Chapter 1
Heavy Metal Toxicity in Plants

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Abstract Plants are sessile organisms that must cope with the surrounding soil composition in order to survive and reproduce. Soils often contain excessive levels of essential and non-essential elements, which may be toxic at high concentrations depending on the plant species and the soil characteristics. Many metals share common toxicity mechanisms, and plants deal with these metals using similar scavenging pathways. The impact of metal toxicity is made more complex by competition, since high levels of one metal may imbalance the uptake and transport of others, therefore contributing to the toxicity symptoms. Here, the toxicity symptoms and mechanisms of the most common essential and non-essential heavy metals will be considered.

Keywords Heavy metal · Plant nutrition · Metal pollution · Metal toxicity

1.1 Heavy Metals: Nutrients or Toxic Elements?

Plants acquire mineral elements from soil primarily in the form of inorganic ions. The extended root apparatus and its ability to absorb ionic compounds even at low concentrations makes mineral absorption highly efficient.

Mineral elements can be divided into two groups: essential nutrients and toxic non-nutrient elements. The essential minerals include the *macronutrients* nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorous (P), sulfur (S) and

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silicon (Si), and the *micronutrients* chlorine (Cl), iron (Fe), boron (B), manganese (Mn), sodium (Na), zinc (Zn), copper (Cu), nickel (Ni), and molybdenum (Mo). These are essential components of plant metabolism and structure, and their absence or deficiency reduces fitness and inhibits growth and reproduction. Micronutrients are required in only small quantities and their excessive abundance in the soil (especially Cu, Ni and Zn), due to natural occurrence or introduction by anthropogenic activities, is also detrimental to the majority of plant species. Other minerals such as cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), arsenic (As), silver (Ag), and antimony (Sb) are toxic to plants even at low concentration. These metals, collectively defined as *heavy metals* since their density is higher than 5.0 g cm^{-3}, are not considered to be nutrients because they have no known function in plant metabolism and appear to be more or less toxic to both eukaryotic and prokaryotic organisms (Sanità di Toppi and Gabbrielli 1999). Only recently, a carbonic anhydrase has been shown to bind Cd as a cofactor in the marine diatom *Thalassiosira weissflogii* (Lane and Morel 2000).

When studying heavy metal toxicity in plants, researchers must take into account the nature of the pollution phenomenon. First, the stress caused by contaminated soils is permanent, and therefore long-term rather than short-term molecular responses must be considered. Most studies have been carried out in hydroponic or in vitro culture, and have involved the application of extremely high metal concentrations in the growth media. This seldom resembles actual environment and represents the consequences of acute stress caused by a single metal species. Second, the toxicity of a heavy metal depends on its oxidation state, e.g., Cr(VI) is considered the most toxic form of Cr, and usually occurs associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions. Cr(III) is less mobile, less toxic, and predominantly bound to organic matter in soil and aquatic environments (Shanker et al. 2005). Third, the ability of heavy metals to persist in the soil in the form that is bioavailable to roots (i.e., soluble and ready for absorption) is influenced by their adsorption, desorption, and complexation in the soil matrix, processes that are strongly influenced by soil pH, composition, and structure. Heavy metals tend to be more mobile in acidic soils. Finally, heavy metal toxicity is species dependent. For instance, metal-tolerant plants and certain plants known as hyperaccumulators [able to accumulate at least 100 mg g^{-1} (0.01% dry weight) Cd, As, and some other trace metals, 1,000 mg g^{-1} (0.1% dry weight) Co, Cu, Cr, Ni, and Pb and 10,000 mg g^{-1} (1% dry weight) Mn and Ni (Reeves and Baker 2000; Watanabe 1997) have defense mechanisms that avoid damage caused by heavy metal-induced stress, although the duration and magnitude of exposure and other environmental conditions, contribute to heavy metal sensitivity (Sanità di Toppi and Gabbrielli 1999).

Metal toxicity is also greatly influenced by the coexistence of many metals in the soil, which could have both *synergic* and *antagonistic* effects depending on the relative concentrations and other soil properties (i.e., presence of nutrient elements). For example, Ca^{2+} strongly inhibits the uptake of Ni in *Arabidopsis bertolonii*, whereas the opposite effect is seen in *Berkheya coddii* (Gabbrielli and Pandolfini 1984). Ni can induce Fe deficiency either by retarding its uptake or trapping Fe in the roots (Mysliwa-Kurdziel et al. 2004), and these effects can be

partially overcome by supplementation with Mg (or Fe) ions suggesting a competitive interaction (Le Bot et al. 1990). In *Pisum sativum*, Mn toxicity can be reduced by applying indole acetic acid (IAA). This also promotes seedling growth, and it has been suggested that IAA protection is mediated by regulation of both the ammonium content and the activities of enzymes involved in ammonium assimilation (Gangwar et al. 2011). Mn toxicity is also reduced in the presence of Si. The biochemical and physiological basis of this phenomenon is poorly understood and may involve the modification of metabolic stress responses (Führs et al. 2009) and a change in the apoplastic Mn-binding properties that lead to a reduction in the concentration of Mn in the apoplast (Horst et al. 1999). As already stated, different species respond to combinations of ions in different ways. As an example, pea plants are protected from Cd toxicity by Ca, which limits Cd accumulation, whereas in *Brassica juncea*, Ca promotes the accumulation of As but reduces its toxicity (Rai et al. 2011a).

Fertilization is also known to influence heavy metal toxicity. The addition of phosphate reduces As toxicity in field-grown *Medicago truncatula* and *Hordeum vulgare* without modifying the specific uptake of As(V), and this may be due to the higher phosphate concentration into cells that outcompetes As in metabolic reactions (Christophersen et al. 2009). The accumulation and translocation of As in rice plants is inhibited when sulfur is abundant but enhanced when its availability is limited. This may reflect the prominent role of sulfur, which is a component of PCs and GS (both of which form complexes with heavy metals) and the impact of its availability on the synthesis of thiolic compounds, elements that ultimately affect As accumulation and metabolism (Zhang et al. 2011).

1.2 Toxicity Mechanisms of Heavy Metals

Heavy metal toxicity in plants occurs through four major mechanisms:

(1) Induction of oxidative stress and changes in the cell membrane permeability and integrity. Many heavy metals induce the formation of ROS such as H_2O_2, $O_2^{\cdot -}$ and $OH^{\cdot}$, which may be a direct process (via the Fenton and Haber–Weiss reactions, as shown in Fig. 1.1) or as secondary effect due to their toxicity into the cell. ROS have a negative impact on plant cells, for instance by inhibiting water channel and transporter proteins and enhancing lipid peroxidation. The latter alters membrane fluidity, stability, and structure, inhibiting membrane-dependent processes such as electron flow in chloroplasts and mitochondria. ROS are counteracted by the activation of *antioxidant enzymes* such as SOD, APX, GPX, CAT, and GSR, whose reaction mechanisms are shown in Fig. 1.2.

(2) Reaction with sulfhydryl groups (–SH). Heavy metals have a strong affinity for –SH groups [Cd, for example, shows a threefold higher affinity for –SH groups than Cu (Schützendübel and Polle 2002)] and therefore bind to structural

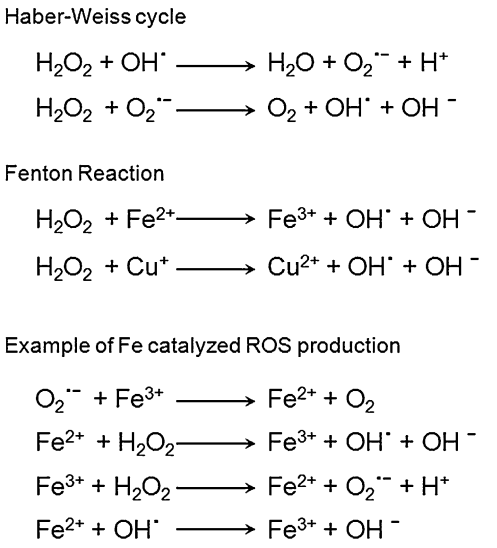

Fig. 1.1 Generation of ROS by heavy metals, including examples of reactions catalyzed by Fe (Halliwell and Gutteridge 1984)

proteins and enzymes containing them. This can prevent correct folding, interfere with catalytic activity, and perturb enzyme-mediated redox regulation (Hall 2002).

(3) Similarity to biochemical functional groups. As(V) in arsenate (AsO_4^{3-}), for example, is an analog of the micronutrient phosphate (PO_4^{3-}) and competes with it in many cellular functions. AsO_4^{3-} displaces phosphate in ATP, leading to the formation of the unstable complex ADP-As that interferes with the energy flows in the cell (Meharg and Hartley-Whitaker 2002).

(4) Displacement of essential (cat)ionic cofactors in enzymes and signaling components. Metal ions in the active sites of enzymes can be displaced by heavy metal ions resulting in the loss of activity, e.g., the displacement of Cu, Zn, Fe, and Mn cofactors from superoxide dismutase by Cd. The displacement of the ionic cofactors from signaling proteins (e.g., calmodulin and transcription factors) results in aberrant proteins that may perturb gene expression. This process can also interfere with homeostatic pathways for essential metal ions (Roth et al. 2006). For example, the displacement of Cu and Fe from proteins releases free ions that may cause oxidative damage, e.g., via Fe/Cu-catalyzed Fenton reactions (DalCorso et al. 2008).

