>2</sub> fixation, and Zn, Mn and Mg contents of *Lolium perenne* under zinc stress". *Journal of Plant Physiology* 158, No. 9: 1137-1144.
- Muthuchelian, Krishnasamy, Massimo Bertamini, and Namachevayam Nedunchezian. 2001. "Triacontanol can protect *Erythrina variegata* from cadmium toxicity". *Journal of Plant Physiology* 158, No. 11: 1487-1490.
- Nagajyoti, PC, KD Lee, and TVM Sreekanth. 2010. "Heavy metal, occurrence and toxicity for plants: a review". *Environmental Chemistry Letters* 8, No. 3: 199-216.
- Nouairi, Issam, Wided Ben Ammar, Nabil Ben Youssef, Douja Daoud Ben Miled, Mohamed Habib Ghorbal, and Mokhtar Zarrouk. 2009. "Antioxidant defense system in leaves of Indian mustard (*Brassica*

- juncea) and rape (*Brassica napus*) under cadmium stress". *Acta Physiologiae Plantarum* 31, No. 2: 237-247.
- Opdenakker, Kelly, Tony Remans, Jaco Vangronsveld, and Ann Cuypers. 2012. "Mitogen-activated protein (MAP) kinases in plant metal stress: regulation and responses in comparison to other biotic and abiotic stresses". *International Journal of Molecular Sciences* 13, No. 6: 7828-7853.
- Papoyan, Ashot, and Leon V Kochian. 2004. "Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance. Characterization of a novel heavy metal transporting ATPase". *Plant Physiology* 136, No. 3: 3814-3823.
- Paradiso, Annalisa, Rosalia Berardino, Maria C de Pinto, Luigi Sanità di Toppi, Maria M Storelli, Franca Tommasi, and Laura De Gara. 2008. "Increase in ascorbate–glutathione metabolism as local and precocious systemic responses induced by cadmium in durum wheat plants". *Plant and Cell Physiology* 49, No. 3: 362-374.
- Pena, Liliana B, Roberto A Barcia, Claudia E Azpilicueta, Andrea AE Méndez, and Susana M Gallego. 2012. "Oxidative post translational modifications of proteins related to cell cycle are involved in cadmium toxicity in wheat seedlings". *Plant Science* 196: 1-7.
- Pence, Nicole S, Paul B Larsen, Stephen D Ebbs, Deborah LD Letham, Mitch M Lasat, David F Garvin, David Eide, and Leon V Kochian. 2000. "The molecular basis for heavy metal hyperaccumulation in *Thlaspi caerulescens*". *Proceedings of the National Academy of Sciences* 97, No. 9: 4956-4960.
- Peng, Hong-Yun, Xiao-E Yang, Li-Yang Jiang, and Zhen-Li He. 2005. "Copper phytoavailability and uptake by *Elsholtzia splendens* from contaminated soil as affected by soil amendments". *Journal of Environmental Science and Health* 40, No. 4: 839-856.
- Pető, Andrea, Nóra Lehotai, Jorge Lozano-Juste, José León, Irma Tari, László Erdei, and Zsuzsanna Kolbert. 2011. "Involvement of nitric oxide and auxin in signal transduction of copper-induced morphological responses in *Arabidopsis* seedlings". *Annals of Botany* 108, No. 3: 449-457.
- Pieterse, Corné MJ, Dieuwertje Van der Does, Christos Zamioudis, Antonio Leon-Reyes, and Saskia CM Van Wees. 2012. "Hormonal modulation of plant immunity". *Annual Review of Cell and Developmental Biology* 28: 489-521.
- Pitzschke, Andrea, and Heribert Hirt. 2010. "Mechanism of MAPK-targeted gene expression unraveled in plants". *Cell Cycle* 9: 18-19.



