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REVIEW

Toxic metal ions in photoautotrophic organisms

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Abstract

We summarize the contemporary understanding of the effects of metal stress on various photosynthetic processes in photoautotrophic organisms and of the defence strategies employed by these organisms to avoid such stress. Cadmium is in the centre of interest of this review, as a non-essential element and important environmental pollutant, but Al, Pb, Hg, As, Cu, and Zn are also considered. Toxic metal ions pollute the environment through anthropogenic activities and affect the quality of plant crop. They represent one of the main abiotic stress factors influencing the health of plants and, as a secondary effect, of animals including man. The review summarizes the generally accepted answers to the questions: How do the toxic metal ions enter the photosynthetic organisms? How are they accumulated in plants? Which mechanisms do plants develop to tolerate metal stress and protect themselves?

Additional key words: abiotic stress; Al; As; Cd; Cu; Hg; photosynthesis; phytoremediation; toxic metals; Zn.

Introduction

Metal ions are essential in low concentrations in the maintenance and development of all organisms. Many molecules and reactions in plants require essential elements. Macrobiogenic elements include magnesium for functional chlorophyll (Chl), activation of ATP-synthase, synthesis of nucleic acids and proteins, and activation of enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO), iron for electron transporters or catalysis of Chl biosynthesis, and calcium for signalling pathways or photoactivation of photosystem 2 (PS2). Microbiogenic elements involve manganese (*e.g.* for oxygen evolving complex, OEC), copper (*e.g.* for plastocyanin), and cobalt (*e.g.* for vitamin B_{12}) (Bertrand and Poirier 2005). Zinc and copper are also required as cofactors by many key enzymes involved in metabolic pathways [superoxide dismutase (SOD), δ-aminolevulinic acid (ALA) dehydratase]. Zinc plays a significant structural role as a stabilizer of some proteins, *e.g.* DNA-binding proteins in structural motives of Zn-fingers (Aravind and Prasad 2004). When essential ions are not available, plants develop specific deficiency symptoms. Absence of iron causes a decrease in the photosynthetic rate, chlorosis of leaves, and their abscission.

However, metals present in excessive amounts may interfere with the usual cellular metabolism and become toxic. Uncontrolled binding of metals can result in inactivation of proteins (Clemens *et al.* 2002). Excess metals reach plants as a result of environmental pollution due to mining, smelting, manufacturing, agricultural and waste disposal technologies, and military practices (Bertrand and Poirier 2005). Such metals are released in the biosphere through air, water, and soil, taken up by root systems or leaves, or absorbed by the whole surface of unicellular organisms. They accumulate in various parts of plants and migrate along the food chain into animal and human tissues. The most toxic to higher plants and microorganisms even at low concentrations are the non-essential metal ions such as Cd^{2+}, $Hg^{+,2+}$, $As^{3+,5+}$, AsO_3^{3-}, AsO_4^{3-}, $Pb^{2+,4+}$, Ag^{+}, Al^{3+}, Cr^{6+}, Se^{4+}, SeO_4^{2-},

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Abbreviations: AA – ascorbate; ALA – δ-aminolevulinic acid; AP – ascorbate peroxidase; Asc-GSH cycle – ascorbate-glutathione cycle; Car – carotenoids; CAT – catalase; CDF – cation diffusion facilitators; Chl – chlorophyll; DHA – dehydroascorbate; DHAR – dehydroascorbate reductase; Fd – ferredoxin; GR – glutathione reductase; GSH – reduced glutathione; GSSG – oxidized glutathione; JA – jasmonic acid; LHC2 – light-harvesting complex of photosystem 2; MDHA – monodehydroascorbate radical; MDHAR – monodehydroascorbate reductase; MT – metallothionein; Nramp – natural resistance associated macrophage proteins; OEC – oxygen evolving complex; PC – phytochelatin; PEPC – phospho*enol*pyruvate carboxylase; PS – photosystem; ROS – reactive oxygen species; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SOD – superoxide dismutase; YCF1 – yeast cadmium factor.

SeO_3^{2-}, and Sn^{4+}. Essential elements Cu^{2+}, Zn^{2+}, Co^{2+} are toxic at high concentrations only (Kučera and Sofrová 2004). The most common overall toxicity sequence of elements decreases in order $Hg^{2+}>Cu^{2+}>Cd^{2+}>Ag^{+}>Pb^{2+}>Zn^{2+}$ (Backor and Vaczi 2002). In particular cases, the relative effects of some of them may be reversed. Zn was more toxic than Cu to *Scenedesmus acutus* having different sensitivity to Cr (Abd-El-Monem *et al.* 1998). Coordinate function of uptake, transport, distribution, and regulation of cytosolic concentrations of essential metals in various tissues and compartments within the narrow physiological limits is clearly important to normal plant growth and development. However, organisms have to cope with exposure to unwanted elements, because of imperfect control of accumulation. A lack of specificity of uptake and distribution systems leads to the accumulation of toxic metal ions (Clemens *et al.* 2002).