The large number of targets for heavy metal toxicity means that negative effects tend to be firstly observed in those cells that are exposed first, i.e., cells responsible for the metal uptake. Heavy metals interfere with ionic homeostasis and enzyme activity, and these effects are apparent in physiological processes involving single organs (such as nutrient uptake by the roots) followed by more general processes such as germination, growth, photosynthesis, plant water balance, primary metabolism, and reproduction. Indeed, visible symptoms of heavy metal toxicity

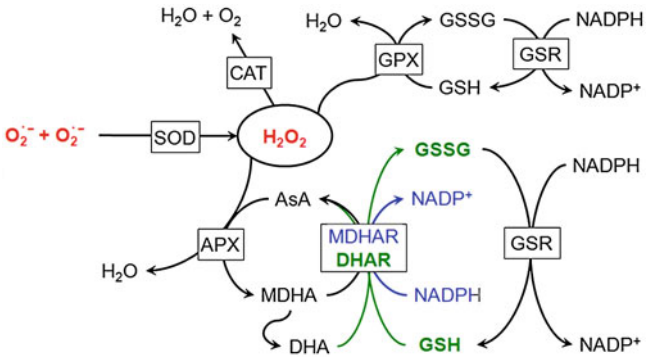

Fig. 1.2 Antioxidant enzymes responsible for the detoxification of H_2O_2 and $O_2^{\cdot -}$. *APX* ascorbate peroxidase, *AsA* ascorbate, *CAT* catalase, *DHA* dehydroascorbate, *DHAR* dehydroascorbate reductase, *GSR* glutathione reductase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *GPX* glutathione peroxidase, *MDHAR* monodehydroascorbate reductase, *NADP* nicotinamide adenine dinucleotide phosphate, reduced (NADPH) and oxidized ($NADP^+$), *SOD* superoxide dismutase

include chlorosis, leaf rolling and necrosis, senescence, wilting and stunted growth, low biomass production, limited numbers of seeds, and eventually death. We now consider the effects of the most relevant heavy metals individually.

1.3 Non-Essential Heavy Metals: Cadmium, Mercury, Lead, Chromium and Arsenic

1.3.1 Cadmium

Cadmium (Cd) is one of the most phytotoxic heavy metals because it is highly soluble in water and promptly taken up by plants. This also represents its main entry into the food chain, making it a threat to human health. Even at low concentrations, the uptake by roots and the transport of Cd to vegetative and reproductive organs have a negative effect on mineral nutrition, homeostasis, growth and development (DalCorso et al. 2010).

In root cells, Cd imbalances water and nutrient uptake, interfering with the absorption of Ca, Mg, K, and P. It inhibits root enzymes involved in nutrient metabolism, such as Fe(III) reductase, nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthetase, leading to Fe(II) deficiency, and reduced nitrogen assimilation and metabolism (glutamine and glutamate synthetases are responsible for the incorporation of ammonium into the carbon skeleton, DalCorso et al. 2008). Nitrogen fixation and primary ammonia assimilation is also inhibited in the nodules of soybean plants in the presence of Cd (Balestrasse et al. 2003). Cd inhibits root growth and lateral root formation, with the concomitant

differentiation of numerous root hairs, for instance, in *Arabidopsis* and tobacco (Farinati et al. 2010). Tomato roots exposed to Cd are thicker and stronger (Chaffei et al. 2004). In shoot tissues, the most evident symptoms of Cd toxicity are leaf roll, chlorosis, water uptake imbalance, and stomatal closure (Clemens 2006). Chlorosis may reflect changes in the Fe:Zn ratio that negatively affect chlorophyll metabolism (Chaffei et al. 2004). Cd causes stomata to close independent of water status probably because its similarity to Ca allows Cd to enter guard cells through voltage-dependent Ca^{2+} channels and to mimic Ca^{2+} activity in the cytosol (Perfus-Barbeoch et al. 2002). Indeed, stomatal closure can be actively driven by Ca^{2+} accumulating in the guard cell cytosol. The increase in cytosolic free Ca^{2+} causes plasma membrane anion and K^{+}_{out} channels to open, resulting in the loss of water and turgor that drives stomatal pore closure (MacRobbie and Kurup 2007).

Both cellular and organellar metabolism are compromised by Cd. In chloroplasts, Cd damages the photosynthetic apparatus, targeting the light-harvesting complex II and the two photosystems (PSI and PSII) which are particularly sensitive. This reduces the chlorophyll and carotenoid content, increases non-photochemical quenching and limits both photosynthetic efficiency and effective quantum yield (Sanità di Toppi and Gabbrielli 1999). Moreover, by inhibiting enzymes involved in CO_2 fixation, Cd reduces carbon assimilation (Perfus-Barbeoch et al. 2002). Cd also affects sulfur metabolism in the chloroplasts by inducing the accumulation of thiolic compounds with a concomitant reduction in leaf ATP-sulfurylase and O-acetylserine sulfurylase activity, i.e., the first and the last enzymes in the sulfate assimilation pathway (Astolfi et al. 2004).

Cd is toxic at the cellular level by interfering with mitosis and inhibiting cell division, due to chromosomal aberrations and inhibition of mitotic processes (Benavides et al. 2005). In *Arabidopsis*, Cd induces mutations, leading to floral anomalies, embryonic malformations, and poor seed production (DalCorso et al. 2008).

Although Cd does not take part in the Fenton and Haber–Weiss reactions, without Cd ions altering their oxidation state (Clemens 2006), exposure can still induce oxidative injuries such as protein carbonylation and lipid peroxidation, disrupting cell homeostasis and interfering with membrane functions (Romero-Puertas et al. 2002; Schützendübel et al. 2001). This appears to reflect a Cd-induced imbalance in the activity of antioxidative enzymes, CAD and SOD *in primis*, leading to the accumulation of ROS, which may be a general effect of redox imbalance or a specific response to heavy metal stress (Romero-Puertas et al. 2004). Other plants induce GDH in response to Cd (Boussama et al. 1999). GDH activity is correlated with the onset of senescence and associated changes in nitrogen metabolism (Masclaux et al. 2000). Similar changes in nitrogen metabolism are observed in plants exposed to Cd so it is possible that the toxic effects of Cd reflect the induction of senescence. In peroxisomes, Cd induces glyoxylate cycle enzymes (malate synthase and isocitrate lyase) as well as peroxisomal peptidases, the latter being well known as leaf senescence-associated factors (Chaffei et al. 2004).

A secondary effect of ROS accumulation is the perturbation of signaling pathways mediated by H_2O_2 and oxygen radicals. Indeed, H_2O_2 plays a role as signal molecule in triggering, for instance, defence mechanisms against both abiotic stresses (Dat et al. 2000; Sharma et al. 1996) and pathogen attack (Bestwick et al. 1998; Thordal-Christensen et al. 1997). Interfering with H_2O_2 accumulation, Cd meddles with the signal transduction pathways in which ROS are involved. Cd^{2+} can also displace the chemically similar Zn^{2+} from zinc finger transcription factors, thus interfering with gene expression (Sanità di Toppi and Gabbrielli 1999). Similarly, Cd^{2+} can displace Ca^{2+} from calmodulin proteins, thus perturbing intracellular calcium level and altering calcium-dependent signaling, e.g., the regulation of stomatal closure discussed above (DalCorso et al. 2008).

1.3.2 Mercury

Mercury (Hg) is generally found only in trace concentrations in soil, and it is tightly bound to organic matter and clay particles or as a sulfide precipitates (Schuster 1991). The predominant source of Hg in the soil is from mining and industrial waste (Zhou et al. 2007). The toxicity of Hg depends on its chemical state (e.g., HgS, Hg^{2+}, Hg^0, and methyl-Hg). The predominant form in agricultural soils is Hg^{2+}, which is not particularly phytotoxic at normal concentrations, but it is soluble, highly reactive, and readily taken up by plants (Han et al. 2006). Alternatively, the uncharged and volatile form Hg^0 can enter leaves via the stomata and diffuse to the mesophyll cells where it is oxidized to Hg(II) (Zhou et al. 2007). The first visible symptoms of Hg toxicity are the profound inhibition of root and shoot growth (Cho and Park 2000). The molecular basis of Hg phytotoxicity remains uncertain but probably reflects: (i) the affinity of Hg for –SH groups; and (ii) the direct generation of ROS via the Fenton reaction, which in turn induces oxidative stress (Fig. 1.1).

Roots show the first signs of Hg toxicity because these are the first tissues to be exposed to the metal. The suppression of root growth by Hg has been observed in tomato seedlings, in *Brassica* spp. and in *Spinacia oleracea* (Cho and Park 2000; Ling et al. 2010). At high concentrations, Hg can bind to water channels in the plasma membrane, interfering with water flow and stomatal functions. When wheat root cells were exposed to $HgCl_2$, the hydraulic conductivity of the membranes was reduced, suggesting that membrane depolarization may inhibit water transport (Zhang and Tyerman 1999). Hg also strongly inhibits photosynthesis by interacting with metal ions in the PSII proteins D1 and D2 and with the Mn-cluster of the OEC (Patra et al. 2004). Oxygen evolution and thylakoid electron transport are also inhibited because Hg depletes the 33-kDa manganese stabilizing protein on the luminal side of PSII (Bernier and Carpentier 1995). PSI is also compromised by Hg, which oxidizes the P_{700} chlorophyll *a* when present as $HgCl_2$ (Sersen et al. 1998). In addition to Hg-induced chlorophyll depletion, these negative effects eventually result in a dramatic reduction in the photosynthetic quantum yield (Cho and Park 2000).

Laboratory experiments with various explants have shown that high concentrations of Hg are genotoxic, causing chromosomal damages, interfering with mitosis and meiosis, and inducing polyploidy (Patra et al. 2004).