- Rady, Mostafa M. 2011. "Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress". *Scientia Horticulturae* 129, No. 2: 232-237.
- Rao, Kudupudi Prabhakara, Gubbala Vani, Kundan Kumar, Dhammaprakash Pandhari Wankhede, Mohit Misra, Meetu Gupta, and Alok Krishna Sinha. 2011. "Arsenic stress activates MAP kinase in rice roots and leaves". *Archives of Biochemistry and Biophysics* 506, No. 1: 73-82.
- Ricachenevsky, Felipe Klein, Paloma Koprovski Menguer, Raul Antonio Sperotto, Lorraine Elizabeth Williams, and Janette Palma Fett. 2013. "Roles of plant metal tolerance proteins (MTP) in metal storage and potential use in biofortification strategies". *Frontiers in Plant Science* 4: 144.
- Rohman, MM, MS Uddin, and M Fujita. 2010. "Up-regulation of onion bulb glutathione S-transferases (GSTs) by abiotic stresses: a comparative study between two differently sensitive GSTs to their physiological inhibitors". *Plant Omics* 3, No. 1: 28-34.
- Romero-Puertas, María C, Francisco J Corpas, María Rodríguez-Serrano, Manuel Gómez, A. Luis, and Luisa M. Sandalio. 2007. "Differential expression and regulation of antioxidative enzymes by cadmium in pea plants". *Journal of Plant Physiology* 164, No. 10: 1346-1357.
- Russo, M, Cristina Sgherri, Riccardo Izzo, and F Navari-Izzo. 2008. "*Brassica napus* subjected to copper excess: Phospholipases C and D and glutathione system in signaling". *Environmental and Experimental Botany* 62, No. 3: 238-246.
- Sánchez-Pardo, Beatriz, Mercedes Fernández-Pascual, and Pilar Zornoza. 2012. "Copper microlocalisation, ultrastructural alterations and antioxidant responses in the nodules of white lupin and soybean plants grown under conditions of copper excess". *Environmental and Experimental Botany* 84: 52-60.
- Sasabe, Michiko, Véronique Boudolf, Lieven De Veylder, Dirk Inzé, Pascal Genschik, and Yasunori Machida. 2011. "Phosphorylation of a mitotic kinesin-like protein and a MAPKKK by cyclin-dependent kinases (CDKs) is involved in the transition to cytokinesis in plants". *Proceedings of the National Academy of Sciences* 108, No. 43: 17844-17849.
- Schäfer, CH Simper, and B Hofmann. 1992. "Glucose feeding results in coordinated changes of chlorophyll content, ribulose-1,5-bisphosphate carboxylase-oxygenase activity and photosynthetic potential photoautotrophic suspension cultured cells of *Chenopodium rubrum*". *Plant, Cell & Environment* 15(3): 343-350.

- Semane, Brahim, Ann Cuypers, Karen Smeets, Frank Van Belleghem, Nele Horemans, Henk Schat, and Jaco Vangronsveld. 2007. "Cadmium responses in *Arabidopsis thaliana*: glutathione metabolism and antioxidative defence system". *Physiologia Plantarum* 129, No. 3: 519-528.
- Sharma, Shanti S., and Karl-Josef Dietz. 2009. "The relationship between metal toxicity and cellular redox imbalance". *Trends in Plant Science* 14, No. 1: 43-50.
- Singh, Vijay Pratap, Prabhat Kumar Srivastava, and Sheo Mohan Prasad. 2013. "Nitric oxide alleviates arsenic-induced toxic effects in ridged Luffa seedlings". *Plant Physiology and Biochemistry* 71: 155-163.
- Smékalová, Veronika, Anna Doskočilová, George Komis, and Jozef Šamaj. 2013. "Crosstalk between secondary messengers, hormones and MAPK modules during abiotic stress signalling in plants". *Biotechnology Advances* 32, No. 1: 2-11.
- Srivastava, Garima, Sushil Kumar, Gunjan Dubey, Vagish Mishra, and Sheo Mohan Prasad. 2012. "Nickel and ultraviolet-B stresses induce differential growth and photosynthetic responses in *Pisum sativum* L. seedlings". *Biological Trace Element Research* 149, No. 1: 86-96.
- Srivastava, Sudhakar, Ashish Kumar Srivastava, Penna Suprasanna, and SF D'souza. 2013. "Identification and profiling of arsenic stress-induced microRNAs in *Brassica juncea*". *Journal of Experimental Botany* 64, No. 1: 303-15.
- Stiborova, Marie. 1988. "Cd<sup>2+</sup> ions affect the quaternary structure of ribulose-1,5-bisphosphate carboxylase from barley leaves". *Biochemie und Physiologie der Pflanzen* 183, No. 5: 371378.
- Sundaramoorthy, Perumal, Alagappan Chidambaram, Kaliyaperumal Sankar Ganesh, Pachikaran Unnikannan, and Logalakshmanan Baskaran. 2010. "Chromium stress in paddy: (i) nutrient status of paddy under chromium stress; (ii) phytoremediation of chromium by aquatic and terrestrial weeds". *Comptes rendus biologiques* 333, No. 8: 597-607.
- Talke, Ina N, Marc Hanikenne, and Ute Krämer. 2006. "Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*". *Plant Physiology* 142, No. 1: 148-167.
- Tamás, Ladislav, Igor Mítrík, Jana Huttová, L'ubica Halušková, Katarína Valentovičová, and Veronika Zelinová. 2010. "Role of reactive oxygen species-generating enzymes and hydrogen peroxide during cadmium,

