

Heavy metal toxicity and mitigation strategies in plants: a review

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ABSTRACT

Heavy metal pollution is one of the major ecological concerns which pose threat to plants as well as animals. Heavy metals are readily taken up by plants from soil and water through roots. In plants, an increase in heavy metal concentration causes phytotoxic effects i.e. altered growth, physiological, biochemical and molecular processes. These effects of heavy metals are due to the generation of reactive oxygen species (ROS) which disturbed balance of cellular redox and finally damage the biomolecules: DNA, lipids and proteins. Although in order to counteract heavy metal toxicity plants have developed several strategies, however, at higher level of contamination plant could not able to cope with oxidative stress and ultimately reduction in plant growth observed. In this review, we have summarized responses of plants to heavy metals toxicity and its mitigating strategies.

Keywords: Antioxidants . Biotechnology . Chelating agents. Crop plants . Heavy metal toxicity . Nanotechnology . Phytohormones . Reactive oxygen species

INTRODUCTION

Population explosion and rapid development result into industrialization, urbanization and other human activities which create disturbances in the ecosystems. Heavy metal pollution is one of the major ecological concerns due to its direct impact on plant productivity. Environmental contamination by heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), mercury (Hg), zinc (Zn), manganese (Mn), cobalt (Co) and metalloids, i.e. arsenic (As) poses threat to plants as well as animals (Table 1). Heavy metals are bioavailable to plants through their uptake from soil and water. An increase in the concentration of heavy metals causes phytotoxic effects on growth, physiological, biochemical and molecular processes of plants (Yang et al. 2005; Singh et al. 2013; Singh and Prasad 2014). Heavy metal stress causes reduction of molecular oxygen and produces intermediate products such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^{\cdot}) by disturbing electron transport chains in the chloroplasts and

mitochondria, and are more toxic and reactive than O₂ (Heyno et al. 2008). The ROS are involved in the free radical chain reaction of membrane lipids and proteins and thus, causing oxidative stresses (Gill and Tuteja 2010).

Despite the phytotoxicity of heavy metals, several plants growing in metals polluted soil are able to exclude/hyperaccumulate heavy metals, and acquire a wide range of adaptive strategies (Sharma, 2012; Ahammed et al. 2013; Singh and Prasad 2014). In order to reduce heavy metal contamination, plants have developed several strategies. The defense systems is composed of antioxidants including enzymes such as superoxide dismutase (SOD, E.C. 1.15.1.1), catalase (CAT, E.C. 1.11.1.6), peroxidase (POD, E.C. 1.11.1.7), ascorbate peroxidase (APX, E.C. 1.11.1.11), glutathione peroxidase (GPX, E.C. 1.11.1.7) and glutathione reductase (GR, E.C.1.6.4.2) and non-enzymatic antioxidants such as ascorbate (AsA), glutathione (GSH) and tocopherols to suppress the ROS (Gill and Tuteja, 2010; Kumar et al. 2012; Shu et al. 2012). Some of the non-enzymatic antioxidants are intermediates of ascorbate–glutathione cycle which play major role in H₂O₂ scavenging pathway operating in the chloroplasts as well as in the cytosol. Although plants have evolved a defense system to scavenge ROS, however, at the higher level of contamination plant could not able to cope with oxidative stress and ultimately plant growth reduced. In this review, we have summarized the heavy metal toxicity on plants and its mitigating strategies with the recent advancement.

2. Uptake and transport of heavy metals in plants

Heavy metals are taken up by the plants through their roots and transported to their aerial parts. Heavy metal uptake depends on several factors such as soil pH, temperature, organic contents, presence of chelating agents etc. Among various soil factors, soil pH being the most important factor to affect the availability of heavy metals. In case of Cd, a linear trend between soil pH and Cd uptake was noticed. For instance, Kirkham (2006) observed that the decrease of soil pH leads to the increasing concentration of Cd in plant. In soils, lead (Pb) forms precipitate with phosphates, sulfates and chemicals in the rhizosphere due to which it has low solubility and availability to plants as earlier reported by Blaylock and Huang (2000). Kim et al. (2002) reported that Ca²⁺ supplied in the medium reduces Pb uptake and toxicity thereby suggesting the entry of Pb in root cells *via* Ca²⁺/Mg²⁺ gated channel. The Ni uptake in plants is carried out by root systems *via* active transport as well as passive diffusion (Seregin and Kozhevnikova 2006). In plants, the uptake of Ni depends on Ni concentrations (Cataldo et al. 1978), the acidity of soil or solution (Antoniadis et al. 2008), plant metabolism (Aschmann and Zasoski 1987), the presence of other metals (Podar et al. 2004) and composition of organic matter (Jean et al. 2008). For example, the uptake of Ni increased with increasing pH up to 5.0, and then decreased as pH is increased up to 8.0 in *Lathyrus sativus* (Panda et al. 2007). Although in case of *Berkheya coddii* the uptake of Ni has been found to be inhibited by Ca²⁺ and Mg²⁺ (Robinson et al. 1999), however, both Ca²⁺ and Mg²⁺ are non-competitive inhibitors of Ni influx in excised barley roots (Korner et al. 1987). Besides this, other factors such as length of season, method of seed sowing, and soil geochemical properties are also reported which can influence the uptake of Ni (Antoniadis et al. 2008).

In plants, uptake of heavy metals is immobilized within the roots but a portion is translocated to the aerial portions of the plant including shoot, leaves and seeds. Though

transportation of Cd occurs symplastically through the root cortex to the stele, and from root stele to the shoots *via* the xylem, however, the phloem may also be involved in transportation (Tudoreanu and Phillips 2004). Similarly, Pb can be transported *via* phloem to the aerial parts. In case of Ni, over 50% of the Ni absorbed by plants is immobilized within the roots (Cataldo et al. 1978) probably may be due to sequestration in the cation exchange sites of the walls of the xylem parenchyma cells and immobilization in the vacuoles of roots (Seregin and Kozhevnikova 2006). Moreover, about 80% of Ni in the roots is present in the vascular cylinder, while less than 20% is present in the cortex suggesting a high mobility of Ni in the xylem and the phloem (Page and Feller 2005).