Hg has a global impact on the redox state of the cell because it catalyzes the formation of ROS. In tomato seedlings, exposure to Hg induces the formation of H_2O_2 (Cho and Park 2000), whereas alfalfa leaves exposed to Hg^{2+} produce excess levels of both H_2O_2 and $O_2^{\cdot-}$ (Zhou et al. 2008). This increase in ROS affects many other cellular functions by damaging nucleic acids and proteins, and by inducing lipid peroxidation thus modifying membrane integrity and permeability (Patra et al. 2004). In tomato, the production of ROS correlates with an increased activity of CAT, SOD, and PRX enzymes, in both roots and shoots (Cho and Park 2000).

1.3.3 Lead

Lead (Pb) is one of the most abundant heavy metals in both terrestrial and aquatic environments, predominantly arising from human activities such as mining, smelting, the use of fuels and explosives, and the disposal of Pb-enriched municipal sewage sludge. Together with Cd, Pb is also considered one of the most serious hazards to human health, since it is readily taken up by plants and therefore can easily enter the food chain. Pb toxicity causes similar symptoms to other heavy metals, namely growth inhibition, chlorosis, and (in the most severe cases) death.

Roots that absorb Pb respond by reducing their growth rate and changing their branching pattern. In *Picea abies*, the emergence and growth of secondary roots are particularly sensitive to Pb toxicity (Godbold and Kettner 1991). In maize, Pb perturbs the organization of the microtubule network of the root meristem, resulting in a shorter branching zone with more compact lateral roots emerging nearer to the root tips (Eun et al. 2000). The inhibition of root growth by Pb also affects nutrient uptake and nitrogen assimilation. For example, the enzymes nitrate reductase and glutamine synthetase are inhibited by Pb in *Cucumis sativus* and *Glycine max*, respectively (Sharma and Dubey 2005). Pb also nonspecifically blocks the uptake of other cations such as K, Ca, Mg, Mn, Zn, Cu, and Fe, probably by modifying the activity and permeability of membranes or binding them to ion carriers, making them unavailable for uptake and transport into the plant (Patra et al. 2004).

High concentrations of Pb cause a water deficit, reducing the transpiration rate, altering the osmotic pressure of the cell sap and the water potential of the xylem. These effects contribute to an overall negative change in the plant water status (Parys et al. 1998).

Pb interacts with –SH groups like many other heavy metals, but it can also interact with –COOH groups, inhibiting enzymes and altering protein conformation (Sharma and Dubey 2005). Pb can also displace metal cofactors from metalloenzymes, which includes Mn in the OEC and Mg in the chlorophyll porphyrin ring, thus interfering with photosynthesis and electron transport

by reducing oxygen evolution and chlorophyll levels and altering the thylakoid membrane structure (Patra et al. 2004). Key chlorophyll biosynthesis enzymes are also strongly inhibited by Pb, as well as many enzymes in the Calvin cycle (e.g., RuBisCO, phosphoenol pyruvate carboxylase, and ribulose 5-phosphate kinase) thus reducing the rate and efficiency of CO_2 fixation (Sharma and Dubey 2005).

One unique effect of Pb is the disruption of the cell cycle by interfering with the alignment of microtubules on the mitotic spindle. This effect cannot be replicated with, e.g., Al and Cu, even at concentrations sufficient to inhibit root growth (Eun et al. 2000).

Pb is not a redox metal so cannot generate ROS directly, but oxidative stress is caused indirectly as shown by the increased lipid peroxidation in rice and pea plants exposed to the metal (Malecka et al. 2001). This is countered by the activation of antioxidant enzymes such as SOD and PRX, but whereas CAT activity increases in pea plants, it declines in rice, perhaps explaining in part why there is an increase in lipid peroxidation (Malecka et al. 2001, Verma and Dubey 2003). This complexity of antioxidant enzyme activity in plants under metal stress may reflect the presence of diverse isoforms which have different spatiotemporal expression profiles, different intracellular locations, and different environmental triggers for activation and inactivation (Scandalios 1990).

1.3.4 Chromium

Chromium (Cr) has received comparatively little attention from plant scientists perhaps because it is ubiquitous in the environment and, due to its complex electron chemistry, it exists in many oxidation states upon which its toxicity depends. Cr pollution results from human activities such as leather processing and finishing, the production of refractory steel, electroplating, wood preservation, and the manufacture of specialty chemicals and cleaning agents such as chromic acid. There is no evidence that Cr has a specific biological in plants, and its absorption involves the use of Fe, S, and P transporters and carriers; Cr thus competes with these essential nutrients for binding sites. Cr ions with different oxidation state appear to be absorbed by different mechanisms (Shanker et al. 2005). Cr stress inhibits germination in *Phaseolus vulgaris*, possibly by promoting the activity of proteases while suppressing the activity of amylases and perturbing the subsequent transport of sugars to the embryo axes (Zeid 2001). In adult plants, Cr toxicity inhibits shoot growth, reduces the number of leaves as well as the leaf area and biomass, reduces the productivity of crops, causes burns on the leaf margins and tips, and induces chlorosis and necrosis (Sharma and Sharma 1993; Singh 2001; Jain et al. 2000). Eventually, the global plant fitness is compromised, giving reduced plant biomass production and productivity, relevant aspects for crops and agronomy-important species.

A well-documented effect of Cr toxicity is the inhibition of primary root growth (observed as reduced root length) and the suppression of new lateral root primordia

(Prasad et al. 2001). The application of Cr inhibited root elongation in *Caesalpinia pulcherrima,* wheat, and *Vigna radiata* (Shanker et al. 2005) possibly by disrupting cell division through chromosomal damage (Panda and Choudhury 2005). Cr stress also induces changes in root morphology, increasing the number of root hairs and the relative proportion of pith and cortical tissue layers (Suseela et al. 2002). The negative effects of Cr on root growth and development combined with the tendency of Cr to compete with essential nutrients for uptake and transport means that Cr has a significant impact on nutrient acquisition. Although there is some variation depending on the plant species and tissue, Cr(VI) seems to have the most potent effect on the uptake of nutrients such as K, Mg, P, Fe, N, Zn, Cu, Mo, and Mn (Shanker et al. 2005). As well as reducing root growth and competing with these essential nutrients for uptake, Cr may also inhibit the activity of H^+ATPases in the plasma membrane, which is required for proton export from the roots and hence acidification of the rhizosphere and the subsequent mobilization of metal ions. Inhibition would therefore result in a general reduction in nutrient bioavailability in the soil (Shanker et al. 2005).

The impact of Cr on plant water status in unknown, although Cr does induce the typical symptoms of water deficit and reduced transpiration, such as turgor loss, plasmolysis, and diminished tracheary vessel diameter (Shanker et al. 2005).

Both photosystems are inhibited by Cr(VI) although the mechanisms are still under investigation. Exposure to Cr(III) and Cr(VI) reduces the chlorophyll content of bean seedlings and wheat plants by displacing Mg from the chlorophyll molecule (Samantaray et al. 2001; Sharma and Sharma 1996). Cr stress also disrupts the ultrastructure of the chloroplast, particularly the arrangement of thykaloid membranes, probably reducing the size of the antenna complexes (Panda and Choudhury 2005; Shanker et al. 2005).

Cr can also inhibit certain enzymes in a species-dependent manner, e.g. nitrate reductase (Panda and Patra 2000) and root Fe(III) reductase (Barton et al. 2000), the latter affecting Fe nutrition in the plant. In mitochondria, Cr may hamper the electron transport interfering with the Cu and Fe ions contained in many electron-carrier proteins. The severe inhibition of mitochondrial cytochrome oxidation, for instance, could be due to the extreme susceptibility of complex III and IV to Cr(VI) (Dixit et al. 2002).

Finally, Cr shares the ability of other heavy metals to induce the formation of ROS in plant cells. Cr is not considered a redox metal, but studies have shown that it can participate in Fenton reactions (Panda and Choudhury 2005). Sorghum plants treated with either Cr(VI) or Cr(III) increased H_2O_2 content in roots and leaves, correlated with an increase in lipid peroxidation (Panda and Choudhury 2005; Shanker and Pathmanabhan 2004). Antioxidant enzyme activities are also modulated by Cr, apparently in a dose-dependent manner. For example, low levels of Cr induce SOD activity in pea plants, whereas higher concentrations inhibit both CAT and SOD (Dixit et al. 2002; Jain et al. 2000).

1.3.5 Arsenic

Arsenic (As) is a profoundly toxic heavy metalloid that originates from both geogenic sources and anthropogenic activities such as mining, the combustion of fossil fuels, and use of As-based pesticides and wood preservatives (Tu and Ma 2005). It is widely distributed in the environment and recognized as a significant threat to human health. The chemistry of As in the soil is complex because it can be present in both organic and inorganic forms, but most As is present as the oxidized mineral arsenate, AsO_4^{3-} As(V), and its reduced form arsenite, AsO_3^{3-} As(III). The bioavailability of As depends on the soil characteristics, including its redox potential, pH, and composition, the presence of other minerals (particularly Fe and Al oxides and hydroxides), and the abundance of microbes that can reduce As(V) to As(III) (Smith et al. 2010). Arsenate is chemically similar to phosphate and it is probably taken up into many plants via phosphate transporters (Pigna et al. 2009). In contrast, arsenite is more abundant and mobile in soils with a low redox potential, and is thought to be acquired via aquaporin transporters in the plasma membrane of root cells (Vromman et al. 2011).