Metal uptake

Metals penetrate into unicellular organisms through the whole surface of the cell. In higher plants and lichens the main contact surface of the organisms is constituted by the root and rhizine systems (Wisniewski and Dickinson 2003). The uptake of metal ions comprises two phases. The first phase consists of a rapid and non-specific binding of the cations to negatively charged cell wall components (sugar residues) located at the cell surface (Prasad 1995) and mobilization of the soil-bound metals by secretion of organic acids (malate, citrate) (Clemens *et al.* 2002). The potential mobilization of metals in soil depends primarily on metal content, its solubility in water, soil pH, redox potential, and other soil characteristics (Bertrand and Poirier 2005). The actual bioavailability of some metals is limited because of low solubility in water and strong binding to soil particles. Iron, the most extreme example, is mainly present as Fe^{3+}, which forms insoluble hydroxides. With the notable exception of Fe, which is solubilised by either reduction to Fe^{2+} by ferric chelate reductase or secretion of Fe^{3+} chelators, little is known about active mobilization of trace elements by plant roots (Clemens *et al.* 2002).

The second phase consists of energy-dependent intracellular uptake of the metal ions across the plasma membrane mediated by transport systems and intracellular high-affinity binding sites (Prasad 1995). The membrane potential in root epidermal cells, that is negative on the inside of the plasma membrane, provides a strong driving force for the uptake of cations through transporters (Clemens *et al.* 2002). Most of metals enter the cell as cations (*e.g.* Zn^{2+}), whereas others cross the plasma membrane as anionic groups (*e.g.* AsO_4^{3-}) or are included in small organic compounds (*e.g.* CH_3Hg^+) (Mendoza-Cozatl and Moreno-Sanchez 2005). The metals are taken up as hydrated ions and in metal-chelate complexes (Clemens *et al.* 2002). Uptake of metal ions takes place through channel proteins and/or H^+-coupled carrier proteins. At the protein level, five families have been implicated in heavy metal transport:

(*1*) CPx-type ATPases, a subgroup of P-type ATPases, pump essential and non-essential metals such as Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} across the plasma membrane.

(*2*) Cation/H^+ antiporters – physiologically exchange Na^+ or H^+ with Ca^{2+} or Cd^{2+}.

(*3*) Nramp family (natural resistance associated macrophage proteins) – H^+-coupled transporters involved in the transport of Cd^{2+}, Mn^{2+}, and Cu^{2+}.

(*4*) CDF family (cation diffusion facilitators) – involved in Zn^{2+}, Co^{2+}, Mn^{2+}, and Cd^{2+} transport in bacteria, fungi, plants, and animals.

(*5*) ZIP family – plant transporters of Fe^{2+}, Mn^{2+}, Cd^{2+}, and Zn^{2+} with biochemical properties similar to CDF but with different topology and highly conserved histidine motif for metal binding.

Most of the transporters thought to be involved in the uptake of micronutrients are in the ZIP and the Nramp family. CDF and ZIP proteins have also been implicated in the intracellular transport of heavy metals to the vacuole (Mendoza-Cozatl and Moreno-Sanchez 2005). For more information about CPx-type ATPases, Nramp, and CDF family see review of Williams *et al.* (2000).

The amounts of metal absorbed by a plant depend on the concentration and chemical form of the metal in the soil, metal mobilization, and efficiency of uptake from the soil to the root, sequestration within the root, efficiency of xylem loading and transport from the root to the shoot, sequestration and storage in leaf cells, and the presence of other ions in the environment. In the case of strongly adsorbed ions, absorption depends more upon the amount of roots produced. In general, effects are more pronounced at higher concentrations and at longer duration of exposure. Once absorbed, the metal can be either stored in the root or exported to other organs. In soybean, about 98 % of the accumulated Cd is strongly retained by roots, and only 2 % is transported to the shoot system (Prasad 1995). The xylem is important for metal distribution through the whole plant. The metal transporters involved in xylem loading and distribution between leaf cells have yet to be identified (Clemens *et al.* 2002).