- mercury and osmotic stresses in barley root tip". *Planta* 231, No. 2: 221-231.
- Thounaojam, Thorny Chanu, Piyalee Panda, Purabi Mazumdar, Devanand Kumar, Gauri Dutta Sharma, Lingaraj Sahoo, and Panda Sanjib. 2012. "Excess copper induced oxidative stress and response of antioxidants in rice". *Plant Physiology and Biochemistry*, 53: 33-39.
- Tikkanen, Mikko, and Eva-Mari Aro. 2012. "Thylakoid protein phosphorylation in dynamic regulation of photosystem II in higher plants". *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1817, No. 1: 232-238.
- Usha, B, Gayatri Venkataraman, and Ajay Parida. 2009. "Heavy metal and abiotic stress inducible metallothionein isoforms from *Prosopis juliflora* (SW) DC show differences in binding to heavy metals in vitro". *Molecular Genetics and Genomics* 281, No. 1: 99-108.
- Van der Zaal, Bert J, Leon W Neuteboom, Johan E Pinas, Agnes N Chardonnens, Henk Schat, Jos AC Verkleij, and Paul JJ Hooykaas. 1999. "Overexpression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation". *Plant Physiology* 119, No. 3: 1047-1055.
- Vázquez, Miriam Núñez, Yanelis Reyes Guerrero, Lisbel Martínez González, and Walfredo Torres de la Noval. 2013. "Brassinosteroids and Plant Responses to Heavy Metal Stress. An Overview". *Open J. Metal* 3, 3: 34.
- Verbruggen, Nathalie, Christian Hermans, and Henk Schat. 2009. "Mechanisms to cope with arsenic or cadmium excess in plants". *Current Opinion in Plant Biology* 12, No. 3: 364-372.
- Wang, Chong-qing, Wang Tao, MU Ping, Zi-chao LI, and Yang Ling. 2013. "Quantitative trait loci for mercury tolerance in rice seedlings". *Rice Sci.* 20, No. 3: 238-242.
- Weber, Michael, Aleksandra Trampczynska, and Stephan Clemens. 2006. "Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd<sup>2+</sup>-hypertolerant facultative metallophyte *Arabidopsis halleri*". *Plant, Cell & Environment* 29, No. 5: 950-963.
- Whiting, Steven N, Jonathan R Leake, Steve P McGrath, and Alan JM Baker. 2000. "Positive responses to zinc and cadmium by roots of the hyperaccumulator *Thlaspi caerulescens*". *New Phytologist* 145, No. 2: 199-210.
- Wilkinson, Sally, Guzel R Kudoyarova, Dmitry S Veselov, Tatyana N Arkhipova, and William J Davies. 2012. "Plant hormone interactions:

- innovative targets for crop breeding and management". *Journal of Experimental Botany* 63, No. 9: 3499-3509.
- Williams, Lorraine E, Jon K Pittman, and JL Hall. 2000. "Emerging mechanisms for heavy metal transport in plants". *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1465, No. 1-2: 104-126.
- Wójcik, M, and A Tukiendorf. 2011. "Glutathione in adaptation of *Arabidopsis thaliana* to cadmium stress". *Biologia plantarum* 55, No. 1: 125-132.
- Xiong, Jie, Guanfu Fu, Longxing Tao, and Cheng Zhu. 2010. "Roles of nitric oxide in alleviating heavy metal toxicity in plants". *J. of Biochemistry and Biophysics* 497, No. 1-2: 13-20.
- Xu, Jin, Wenying Wang, Jianhang Sun, Yuan Zhang, Qing Ge, Ligu Du, Hengxia Yin, and Xiaojing Liu. 2011. "Involvement of auxin and nitric oxide in plant Cd-stress responses". *Plant and soil* 346, No. 1-2: 107-119.
- Yadav, SK. 2010. "Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants". *South African Journal of Botany* 76, No. 2: 167-179.
- Yan, An, Yamin Wang, Swee Ngim Tan, Mohamed Lokman Mohd Yusof, Subhadip Ghosh, and Zhong Chen. 2020. "Phytoremediation: a promising approach for revegetation of heavy metal-polluted land". *Frontiers in Plant Science* 11.
- Yang Z, Chu C. 2011. "Towards understanding plant response to heavy metal stress." In Shanker A, Venkateswarlu B (eds.), *Agricultural and Biological Sciences: Abiotic Stress in Plants-Mechanisms and Adaptations*, 59-78.
- Yang, Hai-Ling, Ying-Ru Zhao, Cai-Ling Wang, Zhi-Ling Yang, Qing-Yin Zeng, and Hai Lu. 2009. "Molecular characterization of a dehydroascorbate reductase from *Pinus bungeana*". *Journal of Integrative Plant Biology* 51, no. 11: 993-1001.
- Yang, Tianbao, Gul Shad Ali, Lihua Yang, Liqun Du, ASN Reddy, and BW Poovaiah. 2010. "Calcium/calmodulin-regulated receptor-like kinase CRLK1 interacts with MEKK1 in plants". *Plant Signaling & Behavior* 5, No. 8: 991-994.
- Yang, Tianbao, Shubho Chaudhuri, Lihua Yang, Liqun Du, and BW Poovaiah. 2010a. "A calcium/calmodulin-regulated member of the receptor-like kinase family confers cold tolerance in plants". *Journal of Biological Chemistry* 285, No. 10: 7119-7126.
- Yang, Xiaoe, Ying Feng, Zhenli He, and Peter J Stoffella. 2005. "Molecular mechanisms of heavy metal hyperaccumulation and

- phytoremediation". *Journal of Trace Elements in Medicine and Biology* 18, No. 4: 339-353.
- Yannarelli, Gustavo G, Ana J Fernández-Alvarez, Diego M Santa-Cruz, and María L Tomaro. 2007. "Glutathione reductase activity and isoforms in leaves and roots of wheat plants subjected to cadmium stress". *Phytochemistry* 68, No. 4: 505-512.
- Yuan, Hong-Mei, Heng-Hao Xu, Wen-Cheng Liu, and Ying-Tang Lu. 2013. "Copper regulates primary root elongation through PIN1-mediated auxin redistribution". *Plant and Cell Physiology* 54, No. 5: 766-778.
- Zhang, Yuexuan, Zhiling Yu, Xinrong Fu, and Chun Liang. 2002. "Noc3p, a bHLH protein, plays an integral role in the initiation of DNA replication in budding yeast". *Cell* 109, No. 7: 849-860.
- Zhao, Feng-Yun, Wen Liu, and Shi-Yong Zhang. 2009. "Different Responses of Plant Growth and Antioxidant System to the Combination of Cadmium and Heat Stress in Transgenic and Non-transgenic Rice". *Journal of Integrative Plant Biology* 51, No. 10: 942-950.
- Zobel, Richard W, Thomas B Kinraide, and Virupax C Baligar. 2007. "Fine root diameters can change in response to changes in nutrient concentrations". *Plant and Soil* 297, No. 1-2: 243-254.

The Life of Plants in a Changing Environment

Edited by Rishikesh Upadhyay

This book first published 2022

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Copyright © 2022 by Rishikesh Upadhyay

All rights for this book reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the copyright owner.

ISBN (10): 1-5275-7676-0

ISBN (13): 978-1-5275-7676-6