Under natural conditions, arsenic (As) is found to be associated with iron oxides in the forms of $\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}(\text{s})$ or $\text{Fe}_3(\text{AsO}_4)_2 \cdot 8\text{H}_2\text{O}(\text{s})$ (Courtin-Nomade et al. 2002). Therefore, As adsorption in soils increases with the iron oxide content (You et al. 2001). Arsenic exists mainly in four oxidation states: arsenate (As^{V}), arsenite (As^{III}), arsenic (As^0), and arsine ($\text{As}^{-\text{III}}$), and its solubility depends on the pH and ionic environment (Gupta et al. 2011). Under normal pH conditions (pH 4-8), most arsenic acid (H_3AsO_4) is dissociated as the oxyanions H_2AsO_4^- or HAsO_4^{2-} with the dissociation constants (pK_a) of 2.2, 6.97, and 11.5, which are the chemical analogs of phosphate ions (Pi) and so, is easily transported across the plasmalemma by Pi transporter (PHT) protein (Wu et al. 2011). In contrast to arsenate, arsenous acid (H_3AsO_3 , $\text{pK}_a = 9.2, 12.1, \text{ and } 13.4$) is undissociated at normal pH conditions (>94% undissociated at pH <8.0). Therefore, plant roots take up As^{III} mainly as the neutral molecule [$\text{As}(\text{OH})_3$]. The As^{III} species are transported through the nodulin 26-like intrinsic (NIPs) aquaporins or water channels (Mosa et al. 2012). Once As enters inside the plant cell, it can move easily from one cellular compartment to another, crosses internal membranes *via* various Pi transporters. As^{V} can be found in the xylem, most likely be loaded in to the xylem vessels by PHT proteins (Mendoza-Cózatl et al. 2011; Wu et al. 2011). In *Arabidopsis*, about 3% of the As taken up by the roots was translocated to the shoots (Quaghebeur and Rengel, 2003). Similar results have been reported for other plants (Lomax et al. 2012). However, more efficient root to shoot translocation has been evolved in hyperaccumulator plant, in which it seems to be a crucial mechanism of hypertolerance such as *P. vittata*. In this case, As is not immobilized in the roots but is instead rapidly transported as As through the xylem to the fronds (Su et al. 2008).

3. Heavy metal toxicity in plants

3.1 Effect of heavy metals on growth and development

Reduction in growth is one of the most common physiological consequences of heavy metals toxicity in plants (Hu et al. 2013) (Fig. 1). The primary effect of heavy metals toxicity in plants is rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip (Eun et al. 2000). Cadmium inhibits the lateral root formation while the main root becomes brown, rigid and twisted because of the disordered division and abnormal enlargement of epidermal and cortical cell layers in the apical region (Yadav 2010; Rascio and Navari-Izzo 2011). The changes in the leaf such as alterations in chloroplast ultrastructure and low chlorophylls which lead chlorosis, and restricted photosynthetic activity (Lee et al. 2010; Miyadate et al. 2011).

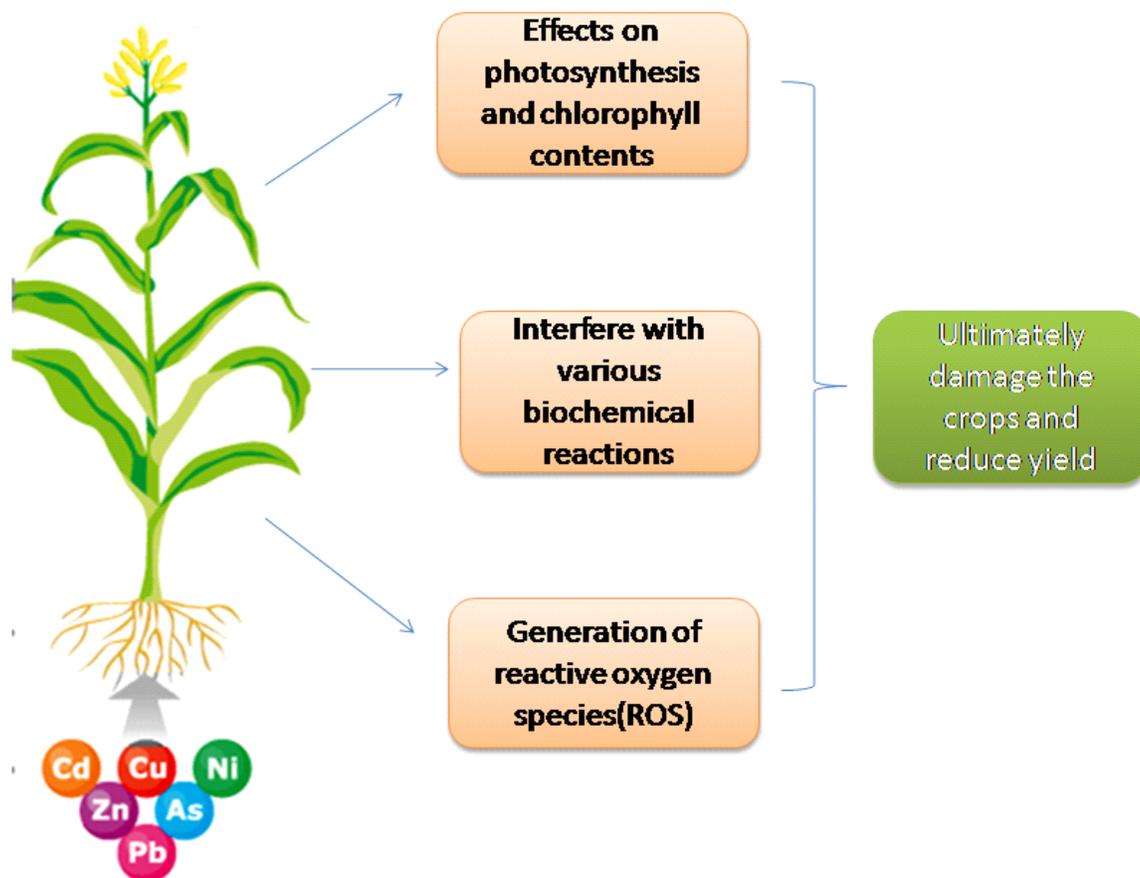


Fig. 1 Effects of heavy metals on various physiological and biochemical processes of crops that ultimately reduce their yield.