The penetration from air to the aerial parts of plant body, particularly leaves, represents a substantial part of the uptake for some toxic metals. The absorption of organic and inorganic mercury from soil by plants is low. Hg emitted to the atmosphere thus seems to contribute significantly to the Hg content in crops (Patra *et al.* 2004). Hg-containing pesticide spray residues are absorbed by plant leaves and migrate to humus through fallen leaves, where they are taken up by plants and translocated to edible portions. However, the removal of Hg^{2+} from organic Hg compounds catalysed by organomercurial lyase and reduction of Hg^{2+} to the elemental form Hg^0 by mercuric ion reductase using NADPH as an electron donor results in transport through the leaves and volatilization (Prasad 1995).

The main exposure to lead takes place through the air, too (Graeme and Pollack 1998). Pb gets deposited on the leaves and other exposed surfaces of plants. Plant roots are able to extract some of this metal from soil but translocation to aerial part is generally limited. The roots may accumulate higher amount of the metal than the shoot and leaves (Singh *et al.* 1997).

Heavy metal tolerance in plants

Plants growing on metal-contaminated sites need to develop some degree of tolerance to metal toxicity in order to survive. Metal tolerance and homeostasis in cells is achieved by controlling transport processes (Meharg 2005) and by metal compartmentation with specific metal-binding biomolecules such as phytochelatins (PCs) and metallothioneins (MTs) (Clemens *et al.* 2002, Hall 2002). The common mechanisms for metal tolerance proposed are:

(*1*) Metal immobilization – the most common mechanism of plant adaptation by which a plant is protected externally from the influence of the stress is avoidance of metal stress by metal exclusion, *e.g.* deposition in cell wall components by extracellularly secreted organic compounds (polysaccharides, amino acids, organic acids).

(*2*) Organic ligand exudation (mobilization) – secretion of organic acids (citrate, malate, oxalacetate), acidification of the rhizosphere, and reduction of some elements to enhance metal accumulation.

(*3*) Metal ion efflux – joining metal uptake to achieve ionic balance, *e.g.* loss of K^+ (Prasad 1995).

(*4*) Chelation – phytochelatin and metallothionein synthesis.

(*5*) Compartmentation (sequestration) – involves transport of toxic ions into a vacuole.

Ad (*1*): Formation of organo-copper complexes by the herb *Silene vulgaris* in the cell walls as well as in the medium near the cells acts as a barrier to Cu uptake and prevents Cu from affecting cell metabolism (Ernst *et al.* 2000).

Ad (*2*): Most of Al occurs as harmless oxides and aluminosilicates. However, when soils become acidic as a result of natural processes or human activities, Al is solubilized into the toxic trivalent cation, Al^{3+}. Al toxicity is a major limiting factor of plant productivity on acidic soils, which now account for about 40 % of the Earth's arable land. Transgenic tobacco plants, that express bacterial citrate synthase, show elevated citrate secretion, which results in protection of plant roots from Al toxicity (de la Fuente *et al.* 1997, López-Bucio *et al.* 2000). Pellet *et al.* (1996) demonstrated in Al-sensitive and Al-resistant wheat cultivars differing in malate and phosphate exudation from root apices, that these features both confer the Al resistance and that the malate exudation is triggered by Al^{3+}. There may be a specific transporter for the organic acid anions in the plasma membrane, probably an anion channel (Ryan *et al.* 1997, Kollmeier *et al.* 2001, Piñeros and Kochian 2001, Zhang *et al.* 2001). Al^{3+} activates an anion channel in the plasma membrane that is permeable to malate and chloride. It is not known how Al activates these anion channels but three possibilities have been proposed: (*a*) Al interacts directly with the channel protein to trigger its opening. (*b*) Al interacts with a specific receptor on the membrane surface or with the membrane itself to initiate a secondary-messenger cascade which then activates the channel. (*c*). Al enters the cytoplasm and activates the channel either directly or indirectly *via* secondary messengers (Ma *et al.* 2001).

Ad (*4*): Inside cells, polypeptides such as MTs and PCs participate in excess metal storage and detoxification together with organic molecules of low molecular mass, mainly organic acids, amino acids, and their derivatives. When these systems are overloaded, oxidative stress defence mechanisms are activated (Bertrand and Poirier 2005). MTs are cysteine-rich, gene-encoded, 8–14 kDa polypeptides that sequester excess amounts of certain toxic ions *via* thiol groups and thus keep them from circulating in the cytosol. PCs [γ-glutamyl-cysteinyl glycine complexes $(\gamma\text{Glu-Cys})_n\text{Gly}$, $n = 2–11$, 1.5–4.0 kDa cytosolic peptides] are synthesized from glutathione (GSH) in the presence of phytochelatin synthase (γGlu-Cys dipeptidyl transferase) in higher plants. PC synthase removes a γGlu-Cys residue from one molecule of GSH (γGlu-Cys-Gly) and couples it to another GSH. PC synthesis is induced by toxic metal ions and proceeds as long as the metal ions are available. The production of PCs and MTs is the principal mechanism of metal detoxification in higher plants, fungi, and apparently also in certain animals. The PC-metal complexes, less toxic to cellular plant metabolism than free metal ions, are transported to the vacuole. In *Datura innoxia*, 80 % of Cd is bound to low molecular mass PCs and about 15 % to high molecular mass MTs. About 60 % of Cu is bound to MTs and only about 25 % to PCs. These collective results suggest co-occurrence of MTs and PCs in plants. Sulphate salts afford protection to Cd toxicity by enhancing sulphate uptake, leading to increased synthesis of GSH (Prasad 1995).