As interferes with cell metabolism by reacting with –SH groups on proteins and replacing phosphate, and inhibits plant growth (Tu and Ma 2005). The symptoms of As toxicity include poor seed germination and profound growth inhibition (Smith et al. 2010). In wheat seeds, for example, germination is considerably affected by both arsenite and arsenate, probably reflecting the inhibition of both α- and β-amylase (Liu et al. 2005). Maize plants treated with toxic concentration of As(V) and As(III) produced stunted roots that were thicker and stiffer than normal, and that had a significantly lower mitotic index; micronuclei and chromosome aberrations were also observed in the root meristems (Duquesnoy et al. 2010). In some species, the effect of As on root growth depends on its concentration. For example, root growth in *Artemisia annua* is stimulated at low As concentrations but inhibited at higher concentrations (Rai et al. 2011b).

The reduction in root growth combined with changes in the selectivity and permeability of cell membranes prevent the uptake of water and nutrients resulting in water imbalance and nutrient deficiency, the severity depending on the species (Paivoke and Simola 2001). For example, As significantly increases the accumulation of N, P, K, Ca, and Mg in the shoots of hydroponically grown *Phaseulus vulgaris* plants (Carbonell-Barrachina et al. 1997), but reduces the uptake of macronutrients such as K, Ca, and Mg, and micronutrients such as B, Cu, Mn, and Zn, in tomato plants (Carbonell-Barrachina et al. 1994). Similarly, arsenite reduces the uptake of Si, Mn, Zn, Cu, P, and K in rice plants and the translocation of these minerals to the shoot, possibly by interacting with the –SH groups of transporters (Hoffmann and Schenk 2011). Interestingly, in some hyperaccumulator species, such as *Pteris vittata*, low levels of arsenate stimulate phosphate accumulation in the fronds and significantly enhance growth (Tu and Ma 2005). The water content, water potential, and stomatal conductance of *Atriplex atacamensis* (Phil) leaves and roots were significantly reduced after prolonged exposure to As (Vromman et al. 2011).

Following absorption, As is thought to interfere with essential phosphate metabolism because the corresponding enzymes can also reduce As(V) to As(III) (Smith et al. 2010). Moreover, As(V) can be reduced *nonenzymatically* by glutathione (at least in vitro; Meharg and Hartley-Whitaker 2002) which is abundant in plants. Although As is not redox-active, it can stimulate the production of ROS through the conversion of arsenate to arsenite (Meharg and Hartley-Whitaker 2002), and can thus induce lipid peroxidation and cellular damages (Gunes et al. 2009). Maize leaves and roots exposed to As(V) produce antioxidant enzymes such as APX in response to the oxidative stress, whereas SOD activity declines. Conversely, higher levels of CAT activity were measured in maize shoots and roots exposed to high concentrations of As(III) (Duquesnoy et al. 2010). In *Bacopa monnieri* plants exposed to moderate levels of As, the activities of GSR, SOD, GPX, APX, and CAT were stimulated in a differential but coordinated manner in the leaves and roots, presumably representing a global response to As toxicity (Mishra et al. 2011). *Artemisia annua* plants treated with As showed a dose-dependent increase in the activities of SOD, APX, GSR, and GPX followed by a gradual decline at higher concentrations, again suggesting a coordinated response to the oxidative stress caused by As toxicity (Rai et al. 2011b).

1.4 Essential Metal Ions: Nickel, Copper, Iron, Manganese, Zinc, and Selenium

1.4.1 Nickel

Nickel (Ni) is abundant in rocks as a free metal and as a complex with other metal ions such as Fe. Like other heavy metals, anthropogenic activities such as mining, smelting, burning fossil fuels, vehicle emissions, waste disposal, electroplating, and the manufacture and disposal of batteries contribute to the release of Ni into the environment (Alloway 1995; Salt et al. 2000). Like Cr, Ni exists in many oxidation states that complicate the investigation of toxicity mechanisms in plants. However, Ni^{2+} is the prevalent oxidation state in soils because it is stable over a wide range of pH and redox conditions (Yusuf et al. 2011).

Unlike the metals discussed above, Ni is an essential micronutrient because it is required as a cofactor in enzymes such as urease, where it usually coordinates with cysteine residues (Dixon et al. 1980). Ni deficiency therefore reduces urease activity, disrupts nitrogen metabolism, and leads to the accumulation of toxic amounts of urea, which manifests as chlorosis and necrosis (Yusuf et al. 2011). These effects are particularly severe in species that develop symbiotic relationships with nitrogen-fixing bacteria, because amino acid metabolism and the ornithine cycle are also compromised (Eskew et al. 1983). Low levels of Ni thus promote growth and development in many crops, including oilseed rape, cotton, sweet pepper, tomato, and potato (Gerendas and Sattelmacher 1999; Welch 1981).

Although Ni is an essential nutrient, excess amounts are toxic in many species and the effects are already apparent during germination in species such as pigeon pea, maize, wheat, and *B. juncea* (Rao and Sresty 2000; Bhardwaj et al. 2007; Gajewska and Sklodowska 2008; Sharma et al. 2008). Later in development, the inhibition of root growth is a prevalent symptom of Ni toxicity, as seen in *B. juncea* plants and wheat seedlings (Alam et al. 2007; Gajewska et al. 2006). The uptake of nutrients is also affected by Ni excess, and its chemical similarity to nutrients such as Ca, Mg, Mn, Fe, Cu, and Zn suggests that Ni may compete with these minerals for uptake and subsequent utilization (Chen et al. 2009). Excess Ni may therefore induce deficiency symptoms for other nutrients, e.g., in barley plants where toxic levels of Ni reduce the absorption of Ca, Fe, K, Mg, Mn, P and Zn (Brune and Deitz 1995). Excess Ni also reduces the level of nitrogen in the leaves and roots of *Cicer arietinum* and *Vigna radiata* plants (Athar and Ahmad 2002). Ni exposure reduces the phosphorus content of *Helinathus annus* and *Hyptis suaveolens* plants (Pillay et al. 1996).

Like other heavy metals, Ni also disrupts the water balance in plants, perhaps reflecting the cumulative effects of Ni toxicity. Indeed, Ni treatment reduces the transpiration rate, leaf growth, and the leaf blade area in wheat (Bishnoi et al. 1993; Chen et al. 2009), and increases the level of endogenous ABA in *Brassica oleracea* leaves, the plant hormone that promotes stomatal closure (Molas 1997).

Ni has a substantial impact on photosynthesis because it disrupts the thylakoid membranes and reduces the chlorophyll content (Molas 2002; Ahmad et al. 2007; Alam et al. 2007). Like other metals, Ni can displace Mg from chlorophyll and enzymes such as RuBisCO that contain Mg ion as cofactor (Yusuf et al. 2011). Moreover, both PSI and the PSII appear to be sensitive to Ni in *Spinacea oleracea*, where the analysis of submembrane fractions showed that Ni^{2+} strongly inhibits oxygen evolution by depleting the extrinsic 16 and 2 kDa polypeptides associated with the OEC (Boisvert et al. 2007).

Unlike Fe and Cu (see below), Ni is not a redox-active metal and cannot generate ROS directly, yet the presence of excess Ni nevertheless induces the formation of superoxide anions, hydroxyl radicals, and hydrogen peroxide in many species, including *Alyssum bertolonii* and wheat (Boominathan and Doran 2002; Gajewska and Sklodowska 2007). Interestingly, the prolonged presence of these ROS does not increase the amount of lipid peroxidation in wheat, perhaps due to the concomitant increase in APX and GPX activities (Gajewska and Sklodowska 2007). In a different experiment, the treatment of *Triticum durum* with Ni^{2+} resulted in a significant increase in membrane lipid peroxidation, along with higher levels of H_2O_2 and O_2^- (Hao et al. 2006). Similarly, H_2O_2 levels rose significantly following the exposure of both *Alyssum bertoloni* and *Nicotiana tabacum* to Ni, although there was little oxidative damage in *A. bertolonii* roots reflecting the much higher endogenous activities of CAT and SOD in this species (Boominathan and Doran 2002). The induction or repression of antioxidant enzymes is species dependent and also reflects the magnitude of the stress. For example, while in *A. bertolonii*, SOD, CAT, and APX activities decline in response to Ni,

the opposite pattern is observed in wheat and maize (Baccouch et al. 2001; Gajewska et al. 2006).

1.4.2 Copper

Copper (Cu) is an essential nutrient that acts as a structural component in regulatory proteins, as a redox component in chloroplast and mitochondrial electron transport, and as a cofactor in enzymes such as Cu-SOD, cytochrome oxidase, plastocyanin, and laccase, therefore participating in a variety of metabolic processes, such as hormone signaling, cell wall metabolism, and stress response. Cu deficiency symptoms include chlorosis and necrosis at the leaf tip, together with leaf twisting and malformation, reflecting the impairment of photosynthetic electron transfer, the loss of essential pigments, and the degeneration of thykaloids.

Cu in plants exists in two oxidation states, Cu^{2+} and Cu^{+}, and *redox cycling* between these states produces hydroxyl radicals (Li et al. 2002). Moreover, since Cu is a redox-active transition metal it can generate ROS directly via the Fenton or Haber–Weiss reactions (Halliwell and Gutteridge 1984), catalyzing the formation of hydroxyl radicals (OH') via non-enzymatic chemical reactions between H_2O_2 and the superoxide anion (O_2^{-}) (see Fig. 1.1). This enhanced capacity to produce ROS is the primary mechanism of Cu toxicity.

Further visible symptoms of Cu toxicity include stunted growth and reduced initiation and development of lateral roots. Nitrogen metabolism and fixation are disrupted in *Glycine max* plants exposed to excess Cu, whereas nitrate and free amino acid levels become depleted in similarly treated *Vitis vinifera* plants (Llorens et al. 2000).