Rascio et al. (2008) reported that treatment of rice seedlings with Cd led to the inhibition of root growth and alterations in its morphogenesis. In several plant species, *Triticum aestivum* (Kaur et al. 2013), *Sedum alfredii* (Gupta et al. 2010), *Z. mays* L. (Kozhevnikova et al. 2009) and *Pisum sativum* (Malecka et al. 2009), a decrease in the length and dry mass of root has been reported under Pb toxicity (Mun-zuroglu and Geckil 2002). In addition, Kaur et al. (2013) observed distentions and lesions in cell wall of *Triticum aestivum* roots as a result of activation of certain wall-degrading enzymes in response to Pb exposure. The excess amount of Ni in ecosystem severely affects the growth and development of plants (Yusuf et al. 2011a, 2011b). The Ni toxicity in plants includes decreased root and shoot growth and reduction in leaf area (Shaw et al. 2004). The elevated concentration of Ni decreased the growth of *Zea mays* seedling (Bhardwaj et al. 2007) as well as excessive concentration (0.5 mg-L) of Ni in nutrient solution also inhibited the growth of *L. gibba* (Khellaf and Zerdaoui 2010). The Ni toxicity also decreased the compound leaves (Khellaf and Zerdaoui 2010; Mishra and Dubey 2011).

For example, reduced sunflower yield was most probably due to the Ni quantity that accumulates in plant's leaf (Ahmad et al. 2011). Roots are usually the first tissue to be exposed to As, where the metalloids inhibits root extension and proliferation. Upon translocation from root to the shoot, As not only severely inhibits the plant growth by slowing or arresting expansion and biomass accumulation but also causes losses in reproductive capacity, fertility and yield (Garg and Singla 2011).

- *Effect of heavy metals on photosynthesis, chlorophylls and biochemical reactions*

Studies showed inhibition in the photosynthesis after both long-term and short-term exposures of Cd in many plant species such as oilseed rape (*Brassica napus*) (Baryla et al. 2001), sunflower (*Helianthus annuus*) (Di Cagno et al. 2001), *Thlaspi caerulescens* (Kupper et al. 2007), maize, pea and barley (Popova et al. 2008), mungbean (*Vigna radiata*) (Wahid et al. 2008), and wheat (*Triticum aestivum*) (Moussa and El-Gamal 2010). Regarding the site and mechanism of inhibition of Cd, it is generally accepted that the water-oxidising complex of photosystem II (PSII) is affected by Cd *via* replacement of Ca^{2+} in Ca/Mn clusters constituting the oxygen-evolving complex (Sigfridsson et al. 2004). The Cd influences 2 key enzymes of CO_2 fixation: ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase). Siedlecka et al. (1998) showed that Cd ions lowers the activity of RuBPCase by damaging its structure by substituting Mg ions (an important cofactor of carboxylation reactions) and Cd can also shift RuBPCase activity towards oxygenation reactions. Similarly, Pb at high concentrations reduced the synthesis of chlorophyll and uptake of essential ions like Mg and Fe (Bruzynski 1987), and also increased the chlorophyllase activity (Drazkiewicz 1994). Further, Sharma and Dubey (2005) reported that plants exposed to Pb ions showed a decline in the photosynthetic rate as a result of distorted chloroplast, restrained synthesis of chlorophyll, obstructed electron transport, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closing. Accumulation of Ni in lower and upper parts of mung bean significantly decreased the chlorophyll content (Ahmad et al. 2011). The Ni stress showed a significant reduction in photosynthetic pigments in black gram (*Vigna mungo*) (Dubey and Pandey 2011). The 200 μM of Ni stress significantly decreased the stomatal conductance (gs) especially in emerging leaves where the stomatal conductance reduced from 0.40 to 0.03 $\text{mol m}^{-2} \text{s}^{-1}$ in *Populus nigra* and this decline in gs resulted into a direct decrease in the photosynthesis (Velikova et al. 2010). Alam et al. (2007) showed that Ni stress (100 μM) decreased net photosynthetic rate and chlorophyll contents in *Brassica juncea*. Photosynthetic rate in five cultivars of *T. aestivum* was significantly decreased under Ni stress (Yusuf et al. 2011). An important mode of action of As toxicity may be the replacement of Pi in crucial biochemical reactions. As-sensitive reactions include cellular metabolism (i.e. glycolysis, oxidative phosphorylation), biosynthesis (i.e. phospholipid metabolism), information storage and retrieval (i.e. DNA and RNA metabolism), and cellular signaling (i.e. protein phosphorylation/dephosphorylation). Due to the irreversible nature of most Pi-liberating reactions few enzymes use Pi as a substrate therefore, few enzymes are expected to use As^{V} directly as a substrate (Tawfik and Viola 2011).

3.3 Effect of heavy metals on generation of reactive oxygen species (ROS)

It is well known that exposure of plants to heavy metals generates ROS in plant tissues (Mishra et al. 2008). In normal condition, the ROS expeditiously cleaned by antioxidant system (Dubey and Pandey 2011) but ROS at higher concentrations are

extremely toxic to the plants. A cell comes into the condition of ‘oxidative stress’ due to the overproduction of ROS and disturbances in antioxidant defense caused by heavy metals toxicity (Sharma 2012) in the cell compartments, associated with electron transport chains in the chloroplasts, mitochondria and peroxisomes (del Río and Puppò 2009; Sandalio et al. 2012). The enhanced production of ROS under heavy metal stress can pose a serious threat to the cell by causing peroxidation of lipids, damage to nucleic acids, enzyme inhibition, oxidation of proteins and activation of programmed cell death (PCD) ultimately leading to the cell death (Mishra et al. 2011; Srivastava and Dubey 2011) (Fig. 2).