Ad (*5*): In plants and some yeasts, Cd^{2+} is accumulated in the vacuole. In plants, Cd^{2+} may be transported as free ion or as a PC-Cd complex. In yeast, Cd^{2+} is also transported as a free ion and, depending on the species, it may also be transported as a PC-Cd complex or as a bis(glutathionato)-Cd complex. The transport of free Cd^{2+} ion requires H^+ gradient across the vacuole membrane. The transport of thiol-metal complexes into vacuoles requires an ABC-type transporter specific for each kind of complex. Expression of the *Saccharomyces cerevisiae* protein YCF1 identified in yeast confers salt tolerance in *Arabidopsis thaliana* (Koh *et al.* 2006). Finally, for a complete Cd^{2+} inactivation and storage inside the vacuole, thiol-Cd complexes, Cd^{2+} and inorganic sulfide (S^{2-}) form high molecular mass complexes around a CdS core. Cells unable to form these complexes become Cd^{2+}-sensitive. *Euglena gracilis* lacks a plant-like vacuole and thus more than 60 % of the accumulated Cd^{2+} is located inside the chloroplast. PCs are also present inside the chloroplast, suggesting that this organelle is involved in the Cd^{2+} resistance mechanism, but the origin of PCs and the mechanism by which Cd^{2+} is transported into the chloroplast are unknown (Mendoza-Cozatl and Moreno-Sanchez 2005).

Several of these mechanisms may be employed by a plant species against a particular metal, as in the case of arsenate in *Holcus lanatus* (Hartley-Whitaker *et al.* 2001). The adaptive tolerance seems to be controlled by a limited number of genes (Macnair 1993). However, distinct metal-specific mechanisms are involved in the tolerance to individual metals (Hall 2002).

Metal stress

When the metal excess reaches sub-lethal amounts, the production of free radicals (reactive oxygen species, ROS) that are triggered by toxic metals leads to the oxidative stress. Molecules are destroyed and metabolism is highly disturbed (Bertrand and Poirier 2005). Excessive concentrations of metals result in phytotoxicity through: (*1*) reactions of sulfhydryl groups and nitrogen-rich ligands with cations; (*2*) affinity for reacting with phosphate groups of ADP or ATP; and (*3*) replacement and deficiency of essential metal ions (Mendoza-Cozatl and Moreno-Sanchez 2005). The consequences of *Cannabis sativa* exposed to Cd are inhibition of Chl synthesis (chlorosis), decrease in Chl content and Chl *a*/*b* ratio, inhibition or decreased activity of enzymes, high ATP content, lipid peroxidation, increase in plasma membrane permeability, imbalance in ionic transport and homeostasis, loss of turgor, altered stomatal action, and DNA damage. Metal interference with chloroplast replication and cell division causes the reduction in the number of chloroplasts per cell (chlorosis) and a change in cell size. The disorganized thylakoids impair the photosynthetic efficiency: the cells and therefore whole photosynthetic organisms grow slowly (Linger *et al.* 2005). In bean plants exposed to Cd, leaf cell growth and relative water content of leaves decreased by about 10 % compared to the control (Prasad 1995).