One of the most potent effects of Cu toxicity is to inhibit oxygen evolution, accompanied by a significant reduction in photosynthetic yield, which may reflect a specific interaction between Cu ions and Tyr_Z and Tyr_D on the D2 protein of PSII (Sabat 1996; Maksymiec and Baszynski 1999). The photosynthetic machinery is strongly inhibited by excess Cu, resulting in the degradation of stromal lamellae, the loss of grana stacking, and an increase in the number and size of plastoglobules (Yruela 2005). The extrinsic proteins of the OEC (PsbO, PsbP and PsbQ) are degraded in the presence of excess Cu (Yruela 2005) and the redox state of cytochrome b_{559} is compromised (Roncel et al. 2001). Important enzymes of the Calvin cycle are also inhibited by Cu, including RuBisCO and phosphoenol pyruvate caboxylase (Balsberg Pahlsson 1989). Cu stress increases susceptibility to photoinhibition in both isolated thylakoids and intact leaves, due to the Cu-induced reduction of chlorophyll content (Pätsikkä et al. 2002).

1.4.3 Iron

Iron (Fe) is an essential nutrient in plants, with crucial roles in processes such as photosynthetic electron transport, oxidative stress tolerance, mitochondrial

respiration, nitrogen fixation, hormone synthesis and organelle maintenance (Hänsch and Mendel 2009). It exists in soils as both Fe^{3+} and Fe^{2+}, although only the latter is soluble and suitable for absorption by plants. Fe geochemistry is influenced by soil characteristics such as pH, organic matter content, and oxygen levels. Fe^{3+} is reduced to Fe^{2+} by soil microorganisms, root exudates, and chemical reactions in the soil. An interesting feature of Fe toxicity is that it is greatly dependent on the soil type; it is often linked to P and Zn deficiency, water logging, and anoxic conditions (Ponnamperuma et al. 1967).

Fe is a highly reactive redox metal that produces large amounts of hydrogen peroxide and superoxide during the reduction of molecular oxygen. Therefore, excess Fe induces the formation of hydroxyl radicals that can damage many targets, including DNA, proteins, lipids, and sugars. A typical visual symptom of iron toxicity in rice is the *bronzing* of leaves due to the accumulation of oxidized polyphenols (Becker and Asch 2005). In *Nicotiana plumbaginifolia* and pea plants, Fe toxicity induces the formation of brown necrotic spots covering the whole leaf surface (Kampfenkel et al. 1995). In wetland plants, iron oxyhydroxide deposits (*iron plaques*) may form on the roots in Fe-rich soils. These deposits reduce further Fe absorption, thus constituting a protective mechanism against Fe toxicity, but they may also sequester nutrients such as phosphate and therefore result in deficiency symptoms (Batty and Younger 2003). Excess Fe reduces water transpiration and photosynthetic activity (Kampfenkel et al. 1995; Adamski et al. 2011), which manifests, for instance, as a sharp decline in the chlorophyll content of potato leaves (Chatterjee et al. 2006) together with a loss of thylakoid membrane integrity (Adamski et al. 2011), and as a reduction in CO_2 fixation and starch accumulation in *N. plumbaginifolia* plants (Kampfenkel et al. 1995). Fe stress in sweet potatoes inhibits the reduction of plastoquinone but appears not to affect electron flux from plastoquinone to the final electron acceptor (Adamski et al. 2011).

Because Fe participates in the Haber–Weiss and Fenton reactions, one of the main toxicity mechanisms is the direct formation of ROS and the induction of oxidative stress, which has been documented in *N. plumbaginifolia,* rice, sunflowers, and soybean (Kamplenken et al. 1995; Fang et al. 2001). As a response to the increased oxidative stress, activity of APX and GSR were increased by Fe^{2+} excess in rice, as well as the amount of free-radical scavengers, such as mannitol and reduced GS (Fang et al. 2001). Application of Fe^{2+} ions was also found to induce peroxidase activity in rice leaves, which could be mediated by *de novo* synthesis of the enzyme at transcriptional level (Peng et al. 1996). CAT and APX were shown to be induced in *N. plumbaginifolia* plants exposed to excess Fe (Kampfenkel et al. 1995).

1.4.4 Manganese

Manganese (Mn) acts as cofactor in many enzymes, including Mn-superoxide dismutase, catalase, pyruvate carboxylase, phosphoenol pyruvate carboxykinase,

malic enzyme, and isocitrate lyase (Hänsch and Mendel 2009). It also has a critical role in oxygen evolution because four Mn atoms are required in the OEC subunits of PSII. The oxidation state and bioavailability of Mn is strongly dependent on soil pH, with the more-soluble Mn(II) form becoming more abundant below pH 5.5 (thus risking Mn toxicity) and the less-soluble manganic forms Mn(III), Mn(IV), and Mn(VII) becoming more abundant above pH 6.5 (thus risking Mn deficiency). Mn utilization and toxicity is therefore exquisitely sensitive to fertilizer applications, particularly ammonia-based chemicals that cause soil acidification (Dučic and Polle 2005).

In *Zea mays* for instance, Mn deficiency restricted the uptake and transport of NO_3^-, inhibited the activity of enzymes related to N-metabolism, such as nitrate reductase, glutamine synthetase, and glutamic-oxaloace transaminase. Mn deficiency also promotes glutamate dehydrogenase activity, reduces chlorophyll and protein synthesis, and thus inhibits growth and development (Gong et al. 2011).

Excess Mn, for instance in low-drained and acidic soils, is toxic in most plant species, inducing general symptoms such as stunting, chlorosis, crinkled leaves, brown necrotic lesions, and death in the most severe cases (Dučic and Polle 2005). Pea plants exposed to excess Mn had lower root and shoot biomass, lower chlorophyll and carotenoid contents, and lower glutamine synthetase and glutamate synthase activities than controls (Gangwar et al. 2011). In *Vigna radiata* leaves, Mn treatment caused a progressive reduction in the total carotenoid, total chlorophyll and chlorophyll *a* contents, and inhibited the Hill activity of isolated chloroplasts, thus reducing the rates of photosynthesis and of CO_2 uptake (Sinha et al. 2002). The decline in photosynthetic activity following exposure to excess Mn also reflects the production of ROS such as H_2O_2 and $O_2^{\cdot-}$ (Gangwar et al. 2011; Shi and Zhu 2008), which cause lipid preoxidation in the thylakoid membranes and damage enzymes such as RuBisCO (Subrahmanyam and Rathore 2001). In plants exposed to high Mn levels, the activity of SOD, PRX, APX, DHAR and GSR is increased. Excess Mn can also cause deficiencies for other nutrients such as Fe, Mg, Zn and Ca, although the mechanism is unclear (Shi and Zhu 2008).

1.4.5 Zinc

Zinc (Zn) is an essential element and participates in many processes of plant life, such as enzyme activation, metabolism of proteins and carbohydrates, lipids, and nucleic acids. Zn is a cofactor in many plant enzymes with important roles in primary metabolism (e.g., alcohol dehydrogenase, glutamic dehydrogenase, carbonic anhydrase, enzymes involved in electron transport, and antioxidant enzymes) and is also an integral component of several transcription factors (e.g., zinc finger transcription factors) (Chang et al. 2005). Zn deficiency initially manifests as a reduction in internodal growth, which reduces stem length and causes plants to acquire rosette-like *habitus*, and in later stages leaves may develop deficiency symptoms such as chlorosis and necrotic spots (Sharma 2006).

Zn is usually abundant in the mineral component of soils and is present as sulfide, sulfate, oxide, carbonate, phosphate, and silicate, e.g., sphalerite, zincosite, gahnite, smithsonite, hopeite, and willemite (Broadley et al. 2007). Zn levels in soils have also increased through human activities such as mining, smelting, limestone topping, burning fossil fuels, and the use of phosphate-based fertilizers (Nriagu 1996). Under physiological conditions, the relatively stable Zn^{2+} redox state is prevalent in soils although this depends on the soil type, clay and mineral content, moisture content, weathering rates, organic matter content, and microbial populations. The most important parameter is soil pH; Zn is more readily adsorbed on cation exchange sites at high pH, while it is more soluble in acidic soils with low levels of soluble organic matter and these conditions favor Zn toxicity (Broadley et al. 2007).

The initial symptoms of Zn toxicity are chlorosis and even reddening of the leaves in severe cases, due to anthocyanin production (Fontes and Cox 1995). This is followed by the appearance of necrotic brown spots on the leaves of some species, accompanied by stunting and reduced yield (Harmens et al. 1993; Broadley et al. 2007). Zn toxicity also inhibits primary root growth and the emergence of lateral roots (Ren et al. 1993). High levels of Zn can displace Mg from the OEC water splitting site of PSII, thus inhibiting both photosystems and the electron transport chain, as seen in Zn-treated *Phaseolus vulgaris* plants (Van Assche and Clijsters 1986). In *Spinacea oleracea*, plastidial ATP synthesis is also inhibited by Zn toxicity (Teige et al. 1990). Zn is a non-redox metal but it can generate ROS indirectly, leading to defense responses including the induction of antioxidant enzymes such as SOD, CAT, and GPX (Prasad et al. 1999; Chang et al. 2005). The oxidative burst induced by Zn toxicity could also be responsible for the cell death observed in rice root cells, since the application of exogenous ROS scavengers was able to increase cell viability; this result points to a relationship between Zn toxicity and programmed cell death (Chang et al. 2005).