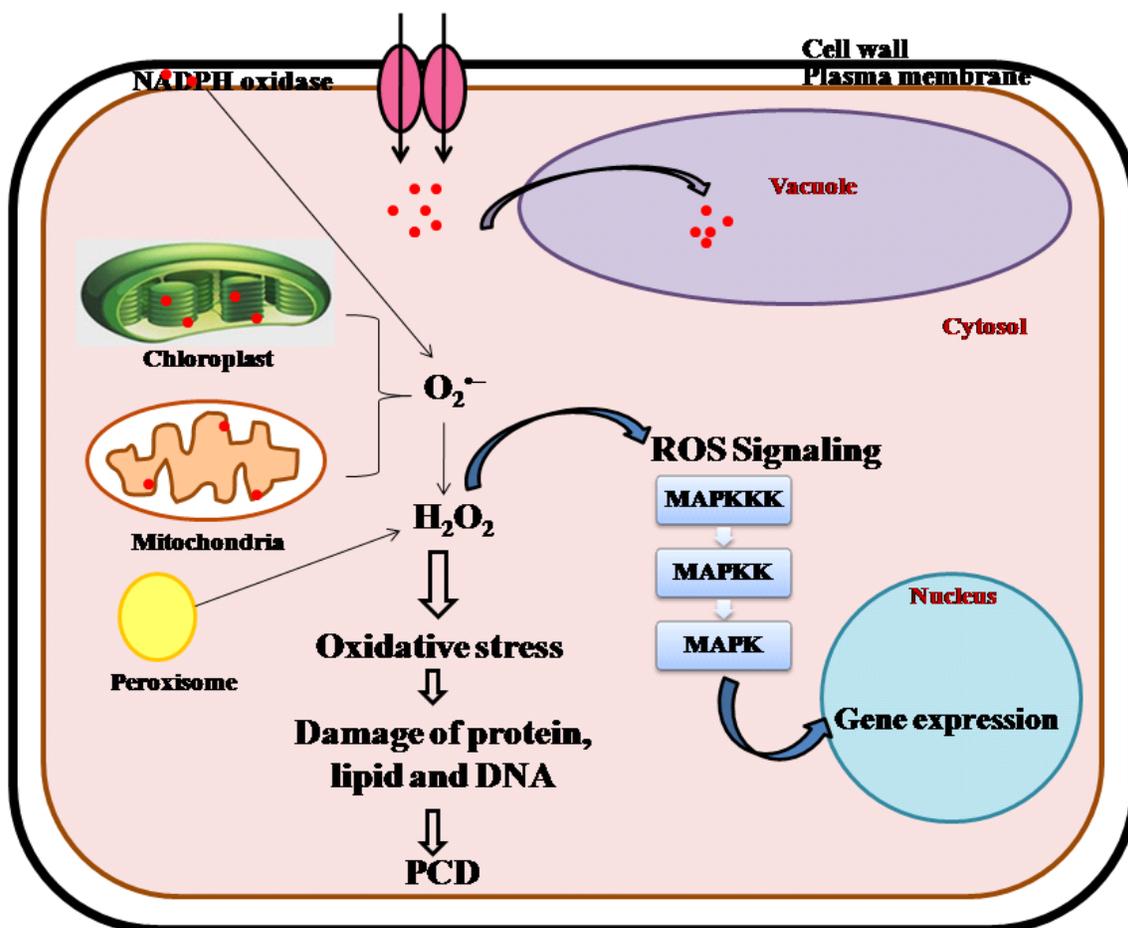


Fig. 2 Heavy metal induced generation of reactive oxygen species (ROS) in chloroplast, mitochondria and peroxisome, and also by plasma-membrane-localized NADPH oxidase. Excess ROS can cause redox imbalances and generate oxidative stress that damages the biomolecules and finally leads to programme cell death. ROS also activate signalling processes i.e. MAPK pathways. Red dots represent the heavy metal.

The Cd is a non-redox metal which is unable to perform single electron transfer reactions and thus does not directly produce ROS such as the superoxide radical ($O_2^{\cdot-}$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), but it can

generate oxidative stress by interfering with the antioxidant defence system (Gratao et al. 2005). The Cd inhibits the photoactivation of PS II by inhibiting electron transfer due to which it could lead to the generation of ROS indirectly by causing disturbances in the chloroplasts. Moreover, Cd may also stimulate ROS production in the mitochondrial electron transfer chain (Heyno et al. 2008). Similarly, Liu et al. (2010) reported that Pb leads the overproduction of ROS such as $O_2^{\cdot-}$ and H_2O_2 in plant cells. Further, studies showed that Pb may induce formation of ROS in different plant species such as *Allium sativum* (Liu et al. 2009), *Zea mays* (Gupta et al. 2009) and *Brassica campestris* (Singh et al. 2011) which results into enhanced H_2O_2 content and lipid peroxidation. It has been reported that after Pb treatment, roots of *Ficus microcarpa* produced high concentrations of H_2O_2 along with an increase in $O_2^{\cdot-}$ accumulation. Passardi et al. (2004) reported that $O_2^{\cdot-}$ is produced in the plasma membrane by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and is converted to H_2O_2 through non-enzymatic pathways or by superoxide dismutase (SOD) activity. Ni has capacity to increase the ROS content as it was observed in wheat (Hao et al., 2006), and *Nicotiana tabacum* and *Alyssum bertolonii* (Boominathan and Doran 2002). Although Ni produces the HO^{\cdot} by Haber-Weiss and Fenton reactions (Kehrer, 2000), however, due to high reduction/oxidation capacity, Ni was not observed as a catalyst in this reaction (Leonard et al. 2004). Gajewska and Sklodowska (2007) reported that Ni in plant tissues may cause cytotoxic damages as H_2O_2 quantity significantly increased in leaf of wheat. Exposure of plants to As^V generates ROS in plant tissues (Mishra et al. 2008) through its intraconversion from its one ionic form to other (Mylona et al. 1998) which induces oxidative stresses such as lipid peroxidation (Tripathi et al. 2012). It is well documented that exposure of plants to As^{III} and As^V induces the production of ROS, including $O_2^{\cdot-}$, HO^{\cdot} and H_2O_2 (Mallick et al. 2011). ROS can damage proteins, amino acids, purine nucleotides and nucleic acids and cause peroxidation of membrane lipids (Møller et al. 2007).