Effects on pigments: A low Chl content (chlorosis) can signalize Fe deficiency or an inhibition of Chl synthesis by the toxic metal. Cd inhibits Chl synthesis through ALA dehydratase and protochlorophyllide reductase by its interference with the sulfhydryl site leading to the lower production of porphobilinogen. Metals such as Cd, Cu, Hg, Ni, Pb, or Zn can replace Mg within Chl leading consequently to a breakdown in photosynthesis (Bertrand and Poirier 2005). According to Prasad (1995), Cd primarily affected the photosynthetic pigments in higher plants: total Chl content and the Chl *a/b* ratio decreased, but carotenoids (Car) were less affected than Chl resulting in a lower Chl/Car ratio. In another study, the Chl *a/b* ratio increased suggesting that Cu affected the light-harvesting complex 2 (LHC2) where Chl *b* is located, rather than the Chl *a* containing reaction centres. This contradicts other studies where the reverse has been found. The effects of Cd and Zn on chlorosis also depend on leaf age (Ebbs and Uchil 2008); sensitivity of genotype to the toxic metal is also very important (He *et al.* 2008, Mihailovic *et al.* 2008). The small increase in β-carotene content in *Quercus robur* exposed to Cu may be due to its resistance to degradation, since it is a relatively stable pigment with a protective role. The activity of xanthophyll cycle (particularly zeaxanthin), implicated as a mechanism that protects the photosynthetic apparatus by the non-photochemical quenching of excited triplet Chl, evidently declines (Wisniewski and Dickinson 2003). A reduction of xanthophyll cycle pigments of nearly 50 % was observed in rape leaves, supporting an unfortunate effect of Cd on zeaxanthin synthesis causing changes in the quenching properties. In addition, tomato and soybean plants also undergo a reduction of Car contents when treated with Cd (Linger *et al.* 2005). This is in contradiction with another study, where an increase of the zeaxanthin content was found, higher than could be accounted for by the light-induced de-epoxidation of violaxanthin (Mendez-Alvarez *et al.* 1999). According to Mendez-Alvarez *et al.* (1999), hydroxylation of β-carotene into zeaxanthin under high irradiance could be an additional source for zeaxanthin that might be involved in non-photochemical quenching.

Metal ions block the electron flow in PS2 leading to the formation of excited triplet Chl ($^3Chl^*$), whose life time is long enough to react with a molecule of oxygen with triplet electronic configuration. During the interaction, Chl gets over to the ground state and oxygen in turn is changed into highly reactive singlet oxygen. Oxidative stress leads to an imbalance in the regeneration and removal of ROS including singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and the most damaging and reactive hydroxyl radical ($OH^{\cdot}$). These reactive molecules are, in the absence of protective mechanisms, capable of damaging membranes, proteins, and nucleic acids leading to altered membrane fluidity, loss of enzyme function, and genomic damage. Thiobarbituric acid reactive substances, the cytotoxic products of lipid peroxidation, are normally the major compounds that indicate the magnitude of oxidative stress. Susceptibility to oxidative stress depends on the overall balance between the factors that increase oxidant generation and the cellular components that exhibit an antioxidant capability (Cho and Seo 2005).

The synchronous action of GSH reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) is part of the antioxidative system that protects various compartments in the cell against ROS. The CAT, localized in peroxisomes and chloroplasts, converts the most stable of all ROS H_2O_2 into H_2O and O_2. Peroxidase decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants in vacuoles, cell walls, and cytosol. Increase in peroxidase activity is generally considered as an indication of plant ageing and might initiate senescence. SOD is a metalloprotein with Zn that catalyzes the dismutation of superoxide to less dangerous H_2O_2 and O_2 in the cytosol, mitochondria, and chloroplasts (Cho and Seo 2005).

The ascorbate-glutathione cycle (Asc-GSH cycle) is essential in removing ROS and maintaining the cellular homeostasis by keeping the reduced forms of GSH and ascorbate in cells on a suitable level and thus to adjust a cellular redox potential. It is localized in various subcellular compartments including the chloroplast, mitochondrion, peroxisome, glyoxisome, and the plasma membrane. The activities of ascorbate peroxidase (AP) and GR are crucial in the operation of Asc-GSH cycle. APX reduces H_2O_2 into H_2O using ascorbate (AA) as the electron donor, resulting in the formation of monodehydroascorbate radical (MDHA). Monodehydroascorbate reductase (MDHAR) reduces MDHA into AA using NAD(P)H as electron donor. Under limiting supply of NAD(P)H, MDHAs are dismutated into AA and dehydroascorbate (DHA). Dehydroascorbate reductase (DHAR) using GSH as an electron donor generates AA from DHA. Oxidized GSH (GSSG) is converted to GSH by NAD(P)H-dependent GR (Drazkiewicz *et al.* 2003, Gorinova *et al.* 2007).