1.4.6 Selenium

Although it has a relatively low density (4.82 g cm^{-3}) and according to the periodic table, it is a non-metal, Se is considered in this article because it shares many biological properties with other minerals (e.g., it exists in the soil in multiple forms and can induce toxicity symptoms depending on availability and abundance). In aerobic soils, inorganic Se is present in numerous oxidation states, the most common being selenite [SeO_3^{2-}, Se(IV)] and selenate [SeO_4^{2-}, Se(VI)], which are the most soluble and the most toxic forms. Elemental selenium (Se^0), which is more prevalent under anaerobic conditions, is insoluble and biologically inert. Inorganic Se is released naturally during the erosion and the leaching of seleniferous minerals, and many human activities also produce inorganic Se, including mining, burning fossil fuels, and glass manufacturing (Di Gregorio et al. 2005). Se is a nutrient, essential in traces for bacteria, animals, and algae, being

a component of few enzymes, such as the glutathione peroxidase, in which it is incorporated as Se-cystein, encoded by the opal codon UGA (Fu et al. 2002). The status of Se as a micronutrient in higher plants remains controversial. Se stimulates the growth of Se-hyperaccumulators such as *Astragalus pectinatus* (Trelease and Trelease 1939), but true seleno-proteins similar to those found in microbes, animals, and algae have not yet been identified (Fu et al. 2002). Se can also regulate the water status of plants subjected to drought stress, increasing the water uptake capacity of roots and inhibiting the stress-induced accumulation of proline (Kuznestov et al. 2003). At low concentrations, Se behaves as an antioxidant in *Lolium perenne*, inhibiting lipid peroxidation and enhancing the activity of GPX (Hartikainen et al. 2000). The foliar application of Se to heat-stressed sorghum plants alleviates oxidative stress by enhancing the antioxidative cycle (Djanaguiraman et al. 2010).

Both selenate and selenite are readily absorbed by the roots of many plant species and are efficiently distributed to other tissues. Here, cellular metabolism converts them into Se-metabolites, which act as analogs of organic sulfur compounds and interfere with the metabolic processes in which these sulfur compounds normally participate. Moreover, the sulfur-containing amino acids cysteine and methionine are replaced by seleno-cysteine and seleno-methionine, which become incorporated into proteins, leading to significant alterations in protein function and structure due to the differences in size and ionization properties between Se and sulfur (Brown and Shrift 1982). High levels of Se trigger a range of toxicity symptoms including stunting, chlorosis, drying of leaves, aberrant protein metabolism, and eventually death. Symptoms vary according to (i) the age of the plant (older plants are more resistant) (ii) the assimilation characteristics of the plant (certain species hyperaccumulate Se); and (iii) the availability of sulfates, which compete with Se and mitigate its toxicity (Terry et al. 2000). Proteomic analysis in rice showed that Se toxicity led to a gradual decline in the chloroplast enzymes involved in the redox cycle (ROS scavenging system) and a corresponding gradual increase in the abundance of ROS and damage to the photosynthetic apparatus, in particular the chlorophyll *a–b* binding proteins and RuBisCO (Wang et al. 2012). This inhibition of photosynthesis combined with the impact of seleno-cysteine and seleno-methionine on protein synthesis and metabolism could therefore explain the reduced growth of rice seedlings caused by excess Se. Finally, it appears that excess Se can also imbalance the uptake of other nutrients, e.g., increasing the intracellular concentration of Ca, Fe, Cu, Mn, and Zn but reducing P levels in *Trifolium repens* (Wu and Huang 1992). Furthermore, due to the chemical similarity between S and Se, SeO_4^{2-} and SO_4^{2-} probably compete for absorption and transport (Grant et al. 2011), reducing the amount of SO_4^{2-} absorbed by the roots (Leggett and Epstein 1956).

References

Adamski JM, Peters JA, Danieloski R, Bacarin MA (2011) Excess iron-induced changes in the photosynthetic characteristics of sweet potato. J Plant Physiol 168:2056–2062

Ahmad MSA, Hussain M, Saddiq R, Alvi AK (2007) Mungbean: a nickel indicator, accumulator or excluder? Bull Environ Contam Toxicol 78:319–324

Alam MM, Hayat S, Ali B, Ahmad A (2007) Effect of 28- homobrassinolide treatment on nickel toxicity in *Brassica juncea*. Photosynthetica 45:139–142

Alloway BJ (1995) Heavy metal in soils. Blackie Academic and Professional, London

Astolfi S, Zuchi S, Passera C (2004) Role of sulphur availability on cadmium-induced changes of nitrogen and sulphur metabolism in maize (*Zea mays* L.) leaves. J Plant Physiol 161:795–802

Athar R, Ahmad M (2002) Heavy metal toxicity in legume-microsymbiont system. J Plant Nutr 25:369–386

Baccouch S, Chaoui A, El Ferjani E (2001) Nickel toxicity induces oxidative damage in *Zea mays* roots. J Plant Nutr 24:1085–1097

Balestrasse KB, Benavides MP, Gallego SM, Tomaro ML (2003) Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. Func Plant Biol 30:57–64

Balsberg Pahlsson AM (1989) Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water Air Soil Pollut 47:287–319

Barton LL, Johnson GV, O'Nan AG, Wagener BM (2000) Inhibition of ferric chelate reductase in alfalfa roots by cobalt, nickel, chromium, and copper. J Plant Nutr 23:1833–1845

Batty LC, Younger PL (2003) Effects of external iron concentration upon seedling growth and uptake of Fe and phosphate by the common reed, *Phragmites australis* (Cav.) trin ex. steudel. Ann Bot 92:801–806

Becker M, Asch F (2005) Iron toxicity in rice—conditions and management concepts. J Plant Nutr Soil Sci 168:558–573

Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34

Bernier M, Carpentier R (1995) The action of mercury on the binding of the extrinsic polypeptides associated with the water oxidizing complex of photosystem II. FEBS Lett 360:251–254

Bestwick CS, Brown IR, Mansfield JW (1998) Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a non-host hypersensitive reaction in lettuce. Plant Physiol 118:1067–1078

Bhardwaj R, Arora N, Sharma P, Arora HK (2007) Effects of 28-homobrassinolide on seedling growth, lipid peroxidation and antioxidative enzyme activities under nickel stress in seedlings of *Zea mays* (L). Asian J Plant Sci 6:765–772

Bishnoi NR, Sheoran IS, Singh R (1993) Influence of cadmium and nickel on photosynthesis and water relations in wheat leaves of differential insertion levels. Photosynthetica 28:473–479

Boisvert S, Joly D, Leclerc S, Govindachary S, Harnois J, Carpentier R (2007) Inhibition of the oxygen-evolving complex of photosystem II and depletion of extrinsic polypeptides by nickel. Biometals 20:879–889

Boominathan R, Doran PM (2002) Ni-induced oxidative stress in roots of the Ni hyperaccumulator, *Alyssum bertolonii*. New Phytol 156:205–215

Boussama N, Ouariti O, Suzuki A, Ghorbel MH (1999) Cd-stress on nitrogen assimilation. J Plant Physiol 155:310–317

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702

Brown TA, Shrift A (1982) Selenium: toxicity and tolerance in higher plants. Biol Rev 57:59–84

Brune A, Deitz KJ (1995) A comparative analysis of element composition of roots and leaves of barley seedlings grown in the presence of toxic cadmium, molybdenum, nickel and zinc concentrations. J Plant Nutr 18:853–868

Carbonell-Barrachina A, Burlò-Carbonell F, Mataix-Beneyto J (1994) Effect of arsenite on the concentration of micronutrients in tomato plants grown in hydroponic culture. J Plant Nutr 17:1887–1903

Carbonell-Barrachina A, Burlò-Carbonell F, Mataix-Beneyto J (1997) Effect of sodium arsenite and sodium chloride on bean plant nutrition (macronutrients). J Plant Nutr 20:1617–1633

Chaffei C, Pageau K, Suzuki A, Gouia H, Ghorbel HM, Mascalaux-Daubresse C (2004) Cadmium toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. Plant Cell Physiol 45:1681–1693

Chang HB, Lin CW, Huang HJ (2005) Zinc-induced cell death in rice (*Oryza sativa* L.) roots. Plant Growth Regul 46:261–266

Chatterjee C, Gopal R, Dube BK (2006) Impact of iron stress on biomass, yield, metabolism and quality of potato (*Solanum tuberosum* L). Sci Hortic 108:1–6

Chen C, Huang D, Liu J (2009) Functions and toxicity of nickel in plants: recent advances and future prospects. Clean 37:304–313

Cho UH, Park JO (2000) Mercury-induced oxidative stress in tomato seedlings. Plant Sci 156:1–9

Christophersen HM, Smith SE, Pope S, Smith FA (2009) No evidence for competition between arsenate and phosphate for uptake from soil by medic or barley. Env Int 35:485–490

Clemens S (2006) Evolution and function of phytochelatin synthases. J Plant Physiol 163: 319–332

DalCorso G, Farinati S, Maistri S, Furini A (2008) How plants cope with cadmium: staking all on metabolism and gene expression. J Integr Plant Biol 50:1268–1280

DalCorso G, Farinati S, Furini A (2010) Regulatory networks of cadmium stress in plants. Plant Signal Behav 5:663–667

Dat JF, Vandenabeele S, Vranovà E, Van Montagu M, Inzè D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57: 779–795

Di Gregorio S, Lampis S, Vallini G (2005) Selenite precipitation by a rhizospheric strain of *Stenotrophomonas* sp. isolated from the root system of *Astragalus bisulcatus*: a biotechnological perspective. Environ Int 31:233–241

Dixit V, Pandey V, Shyam R (2002) Chromium ions inactivate electron transport and enhance superoxide generation in vivo in pea (*Pisum sativum* L. cv: Azad) root mitochondria. Plant Cell Environ 25:687–693