3.4 Effect of heavy metals on mitogen-activated protein kinase signaling pathway

In higher plants oxygen is require for the efficient production of energy by aerobic respiration. During the process of O_2 reduction to H_2O , reactive oxygen species (ROS), namely $O_2^{\cdot-}$, H_2O_2 , and OH^{\cdot} can be formed (Gill and Tuteja 2010). The ROS may also activate signal transduction by the mitogen-activated protein kinase (MAPK) cascade (He et al. 1999). This involves a series of phosphorylation reactions in which three kinases are sequentially activated: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK. By inducing the phosphorylation of many transcription factors, activated MAPKs are eventually involved in the modulation of gene expression (Fig. 2).

4. Defense system of plant against heavy metal toxicity

Heavy metals pollution in air, water and soil is a global hindrance causing a great loss to crop yield, and also exerts hazardous effects on human health when these metals enter into the food chain. Plants like other organisms are known to generate several defense responses to cope with stresses which they are exposed and appear to be inhibitory and/or lethal to non-tolerant individuals. This review also provides an overview of the variety of potential mechanisms that may be involved in the detoxification and tolerance to heavy metals (Fig. 3).

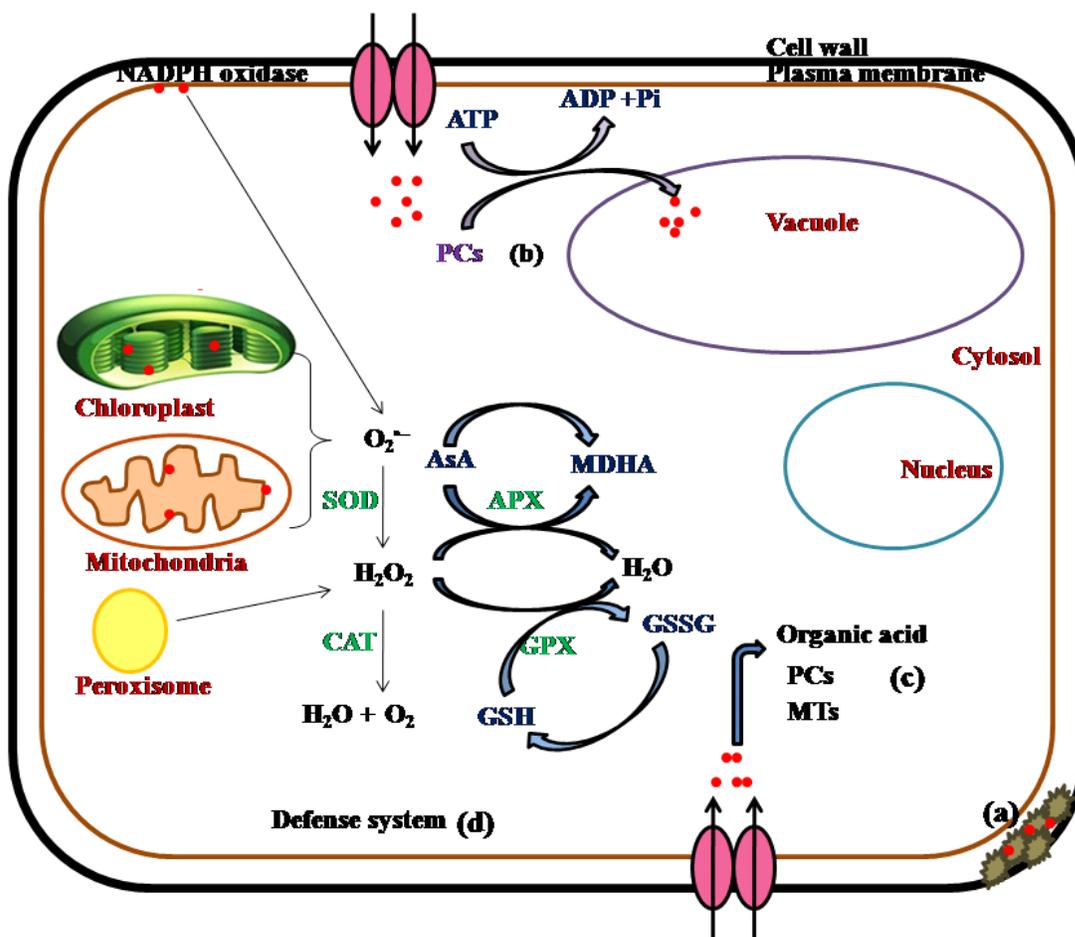


Fig. 3 Defense system of plant against heavy metal. (a) metal ion binding to the cell wall and root exudates, (b) sequestration in the vacuole by phytochelatin (PCs) (c) metal chelation in the cytosol by ligands such as organic acids, PCs and metallothioneins (MTs) and (d) enzymatic antioxidant defense system. Red dots represent the heavy metal.

- *Metal binding to cell wall*

Pectic sites, histidyl groups and extracellular carbohydrates present in cell wall cause immobilization of heavy metals and prevent their uptake in to the cytosol (Rea et al. 1998). Cell wall pectins consisting polygalacturonic acid act as a cation exchanger. The heavy metals are bound to carboxylic groups of polygalacturonic acids restricting the uptake of heavy metals (Ernst et al., 1992). It seems that chemical properties of the cell wall might modulate metal uptake and consequently metal tolerance. The cell wall has little impact on metal tolerance due to the inadequate number of metal absorption sites. However, role of the cell wall in metal tolerance is not completely understood.

- *Organic acids*

As a first line of defense against heavy metals, plant roots secrete exudates into the soil matrix. One of the major roles of root exudates is to chelate metals and to prevent

their uptake inside the cells (Marschner 1995). Organic acids have been intensively studied as they are well known chelators of some metals and can decrease metal toxicity. Roots produce and release root exudates, protons and several metabolites such as citric, fumaric and uronic acids which modify the soil pH and thus interfere with the dissolution processes and formation of soluble metal-organic complexes (Leyval and Berthelin 1991). These acids have ability to form complexes and to chelate metal ions including Pb (Mench et al. 1987). For instance, Ni-chelating histidine and citrate are present in root exudates and reduce the uptake of Ni from soil (Salt et al. 2000). In some cases, the acids are secreted into the rhizosphere and decrease metals activity before they reach into the plants. Organic acids and amino acids can bind heavy metals and may, therefore, be deployed in response to metal toxicity (Rauser 1999). However, a clear correlation between heavy metal accumulation and the production of these compounds has not yet been established. Organic acids such as malate, citrate, and oxalate confer metal tolerance by transporting metals through the xylem and sequestering them into the vacuoles (Rauser 1999). Organic acids within cells detoxify metals by complexing and making them unavailable to the plants. They act as metabolic intermediates in the formation of ATP from carbohydrates, nitrogen metabolism and ionic balance. Hence, metabolic abnormalities in these processes would be reflected by changes in the concentrations of organic acids. Therefore, an increase in organic acids with increasing supply of metals could imply a detoxification mechanism or conversely disruption of metabolism results into the production of organic acids as a stress response to excess metal.