AA takes part in growth processes, electron transport, photoprotection, regulation of photosynthesis, and preservation of the enzyme activities that contain prosthetic transition metal ions. It is also active in regeneration of the lipophilic antioxidant α-tocopherol and in antioxidant processes by reacting directly with ROS. GSH is used in the detoxification of xenobiotics and in PC synthesis. It affects also expression of defence genes and is associated with stress resistance. As a component of the Asc-GSH cycle, GSH is involved in the control of H_2O_2 concentration in plant cells. GSH reacts also non-enzymatically with singlet oxygen, superoxide and hydroxyl radicals (Drazkiewicz *et al.* 2003). Car quench the oxidizing species and triplet state of Chl. The decreased Car content in Cd-treated freshwater macrophyte *Ceratophyllum demersum* and thus an increase in the membrane damage due to the loss of ROS scavenging function has been observed by Aravind and Prasad (2004). In the same plant, at high Pb concentrations the enhanced PC synthesis may result in decrease of GSH content and the consequent oxidative stress (Mishra *et al.* 2006).

Nagalakshmi and Prasad (2001) observed with increasing concentrations of Cu progressive depletion of GSH content in the cells of *Scenedesmus bijugatus.* Cu stress increased the activities of PC synthase, APX, and decreased the activity of GR. This suggests that Cu alters the equilibrium between synthesis and utilization of GSH either due to its antioxidant role or by serving as a precursor in the synthesis of PCs. The increase in the activities of enzymes of GSH metabolism and the activities of antioxidant enzymes suggests that Asc-GSH cycle has a pivotal role in Cu detoxification, apart from the role of PCs in the chelation of metals. In another experiment, *A. thaliana* exposed to Cu stress for a day showed elevated DHAR activity accompanied by the decrease of GSH level to counteract DHA accumulation. In plants exposed to the metal for three days, GR and MDHAR were especially active to maintain large pool of GSH and AA. However, at seven-day exposition, a suitable level of AA was maintained due to enhanced activity of MDHAR or DHAR depending on Cu concentration. Increase of AP and DHAR activities was observed at 5 μM Cu and increase of MDHAR activity occurred at 25 μM Cu. Moreover, decrease of AP activity at 50 μM Cu, but increase of MDHAR and decrease of DHAR activity under exposition to 100 μM Cu were characteristic of plants treated with the metal for seven days (Drazkiewicz *et al.* 2003). According to Cho and Seo (2005), H_2O_2 accumulation is the major reason causing Cd phytotoxicity in *A. thaliana* seedlings. It can result from inefficient quenching by the altered antioxidant enzymes, particularly those involved in the Asc-GSH pathway and lowered CAT activity. Cd-resistant plants respond to an increased ROS production by increasing the synthesis of antioxidant enzymes (SOD, APX, GR) and GSH that can partly counteract the accumulation of H_2O_2. The Asc-GSH cycle might be operating at higher rate in order to detoxify the ROS in these cultivars, or it is essential to maintain reduced GSH pool at higher levels so that it does not become limiting for the synthesis of PCs. These results are in agreement with studies of responses of *Nicotiana tabacum* with injected MT gene to elevated Cd, where the increased SOD and AP activities and the enlarged AA pool indicated a mobilization of the Asc-GSH cycle. The control plants showed the inhibition of photosynthesis due to the decreased CAT activity (Gorinova *et al.* 2007). In another experiment, different *Brassica juncea* cultivars were exposed to various concentrations of Cd and the changes in the rate of lipid peroxidation, Cd accumulation, and activities of CAT, SOD, AP, and GR along with AA and GSH contents were studied. In all the cultivars, a reduction in the CAT activity and AA content was noted. The cultivar with the least increase in the lipid peroxidation rate, the highest Cd and GSH contents, and increased activities of SOD, AP, and GR possessed better Cd-sequestering and antioxidant system. High increase in the content of GSH suggests its possible incorporation in the synthesis of PCs and MTs to sequester Cd (Qadir *et al.* 2004). According to Maksymiec *et al.* (2007), heavy metals may act either directly on oxidative processes or through induction of the signalling pathways. Jasmonic acid (JA) is formed from lipid peroxides as a consequence of enhanced lipoxygenase activity, but the formation may also be a prolonged consequence of heavy metal induction of oxidative stress. JA can enhance the inhibitory effects on *A. thaliana* by Cu and Cd stress. High concentrations of antioxidative enzymes, together with enhanced pigment synthesis may allow a plant to overcome completely the harmful action of a toxic metal and to show normal healthy growth, as shown for spinach and chromium. However, the plant still accumulates fairly high amounts of the metal, a feature that can be useful for phytoremediation but dangerous in case of edible plants (Sinha *et al.* 2007).