Dixon H, Hinds JA, Fihelly AK, Gozala C, Winzor DJ, Blakeley RL, Zerner B (1980) Jack bean urease (EC 3.5.1.5). IV. The molecular size and mechanism of inhibition by hydroxamic acids. Spectrophotometric fixation of enzymes with reversible inhibitors. Can J Biochem 58:1323–1334

Djanaguiraman M, Prasad PV, Seppanen M (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol Biochem 48:999–1007

Dučic T, Polle A (2005) Transport and detoxification of manganese and copper in plants. Braz J Plant Physiol 17:103–112

Duquesnoy I, Champeau GM, Evray G, Ledoigt G, Piquet-Pissaloux A (2010) Enzymatic adaptations to arsenic-induced oxidative stress in Zea mays and genotoxic effect of arsenic in root tips of *Vicia faba* and *Zea mays*. C R Biol 333:814–824

Eskew DL, Welch RM, Cary EE (1983) Nickel: an essential micronutrient for legumes and possibly all higher plants. Science 222:691–693

Eun SO, Yon HS, Lee Y (2000) Lead distrurbs microtubule organization in the root meristem of *Zea mays*. Physiol Plant 110:357–365

Fang WC, Wang JW, Lin CC, Kao CH (2001) Iron induction of lipid peroxidation and effects on antioxidative enzyme activities in rice leaves. Plant Growth Regul 35:75–80

Farinati S, DalCorso G, Varotto S, Furini A (2010) The *Brassica juncea* BjCdR15, an ortholog of *Arabidopsis* TGA3, is a regulator of cadmium uptake, transport and accumulation in shoots and confers cadmium tolerance in transgenic plants. New Phytol 185:964–978

Fontes RLF, Cox FR (1995) Effects of sulfur supply on soybean plants exposed to zinc toxicity. J Plant Nut 18:1893–1906

Fu LH, Wang XF, Eyal Y, She YM, Donald LJ, Standing KG, Ben-Hayyim G (2002) A selenoprotein in the plant kingdom. Mass spectrometry confirms that an opal codon (UGA) encodes selenocysteine in *Chlamydomonas reinhardtii* gluththione peroxidase. J Biol Chem 277:25983–25991

Führs H, Götze S, Specht A, Erban A, Gallien S, Heintz D, Van Dorsselaer A, Kopka J, Braun HP, Horst WJ (2009) Characterization of leaf apoplastic peroxidases and metabolites in *Vigna unguiculata* in response to toxic manganese supply and silicon. J Exp Bot 60:1663–1678

Gabbrielli R, Pandolfini T (1984) Effect of Mg^{2+} and Ca^{2+} on the response to nickel toxicity in a serpentine endemic and nickel accumulating species. Physiol Plant 62:540–544

Gajewska E, Sklodowska M (2007) Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. Biometals 20:27–36

Gajewska E, Sklodowska M (2008) Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. Plant Growth Regul 54:179–188

Gajewska E, Sklodowska M, Slaba M, Mazur J (2006) Effect of nickel on antioxidative enzyme activities, proline and chlorophyll content in wheat shoots. Biol Plant 50:653–659

Gangwar S, Singh VP, Maurya JN (2011) Responses of *Pisum sativum* L. to exogenous indole acetic acid application under manganese toxicity. Bull Environ Contam Toxicol 86:605–609

Gerendas J, Sattelmacher B (1999) Influence of Ni supply on growth and nitrogen metabolism of *Brassica napus* L. grown with NH_4NO_3 or urea as N source. Ann Bot 83:65–71

Godbold DL, Kettner C (1991) Lead influences root growth and mineral nutrition of *Picea abies* seedlings. J Plant Physiol 139:95–99

Gong X, Qu C, Liu C, Hong M, Wang L, Hong F (2011) Effects of manganese deficiency and added cerium on nitrogen metabolism of maize. Biol Trace Elem Res 144:1240–1250

Grant K, Carey NM, Mendoza M, Schulze J, Pilon M, Pilon-Smits EA, van Hoewyk D (2011) Adenosine 5′-phosphosulfate reductase (APR2) mutation in *Arabidopsis* implicates glutathione deficiency in selenate toxicity. Biochem J 438:325–335

Gunes A, Pilbeam DJ, Inal A (2009) Effect of arsenic phosphorus interaction on arsenic-induced oxidative stress in chickpea plants. Plant Soil 314:211–220

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219:1–14

Han FX, Su Y, Monts DL, Waggoner AC, Plodinec JM (2006) Binding, distribution, and plant uptake of mercury in a soil from Oak Ridge, Tennessee, USA. Sci Total Environ 368:753–768

Hänsch R, Mendel RR (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B Cl). Curr Opin Plant Biol 12:259–266

Hao F, Wang X, Chen J (2006) Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Sci 170:151–158

Harmens H, Gusmao NGCPB, Hartog DPR, Verkeij JAC, Ernst WHO (1993) Uptake and transport of zinc in zinc-sensitive and zinc-tolerant *Silene vulgaris*. J Plant Physiol 141: 309–315

Hartikainen H, Xue T, Piironen V (2000) Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant Soil 225:193–200

Hoffmann H, Schenk MK (2011) Arsenite toxicity and uptake rate of rice (*Oryza sativa* L.) in vivo. Environ Pollut 159:2398–2404

Horst WJ, Fecht M, Naumann A, Wissemeier AH, Maier P (1999) Physiology of manganese toxicity and tolerance in *Vigna unguiculata* (L.) Walp. J Plant Nut Soil Sci 162:263–274

Jain R, Srivastava S, Madan VK, Jain R (2000) Influence of chromium on growth and cell division of sugarcane. Indian J Plant Physiol 5:228–231

Kampfenkel K, Van Montagu M, Inzé D (1995) Effects of iron excess on *Nicotiana plumbagnifolia* plants—implications to oxidative stress. Plant Physiol 107:725–735

Kuznetsov VV, Kholodova VP, Kuznetsov VV, Yagodin BA (2003) Selenium regulates the water status of plants exposed to drought. Doklady Biol Sci 390:266–268

Lane TW, Morel FM (2000) A biological function for cadmium in marine diatoms. Proc Natl Acad Sci U S A 97:4627–4631

Le Bot J, Goss MJ, Carvalho MJGPR, Beusichem ML, Kirkby EA (1990) The significance of the magnesium to manganese ratio in plant tissues for growth and alleviation of manganese toxicity in tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*) plants. Plant Soil 124:205–210

Leggett JE, Epstein E (1956) Kinetics of sulfate absorption by barley roots. Plant Physiol 31: 222–226

Li Y, Seacat A, Kuppusamy P, Zweier JL, Yager JD, Trush MA (2002) Copper redox-dependent activation of 2-tert-butyl(1,4)hydroquinone: formation of reactive oxygen species and induction of oxidative DNA damage in isolated DNA and cultured rat hepatocytes. Mutat Res 518:123–133

Ling T, Fangke Y, Jun R (2010) Effect of mercury to seed germination, coleoptile growth and root elongation of four vegetables. Res J Phytochem 4:225–233

Liu X, Zhang S, Shan X, Zhu YG (2005) Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat. Chemosphere 61:293–301

Llorens N, Arola L, Bladé C, Mas A (2000) Effects of copper exposure upon nitrogen metabolism in tissue cultured *Vitis vinifera*. Plant Sci 160:159–163

MacRobbie EA, Kurup S (2007) Signalling mechanisms in the regulation of vacuolar ion release in guard cells. New Phytol 175:630–640

Maksymiec W, Baszynski T (1999) The role of Ca^{2+} ions in modulating changes induced in bean plants by an excess of Cu^{2+} ions. Chlorophyll fluorescence measurements. Physiol Plant 105:562–568

Malecka A, Jarmuszkiewicz W, Tomaszewska B (2001) Antioxidative defense to lead stress in subcellular compartments of pea root cells. Acta Biochim Polon 48:687–698

Masclaux C, Valadier M, Brugière N, Morot-Gaudry J, Hirel B (2000) Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. Planta 211:510–518

Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol 154:29–43

Mishra S, Srivastava S, Dwivedi S, Tripathi RD (2011) Investigation of biochemical responses of Bacopa monnieri L. upon exposure to arsenate. Environ Toxicol. doi:10.1002/tox.20733

Molas J (1997) Changes in morphological and anatomical structure of cabbage (Brassica oleracera L.) outer leaves and in ultrastructure of their chloroplasts caused by an in vitro excess of nickel. Photosynthetica 34:513–522

Molas J (2002) Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni II complexes. Environ Exp Bot 47:115–126

Mysliwa-Kurdziel B, Prasad MNV, Strzalka K (2004) Photosynthesis in heavy metal stressed plants. In: Prasad MNV (ed) Heavy metal stress in plants: from biomolecules to ecosystems. Narosa Publishing House, New Delhi

Nriagu JO (1996) A history of global metal pollution. Science 272:223–224

Paivoke AEA, Simola LK (2001) Arsenate toxicity to Pisum sativum: mineral nutrients, chlorophyll content and phytase activity. Ecotox Environ Safe 49:111–121

Panda SK, Choudhury S (2005) Chromium stress in plants. Braz J Plant Physiol 17:95–102

Panda SK, Patra HK (2000) Nitrate and ammonium ions effect on the chromium toxicity in developing wheat seedlings. P Natl Acad Sci India B 70:75–80

Parys E, Romanowska E, Siedlecka M, Poskuta JW (1998) The effect of lead on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of Pisum sativum. Acta Physiol Plant 20:313–322

Patra M, Bhowmik N, Bandopadhyay B, Sharma A (2004) Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ Exp Bot 52:199–223