- *Phytochelatins (PCs)*

Chelation of metals in the cytosol by high-affinity ligands is a very important mechanism of heavy metal detoxification and tolerance. Potential ligands include amino acids, organic acids and two classes of peptides i.e. phytochelatins (PCs) and the metallothioneins (MTs) (Hall, 2002). The role of PCs and MTs in heavy metal detoxification in plants has been studied thoroughly. Phytochelatins are derived from glutathione (GSH) and possess the general structure (γ-Glu-Cys)_n-Gly (where n=2 to 11) (Hall 2002). Recently, genes encoding for PC synthases have been identified in higher plants and yeast, and it has been shown that the *Arabidopsis* gene could confer substantial increase in metal tolerance in yeast (Clemens et al. 1999). Metallothioneins are sulfur containing proteins inherently being highly flexible in their structure. This flexibility allows different coordination geometries for binding of different metals. Nevertheless, each MT exhibits preferences for a special metal ion due to the coordination residues other than cysteine, and differences in folding and stability depend on the bound metal (Leszczyszyn et al. 2007). On activation in presence of heavy metals, PC synthase enzyme produces PCs by trans-peptidation of γ-glutamyl-cysteinyl dipeptides from GSH. Among the various heavy metals, Cd is the strongest inducer of PCs. In vacuoles, PCs metal complexes become more resistant to proteolytic degradation on incorporation of inorganic sulfide and sulfite ions (Bertrand et al., 2002). It was observed that MTs participate in antioxidant protection and plasma membrane repair mechanism.

- *Antioxidant defense systems*

The increased formation of ROS like O₂^{•-}, [•]OH and H₂O₂ is one of the initial responses to heavy metal stress. In order to mitigate the oxidative damage initiated by

ROS, plants have developed an antioxidant defense system comprised of non-enzymatic antioxidants: non protein thiols, proline, cysteine, ascorbate, glutathione etc., and enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione-S-transferase (GST) (Gill and Tuteja, 2010; Ahemmad et al. 2013). Antioxidants can remove, neutralize, and scavenge ROS from the vicinity of the cell. The first line of defense against ROS-mediated toxicity is achieved by SOD that catalyzes the dismutation of $O_2^{\cdot-}$ into H_2O_2 and O_2 . Heavy metal-mediated enhancement in activity of SOD has been previously observed which may be due to either direct effect of this metal on the SOD gene or an indirect effect mediated *via* an increase in the level of $O_2^{\cdot-}$ (Chongpraditnum et al. 1992). The SOD has a metal cofactor and depending on the metal, SODs can be classified in three different groups, localized in different cell compartments: FeSOD (chloroplasts), MnSOD (mitochondria and peroxisomes) and Cu/ZnSOD (chloroplast and cytosol). As SOD produces H_2O_2 that is subsequently converted to water by peroxidases and catalases, the activities of these enzymes must be carefully balanced. The CAT can have an important role in H_2O_2 detoxification that can diffuse into the peroxisome from other cell locations where it is produced (Mittler, 2002). The CAT catalyses the breakdown of H_2O_2 to water: $H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$. Peroxidases are known to play a significant role under oxidative stress conditions. It has been shown that peroxidase activity can be used as a potential biomarker for sub-lethal metal toxicity in examined plant species (Radotic et al. 2000). In plants, the detoxification of H_2O_2 has been shown to be an important function of the POD that uses ascorbate as the hydrogen donor (Hegedus et al. 2001). They have the general reaction: $H_2O_2 + R(OH)_2 \rightarrow 2H_2O + RO_2$ [R(OH)₂ represents different electron donors]. Guaiacol peroxidase (GPOD) uses phenolic donors and glutathione peroxidase (GPX) uses glutathione. Besides their role as a scavenger of H_2O_2 , cell wall peroxidases are also involved in ROS formation, both as a defense against biotic stresses and as a signaling process against several stresses leading to the activation of other defense mechanisms (Mika et al. 2004). The APX has a much higher affinity to H_2O_2 than CAT suggesting that it has different roles in the scavenging of this ROS, with APX being responsible for maintaining the low levels of H_2O_2 while CAT is responsible for the removal of H_2O_2 excess (Mittler, 2002). The water-water cycle occurs in chloroplasts and is a fundamental mechanism to avoid photooxidative damage (Rizhsky et al., 2003), using SOD and APX to scavenge the $O_2^{\cdot-}$ and H_2O_2 , respectively in the location where they are produced and thus avoiding the deleterious effects on cellular components (Asada, 1999).

Besides enzymatic antioxidants, non-enzymatic antioxidants also play crucial roles in minimizing ROS-mediated damages to cellular components. Some are well known and have been extensively studied (ascorbate and glutathione) while others are thought to be part of defense mechanisms. Ascorbate and glutathione exist in relatively high concentration in the cell compartments (Potters et al. 2002), and are involved in the ascorbate-glutathione cycle as described above, being also a substrate for APX an enzyme important in H_2O_2 removal. Ascorbate is an electron donor that can be oxidized to the radical monodehydroascorbate (MDHA) and this compound can then form dehydroascorbate (DHA) in the ascorbate-glutathione cycle or the Asada-Halliwell pathway. Besides its role as an enzyme substrate, ascorbate also reacts directly with 1O_2 and $O_2^{\cdot-}$ and important in the regeneration of α -tocopherol and certain carotenoids

(Potters et al. 2002). Glutathione is a tripeptide (containing glutamate, cysteine and glycine) that can exist in two predominant forms: the reduced form (usually represented by GSH) and the oxidized form (usually represented by GSSG) (Noctor and Foyer 1998). It is involved in the sulphur metabolism and in defense reactions against oxidative stress (Potters et al. 2002). It can also lead to the synthesis of phytochelatins that are important sequesters for heavy metals (Cobbett and Goldsbrough 2002).