Effects on light reactions: Metal stress impairs electron transport activity by inducing peroxidation and loss of thylakoid membrane integrity. The disorganization of the chloroplast structure and the substitution of Mn by Zn or Cd leads to the inactivation of OEC and as a consequence electron donation to PS2 is inhibited. Induced changes in the arrangement and structure of LHC2 decrease the efficiency of excitation energy capture by PS2 and reduce the rate of photosynthetic oxygen evolution. Photophosphorylation rates decrease due to PS2 dysfunction without evidence for a direct inhibition by the metal of the ATP-synthase (Bertrand and Poirier 2005). Cd affects the lipid structure around LHC2 in *C. demersum* leading to the loss of major fatty acids and lipid peroxidation. This induces the release of several pigment-protein complexes, OEC, and plastocyanin. An impaired plastocyanin blocks further electron transport. This severe extent of peroxidative protein loss is ultimately responsible for the impaired photosynthetic process. Cd forms complexes with aromatic amino acid residues (tryptophan) of D1 protein, which are degraded by a protease leading to an impaired PS2 activity (Aravind and Prasad 2004). These results are in agreement with another study (Kráľová *et al.* 2006) according to which Cd interacts with the tyrosine radicals, the primary donor of PS1, and with the Mn cluster in OEC. The interruption of the photosynthetic electron transport occurs due to these interactions. The formation of Cd^{2+} complexes with amino acid residues constituting photosynthetic polypeptides was suggested as possible mechanism of Cd inhibitory action. Faller and Krieger-Liszkay (2005) proved that Cd^{2+} is a competitive inhibitor of the Ca^{2+} site in the catalytic centre in PS2 during photoactivation inhibiting oxygen evolution in *C. reinhardtii*. This mechanism is a serious candidate for the involvement of Cd^{2+} toxicity in photosynthetic organisms in general, at least in unicellular ones, where Cd^{2+} has ready access to the chloroplast. Ca^{2+} can abolish the toxic effects of heavy metal ions, especially of Cd, in *Kirchneriella lunaris* (Issa *et al.* 1995). Cd may inhibit PS2 (Tůmová and Sofrová 2002, Nováková *et al.* 2004), possibly affecting OEC and photophosphorylation in spinach chloroplasts. PS1 is less affected (Tůmová and Sofrová 2002). Cd-induced protein phosphorylation changes in rice and maize (Prasad 1995). Regulation of energy distribution of PS2 is affected in plants caused by structural alterations of PS2 and/or antenna complexes and impairment of the xanthophyll cycle (Linger *et al*. 2005).

Effects on dark reactions: Inhibition of carbon metabolism due to Zn pollution has been reported in *Datura innoxia.* Increasing stomatal and mesophyll resistance leads to reduced CO_2 uptake, because of a reduced number of stomata or stomata closing (Vaillant *et al.* 2005). Metals may oxidize sulfhydryl groups of proteins and modify the spatial conformation of enzymes, inhibiting enzyme activity or protein function, and lowering the pool of reducing antioxidant agents such as GSH. Metals may also react with nitrogen-rich ligands such as histidine. Metal-sensitive plant enzymes are RuBPCO, PEPC, alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and nitrate reductase. At moderate pollution, the enzymatic activities appear stimulated as part of defence mechanisms, while they seem inhibited at higher concentrations (Williams *et al.* 2000). Heavy metal treatment inhibits RuBPCO capacity in C_3 plants and cyanobacteria (Nováková *et al.* 2004) and PEPC capacity in C_4 plants. Furthermore, high Cd^{2+} concentrations can also lead to irreversible dissociation of small and large subunits of RuBPCO, with the consequence of total inhibition of the enzyme (Bertrand and Poirier 2005). Cu^{2+} and Cd^{2+} affect glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase at an early stage of stress development (Stiborová *et al.* 1986, Sheoran *et al.* 1990, Burzyński and Żurek 2007).

Cd interference with other metal ions: When an essential metal is administered together with a harmful one of similar properties, the damages are less severe than with the toxic element alone (Ernst *et al.* 2000). Moderate Fe excess has a beneficial influence on Cd treated plants resulting in more intensive growth, accumulation of photosynthetic pigments, and more efficient light phase of photosynthesis. Nutrient-medium Fe deficiency results in increased uptake and accumulation of Cd. Strong Fe excess, toxic itself, enhances Cd toxicity (Siedlecka and Krupa 1999). When toxic metals and beneficial elements enter the cell together, the beneficial part may be too weak because of competition. It happens actually for Fe that when Cd is given in excess to a growth medium, it causes Fe deficiency because both metals have partly common pathways.