Pätsikkä E, Kairavuo M, Sersen F, Aro E-M, Tyystjärvi E (2002) Excess copper predisposes photosystem II to photoinhibition in vivo by outcompeting iron and causing decrease in leaf chlorophyll. Plant Physiol 129:1359–1367

Peng XX, Yu XL, Li MQ, Yamauchi M (1996) Induction of proxidase by Fe2+ in detached rice leaves. Plant Soil 180:159–163

Perfus-Barbeoch L, Leonhardt N, Vavaddeur A, Forestier C (2002) Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. Plant J 32:539–548

Pigna M, Cozzolino V, Violante A, Meharg AA (2009) Influence of phosphate on the arsenic uptake by wheat (*Triticum durum* L.) irrigated with arsenic solutions at three different concentrations. Water Air Soil Pollut 197:371–380

Pillay SV, Rao VS, Rao KVN (1996) Effect of nickel toxicity in *Hyptis suareeolens* (L.) Poit. and *Helianthus annuus* L. Indian J Plant Physiol 1:153–156

Ponnamperuma FN, Tianco EM, Loy T (1967) Redox equilibria in flooded soils: the iron hydroxide systems. Soil Sci 103:374–382

Prasad K, Saradhi PP, Sharmila P (1999) Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environ Exp Bot 42:1–10

Prasad MNV, Greger M, Landberg T (2001) *Acacia nilotica* L bark removes toxic elements from solution: corroboration from toxicity bioassay using *Salix viminalis* L in hydroponic system. Int J Phytoremed 3:289–300

Rai AN, Srivastava S, Paladi R, Suprasanna P (2011a) Calcium supplementation modulates arsenic-induced alterations and augments arsenic accumulation in callus cultures of Indian mustard (*Brassica juncea* (L.) Czern.). Protoplasma

Rai R, Pandey S, Rai SP (2011b) Arsenic-induced changes in morphological, physiological, and biochemical attributes and artemisinin biosynthesis in *Artemisia annua*, an antimalarial plant. Ecotoxicology 20:1900–1913

Rao KVM, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea *(Cajanus cajan L.)* Millspauga in response to Zn and Ni stress. Plant Sci 157:113–128

Reeves RD, Baker AJM (2000) Metal-Accumulating Plants. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York

Ren F, Liu T, Liu H, Hu B (1993) Influence of zinc on the growth, distribution of elements, and metabolism of one-year old American ginseng plants. J Plant Nut 16:393–405

Romero-Puertas MC, Palma JM, Gomez LA, del Rio LA, Sandalio LM (2002) Cadmium causes oxidative modification of proteins in plants. Plant Cell Environ 25:677–686

Romero-Puertas MC, Rodriguez-Serrano M, Corpas FJ, Gomez M, del Rio LA, Sandalio LM (2004) Cadmium-induced subcellular accumulation of O_2^- and H_2O_2 in pea leaves. Plant Cell Environ 27:1122–1134

Roncel M, Ortega JM, Losada M (2001) Factors determining the special redox properties of photosynthetic cytochrome b559. Eur J Bioche 268:4961–4968

Sabat SC (1996) Copper iron inhibition of electron transport activity in sodium chloride washed photosystem II particle is partially prevented by calcium ion. Z Naturforsch 51c:179–184

Roth U, Von Roepenack-Lahaye E, Clemens S (2006) Proteome changes in Arabidopsis thaliana roots upon exposure to Cd^{2+}. J Exp Bot 57:4003–4013

Salt DE, Kato N, Kramer U, Smith RD, Raskin I (2000) The role of root exudates in nickel hyperaccumulation and tolerance in accumulator and nonaccumulator species of *Thlaspi*. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. CRS Press LLC, London

Samantaray S, Rout GR, Das P (2001) Induction, selection and characterization of Cr and Ni-tolerant cell lines of *Echinochloa colona* (L) in vitro. J Plant Physiol 158:1281–1290

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Scandalios JG (1990) Response of plant antioxidant defense genes to environmental stress. Adv Genet 28:1–41

Schuster E (1991) The behavior of mercury in the soil with special emphasis on complexation and adsorption processes—A review of the literature. Water Air Soil Pollut 56:667–680
Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365
Schützendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld- Heyser R, Godbold DL et al (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. Plant Physiol 75:887–898
Sersen F, Kralova K, Bumbalova A (1998) Action of mercury on the photosynthetic apparatus of spinach chloroplasts. Photosynthetica 35:551–559
Shanker AK, Pathmanabhan G (2004) Speciation dependant antioxidative response in roots and leaves of *Sorghum* (*Sorghum bicolor* (L) Moench cv CO 27) under Cr(III) and Cr(VI) stress. Plant Soil 265:141–151
Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753
Sharma CP (2006) Plant micronutrients. Science Publishers, Enfield
Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiology 17:35–52
Sharma DC, Sharma CP (1993) Chromium uptake and its effects on growth and biological yield of wheat. Cereal Res Commun 21:317–321
Sharma DC, Sharma CP (1996) Chromium uptake and toxicity effects on growth and metabolic activities in wheat, *Triticum aestivum* L. cv.UP Indian. J Exp Biol 34:689–691
Sharma Y, Leòn J, Raskin I, Davis KR (1996) Ozone-induced responses in *Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defence-related transcripts and induced resistance. Proc Natl Acad Sci U S A 93:5099–5104
Sharma P, Bhardwaj R, Arora N, Arora HK, Kumar A (2008) Effects of 28-homobrassinolide on nickel uptake, protein content and antioxidative defence system in *Brassica juncea*. Biol Plant 52:767–770
Shi Q, Zhu Z (2008) Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. Environ Exp Bot 63:317–326
Singh AK (2001) Effect of trivalent and hexavalent chromium on (spinach Spinacea oleracea L). Environ Eco l19:807–810
Sinha S, Mukherji S, Dutta J (2002) Effect of manganese toxicity on pigment contet, Hill activity and photosynthetic rate of *Vigna radiata* L. Wilczek seedlings. J Envirn Biol 23:253–257
Smith SE, Christophersen HM, Pope S, Smith FA (2010) Arsenic uptake and toxicity in plants: integrating mycorrhizal influences. Plant Soil 327:1–21
Subrahmanyam D, Rathore VS (2001) Influence of Manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. Photosynthetica 38:449–453
Suseela MR, Sinha S, Singh S, Saxena R (2002) Accumulation of chromium and scanning electron microscopic studies in scirpus lacustris l treated with metal and tannery effluent. Bull Environ Contam Toxicol 68:540–548
Teige M, Huchzermeyer B, Schultz G (1990) Inhibition of chloroplast ATPsynthase/ATPase is a primary effect of heavy metal toxicty in spinach plants. Biochem Physiol Pfl 186:165–168
Terry N, Zayed AM, de Souza MP, Tarun AS (2000) Selenium in higher plants. Annu Rev Plant Physiol Plant Mol Biol 51:401–432
Thordal-Christensen H, Zang Z, Wei Y, Collinge DB (1997) Subcellular localization of H_2O_2 accumulation in papillae and hypersensitive response during the barley powdery mildew interaction. Plant J 11:1187–1194
Trelease SF, Trelease HM (1939) Physiological differentiation in *Astragalus* with reference to selenium. Am J Bot 26:530–535
Tu C, Ma LQ (2005) Effects of arsenic on concentration and distribution of nutrients in the fronds of the arsenic hyperaccumulator *Pteris vittata* L. Environ Pollut 135:333–340
Van Assche F, Clijsters H (1986) Inhibition of photosynthesis in Phaseolus vulgaris by treatment with toxic concentrations of zinc: effects on electron transport and photo-phosphorylation. Physiol Plant 66:717–721

Verma S, Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activity of antioxidant enzymes in growing rice plants. Plant Sci 164:645–655

Vromman D, Flores-Bavestrello A, Slejkovec Z, Lapaille S, Teixeira-Cardoso C, Briceño M, Kumar M, Martínez JP, Lutts S (2011) Arsenic accumulation and distribution in relation to young seedling growth in *Atriplex atacamensis* Phil. Sci Total Environ 412–413:286–295

Wang YD, Wang X, Wong YS (2012) Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. J Proteomics. http://dx.doi.org/10.1016/j.jprot.2011.12.030

Watanabe ME (1997) Phytoremediation on the brink of commercialization. Environ Sci Technol 31:182–186

Welch RM (1981) The biological significance of nickel. J Plant Nutr 3:345–356

Wu L, Huang ZZ (1992) Selenium assimilation and nutrient element uptake in white clover and tall fescue under the influence of sulphate concentration and Selenium tolerance of the plants. J Exp Bot 43:549–555

Yruela I (2005) Copper in plants. Braz J Plant Physiol 17:145–156

Yusuf M, Fariduddin Q, Hayat S, Ahmad A (2011) Nickel: an overview of uptake, essentiality and toxicity in plants. Bull Environ Contam Toxicol 86:1–17

Zeid IM (2001) Responses of Phaseolus vulgaris chromium and cobalt treatments. Biol Plant 44:111–115

Zhang WH, Tyerman SD (1999) Inhibition of water channels by $HgCl_2$ in intact wheat root cells. Plant Physiol 120:849–857

Zhang J, Zhao QZ, Duan GL, Huang YC (2011) Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. Environ Exp Bot 72:34–40

Zhou SZ, Huang SQ, Guo K, Mehta SK, Zhang PC, Yang ZM (2007) Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativ*a L. J Inorg Biochem 101:1–9

Zhou SZ, Wang SJ, Yang ZM (2008) Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants. Chemosphere 70:1500–1509