- *Phytohormones*

The regulation of hormone synthesis in presence of heavy metals indicates that plant hormones play a crucial role in the adaptation to metal stress (Peleg and Blumwald 2011). The hormones such as salicylic acid, jasmonic acid, ethylene, gibberellic acid are implicated in plant defense signaling pathways. Jasmonic acid treatment increased the capacity for glutathione synthesis which plays central role in protecting plants from heavy metal stress (Xiang and Oliver 1998). Salicylic acid activates defense related genes either by H₂O₂-mediated signal transduction pathway or by directly affecting mechanisms of metal detoxification (Metwally et al. 2003). Salicylic acid inhibits two major H₂O₂ scavenging enzymes CAT and APX which causes cellular concentration to rise and subsequently H₂O₂ acts as second messenger for triggering defense against metal stress. Heavy metal stress induces ethylene biosynthesis (Milone et al. 2003) that acts as endogenous signal triggering the plant defense response.

- *Stress related proteins*

Following heavy metal exposure, most of the plants trigger the synthesis of sets of novel proteins (Mishra and Dubey 2006). Most of the proteins endowed plasma membranes to act as barrier for metal inflow which lead to metal homeostasis and detoxification (Suzuki et al. 2002). The common stress related Heat Shock Proteins (HSP) found in all groups of living organisms, can be classified according to molecular size and are known to act as molecular chaperones in normal protein folding and assembly. They also function in the protection and repair of proteins under stress conditions (Shin et al. 2012).

5. Mitigating strategies of heavy metal toxicity

Contamination of soil by heavy metals causes a serious negative impact on agricultural production. Plants grown in heavy metal contaminated soil have stunted growth due to the generation of ROS which cause subsequent damages to various cellular components. Therefore, in order to reduce the metal contamination in plants several strategies have been developed which are discussed in this section (Fig. 4).

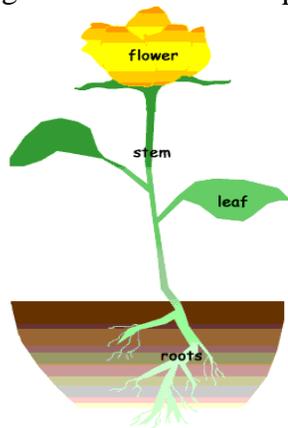


Fig. 4 Mitigation strategies for heavy metal toxicity using various approaches*5.1 By using chelating agents*

Chelating agents which have high affinity for heavy metals could be used to enhance the solubilization of metals in soils through the formation of soluble metal complex chelates. Number of synthetic chelating agents are available including amino polycarboxylates such as EDTA, diethylenetriaminepentaacetic acid (DTPA), ethylenediamine-N,N-bis (2-hydroxyphenylacetic acid) (EDDHA), N,N-ethylenebis-[2-(o-hydroxyphenyl)]-glycine (EHPG) and many others (Bolan et al. 2014). The effectiveness of any chelate in mobilizing heavy metals depends on several factors including metal species, metal (loid):chelate ratio, stability constants, thermodynamic presence of competing cations, stability of the metal-chelate complex, soil pH, extent of metal retention onto soil constituents and the aging of the contaminating metal. The chelating agents after complex formation can be removed from the soil through enhancing phytoextraction in plants. Chelants can desorb metals from the soil matrix, and the mobilized metals move to rhizosphere for uptake by the plant roots (Tahmasbian and Sinegani 2014). Sukumara et al. (2012) have observed that EDTA is one of the most powerful and commonly used chelating agents, forms complexes with many of metal contaminants within the natural environment. It was found that application of EDTA as chelating agent increases the efficiency of an emergent wetland plant species such as *Typha* sp. and floating wetland macrophytes like *Pistia* sp., *Azolla* sp., *Lemna* sp, *Salvinia* sp. and *Eichhornia* sp. in phytoremediation of lead and copper (Sukumara et al. 2012). Zhang et al. (2013) reported that the derivatives: CDTA (trans-1,2-cyclohexanediaminetetraacetic acid), BDTA (benzylidiaminetetraacetic acid), and PDTA (phenyldiaminetetraacetic acid) of EDTA which contain a cyclohexane ring, a benzyl group, and a phenyl group, respectively may helpful in mobilizing heavy metals.

- *By using microorganisms*

Microorganisms are ubiquitous in nature and their implication in metal remediation has been regarded as an environment-friendly, economical and efficient means of environmental restoration (Hryniewicz and Baum 2014). Soil microorganisms can affect trace metal mobility and availability to the plants (Abou-Shanab et al. 2003; Idris et al. 2004). For example, the presence of rhizosphere (defined as the volume of soil adjacent to and influenced by the plant root) (Smalla et al. 2001) bacteria increased the uptake of Cd in *Brassica napus* (Sheng and Xia 2006) and Ni in *Alyssum murale* (Abou-Shanab et al. 2006). *Arbuscular mycorrhizal* (AM) fungi are ubiquitous symbiotic associations found in both natural and heavy metal-contaminated sites (Wang et al. 2007). AM fungi may stimulate phytoextraction by improving plant growth and increasing the total metal uptake (Wang et al. 2007). The microorganisms, which are closely associated with roots, have been termed plant growth promoting rhizobacteria (PGPR). The PGPR include a diverse group of free-living soil bacteria that can improve host plant growth and development in heavy metal contaminated soils by mitigating toxic effects of heavy metals on plants. The association of PGPR with plant roots may exert beneficial effects on plant growth and nutrition by N₂ fixation, production of phytohormones and siderophores, and transformation of nutrient elements (Koo and Cho 2009). With the help

of PGPR, an increase in the growth and reduction in the metal toxicity occurs due to the enhancement in soil nutrients so it is one of the most suitable choices for bioremediation (Zaidi et al. 2003; Khan et al. 2009). Heshmatpure and Rad (2012) have also observed that the *Pseudomonas fluorescence* plant growth-promoting rhizobacterium increases the resistance against high levels of Cd and reduces its adverse effects in *B. napus* L. This rhizobacterium enhances the efficiency of phytoremediation in the presence of Cd.