However, in some cases the beneficial element is an antidote against the toxic element when it is given in excess (Bertrand and Poirier 2005). Zn protects chloroplasts and associated photochemical functions from Cd toxicity in *C. demersum*. Since both Cd and Zn belong to the same group of transition elements with similar electronic configuration and valency, they have similar geochemical and environmental properties. Zn antagonizes Cd toxicity by counteracting the inhibition of photosynthetic pigment synthesis by maintaining the contents of pigments. Zn probably maintains Chl synthesis through sulfhydryl group protection of enzymes from thiol oxidation and disulfide formation either by direct binding to a site close to the sulfhydryl group or by conformational change resulting in apparent stability of the enzymes. It maintains the contents of Car, but the mechanism has not been clarified yet. Zn has a stabilizing and protective effect on the biomembranes against oxidative and peroxidative damage by inducing active antioxidant enzyme system to control the destructive oxygen species. Zn replaces the toxic Cd, maintains the OEC integrity, prevents oxidative burst at antenna Chl molecules, and probably prevents binding of Cd to the major thylakoid proteins like D_1 of the PS2 (Aravind and Prasad 2004).

Phytoremediation

Phytoremediation is a method used to decontaminate and remediate the polluted environment by the plants capable of hyperaccumulating the toxic metal ions in their roots and shoots far more than in either soil or water which surrounds them (Masarovičová *et al.* 1999). In wealthy industrialized countries contamination is often highly localized, and the pressure to use contaminated land and water for agricultural food production or for human consumption is minimal. However, soil and water contamination is widespread in Eastern Europe, and is increasingly recognized as dramatic in large parts of the developing world, primarily in India and China (Prasad 1995). Metal hyperaccumulating plants have been defined as plants that accumulate more than 10 000 mg kg^{-1} Zn or Mn, 1 mg kg^{-1} Co or Cu, and 100 mg kg^{-1} Cd. To date, only *Thlaspi caerulescens* and *Arabidopsis halleri*, both belonging to Brassicaceae, have been identified as Cd-hyperaccumulators. More recently, Zn hyperaccumulator *Sedum alfredii*, which belongs to Crassulaceae, has been found in an old Zn/Pb mining area in China to hyperaccumulate Cd (Zhou and Qiu 2005). Surface exposure and the contaminated soils associated with mining have provided a long period of exposure for plants to evolve physiological tolerance to the toxic effects of metals. Some trees, such as *Salix caprea*, *Betula pubescens*, and *Quercus robur* readily colonize contaminated soils and possess tolerance traits (Wisniewski and Dickinson 2003). The herb *Silene vulgaris* is characteristic for many metal-enriched soils in Europe (Ernst *et al.* 2000). Aquatic plants, *e.g. Lemna trisulca* and *L. minor* (duckweeds) or *Spirodela polyrrhiza*, accumulate and bio-concentrate heavy metals (Prasad *et al.* 2001, Hou *et al.* 2007, Seth *et al.* 2007). Some lichen photobionts accumulate large quantities of metals, *e.g. Trebouxia erici* (Backor and Vaczi 2002). Tolerance of algae to heavy metals has been demonstrated for the green algae *Chlorella* and *Scenedesmus* (Abd-El-Monem *et al.* 1998).

Plants ideal for phytoremediation should posses multiple traits. They must be fast growing, have high biomass, deep roots, be easy to harvest, and should tolerate and accumulate a range of heavy metals in their aerial and harvestable parts (Kramer 2005). To date, no plant has been described that fulfils all these criteria. However, a rapidly growing non-accumulator could be engineered so that it achieves some of the properties of hyperaccumulators (Salt *et al.* 1998). As already mentioned, the tolerance to individual metals is conferred by individual distinct mechanisms (Hall 2002). Co-tolerance is rather rare and thus preparing a species tolerant to a wide range of metals would mean multiple genetic changes (Macnair *et al.* 2000).

Phytoremediation technologies are currently available for only a small subset of pollution problems, such as As and Hg by engineering of transgenic *A. thaliana* plants that over-express an *Escherichia coli* arsenate reductase and PC synthase. A novel approach in phytoremediation is phytovolatilization, whereby the contaminant organic mercury compounds and Hg^{2+} are not primarily accumulated in shoot tissues, but are instead transformed by the plant into a volatile compound Hg^0 that is released into the atmosphere. For this purpose, modified bacterial genes were introduced into several plant species including *Arabidopsis*, tobacco, and poplar, which induce expression of organomercurial lyase and mercuric ion reductase (Kramer 2005). Kramer (2005) also showed that transgenic *A. thaliana* plants expressing a selenocysteine methyltransferase isolated from the Se hyperaccumulator *Astragalus bisulcatus* accumulated methylselenocysteine and contained up to eightfold higher Se contents than wild-type plants, when grown on a soil supplemented with selenite (SeO_3^{2-}).

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