- *By using exogenous plant growth regulators*

Heavy metals cause deleterious effects on plant growth and one of the reasons associated with growth reduction is the degradation of endogenous plant growth regulators (PGRs). As consequence of this, endogenous of level PGR in plant is decreased and ultimately reduced the growth (Chou and Ferjani 2005). Recent research has shown the promising effects of PGRs like auxins, abscisic acid (ABA), cytokinins, gibberellins (GA), brassinosteroids (BRs), jasmonic acid (JA) and polyamines (PAs) in mitigating heavy metal stress. Yusuf et al. (2012) reported that the application of 24-epibrassinolide to the Ni-stressed plants improved growth, nodulation and enhanced the activity of various antioxidant enzymes (*viz.* CAT, POD and SOD) and also the content of proline. Chen et al. (2014) reported that exogenous application of JA inhibited the uptake of Cd to the aboveground part of the seedlings and thus reduced the direct damages of Cd to the photosynthetic organs of the plant. Many workers reported up-regulation of antioxidant enzymes by PGRs could have conferred tolerance to the heavy metal stressed plants resulting in improved growth, nodulation and yield attributes (Creelman and Mullet 1995; Xiang and Oliver 1998; Gangwar et al. 2011; Khan and Khan 2013; Singh and Prasad 2014).

6. Recent advancement

6.1. *By using nanotechnology*

Nanotechnology is manufacturing at the molecular level-building things from nanoscale components, where unique phenomenon enables novel applications. Nanos: Greek term for dwarf and technology: visualize, characterize, produce and manipulate matter of the size of 1-100 nm (Ball et al. 2002). The application of nanotechnology for remediation of contaminants may give promising results in the future. Nanotechnology can provide a way to purify the air and water resources by utilizing nanoparticles as a catalyst and/or sensing systems (Fulekar et al. 2014). Yang et al. (2006) have found that application of nanostructured materials can be used as adsorbents or catalysts to remove toxic and harmful metals from wastewater and air and finally from soil. Liu (2011) also reported an effective remediation of a Pb-laden soil from a shoot range using synthesized apatite nanoparticles. Salam (2013) has used multi-walled carbon nanotubes (CNTs) for successfully removal of Cu^{II} , Pb^{II} , Cd^{II} , and Zn^{II} from aqueous solution. Yu et al. (2013) also used CNTs and their composites to remove metals from contaminated water. Rathore et al. (2013) have found that the application of carbon nanoparticles resulted into 75-92% reduction in Ni contamination from soil and about 99% reduction from water system. Carbon nanoparticle have exceptional adsorption and mechanical properties due to its unique electrical property, highly chemical stability and large specific surface area (Tofiqhy and Mohammad 2011; Salam 2013). Other workers reported that the

nanoparticles of *E. macroclada* can be used in detoxification of heavy metals (especially Pb, Cd, Cu and Zn) from polluted environments (Mohsenzadeh and Rad 2011).

6.2. By using biotechnologically modified plants

An improvement of plant tolerance to heavy metals is one of the major challenges in plant biotechnology. Biotechnological tools include genetic engineering in order to improve the performance of plants in effective removal of metals from the environment. Addition of new genotype and phenotype by transferring the gene from metal-hyperaccumulating plants and microbes increases the remediation property of plants (James and Strand 2009). Aken (2008) has observed that transgenic plants can be safer for phytoremediation purpose. Thomas et al. (2003) have shown that transgenic plants expressing metallothioneins exhibited enhanced tolerance to high metal concentrations. To increase metal uptake, the yeast metallothionein CUP1 was introduced into tobacco plants, and this gene is expressed in plants. Expression of this gene increases phytoextraction of Cu and Cd. By integrating dehaloperoxidase gene from salt marsh worm (*Amphitrite ornata*) into *Arabidopsis* and tobacco model systems, there was an enhancement in remediation property of experimental plants (Czako et al. 2006). Nakamura et al. (2014) have generated transgenic *Nicotiana tabacum* (tobacco) plants overexpressing both serine acetyltransferase (SAT) and cysteine synthase (CS) [*O*-acetylserine (thiol)-lyase], which are committed in the last two steps of cysteine (Cys) biosynthesis, by crossing the respective single-gene transgenic plants. The levels of cysteine and γ -glutamylcysteine (γ -EC) were also increased in the dual transgenic plants, most likely enhancing the metabolic flow of Cys biosynthesis leading to the ultimate synthesis of PCs which detoxify Cd by chelating. These results suggested that the overexpression of two genes, SAT and CS, could be a promising strategy for engineering Cd resistant plants (Nakamura et al. 2014).

Concluding remark

Intensive research during the three decades has yielded significant improvement in our understanding of the physiological response and molecular background of heavy metal stress. Contamination of soils with heavy metals is a critical factor affecting soil properties and plant growth. Roots, being directly exposed, always accumulate greater amounts of heavy metals than shoots. Effects of heavy metals toxicity on aboveground parts include stunting growth, leaf rolling, chlorosis and necrosis, diminished stomatal conductance and gas exchange, perturbed leaf water and nutrient status, hormonal imbalance, produce oxidative stress, and enhanced peroxidation of membrane lipids. Plants exploit various mechanisms to cope with heavy metal stresses which include organic acids exudates, synthesis of metal chelating proteins and expression of enzymatic and nonenzymatic antioxidants. It will be highly important to explore the connection of signalling events with the adaptation and acclimation processes occur under heavy metal stress. Heavy metal toxicity can be mitigated by the exogenous use of metal chelators, PGRs, and microorganisms. Ultimately, strategies to bind heavy metals in soil systems and better understanding of species diversity for heavy metal tolerance, heavy metal responsive genes and the molecular basis of heavy metal tolerance may be important strategies for coping with this ever increasing problem.

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Compliance with ethical standards

Authors comply with the ethical standards as laid down by the journal.

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