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REVIEW

Photosynthetic organisms and excess of metals

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Abstract

When cells get metals in small excess, mechanisms of avoidance occur, such as exclusion, sequestration, or compartmentation. When the excess reaches sub-lethal concentrations, the oxidative stress, that toxic metals trigger, leads to persistent active oxygen species. Biomolecules are then destroyed and metabolism is highly disturbed. At the chloroplast level, changes in pigment content and lipid peroxidation are observed. The disorganized thylakoids impair the photosynthetic efficiency. The Calvin cycle is also less efficient and the photosynthetic organism grows slowly. When an essential metal is given together with a harmful one, the damages are less severe than with the toxic element alone. Combined metals and phytochelatins may act against metal toxicity.

Additional key words: chloroplast; oxidative stress; photosynthesis; thylakoid membrane; xanthophyll cycle.

Introduction

Several metals are essentials for plant development and daily life. Many bio-molecules and reactions in plants require essential elements, *e.g*. Fe for electron transporters and chlorophyll (Chl) biosynthesis, Mg for functional Chl, Mn for photosystem 2 (PS2) activity, Cu for plastocyanin, or Zn for superoxide dismutase. Usually, most of the vital elements are present in a concentration range, which is compatible with life. Fe is an atypical element the content of which is far below those required for microbial or plant growth, because of the low solubility of Fe-containing chemical species (Schmidt 1999, Connolly and Guerinot 2002). When metals are present in excess, they become toxic. Non essential metals (*e.g*. Al, Ag, Cd, Cr, Hg, Pb) are also not favourable. Excess of metals reaches plants as a result of environmental pollution because of anthropic activities such as mining, smelting, manufacturing, agricultural and waste disposal technologies (Foy *et al*. 1978). Some authors give the relative toxicity for different elements: for instance, for algae, the decreasing toxicity is the following: Hg > Cu > Cd > Ag > Pb > Zn. In some cases, the relative effects of two elements are reversed (*e.g*. Backor *et al*. 1998, Abd-El-Monem *et al*. 1998). Moreover, some elements have variable toxicity according to the chemical species they belong to; for instance, the more reduced forms of As, Cr, or Hg are less toxic (Ochiai 1987).

Metals can penetrate into unicellular organisms through the whole surface of the cell. With macroscopic pluricellular organisms, the situation is different because parts of the organisms are especially dedicated to food income or mineral assimilation. For instance in higher plants and lichens, the main contact surface of the organisms is constituted by the root and rhizine systems, respectively (Goyald and Seaward 1981, 1982, Wisniewski and Dickinson 2003). Once absorbed, the metal can be either stored there or exported to other organs. For instance, the xylem is important for metal distribution through the whole plant (Blamey *et al.* 1986, Salt *et al.* 1995).

This review presents the main molecular targets of toxic metals in plant cells and develops their impact on chloroplast components and photosynthetic activities due to imbalanced metabolism and active oxygen species. The beneficial effects of some metal combinations and phytochelatins are finally shown.

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Abbreviations: AOS – active oxygen species; AP – ascorbate peroxidase; Chl – chlorophyll; GR – glutathione reductase; GSH – reduced glutathione; GSSG – oxidized glutathione; HM – heavy metal(s) (the element symbol may include different chemical species); PC – phytochelatin; Pchlide – protochlorophyllide; PS – photosystem; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SOD – superoxide dismutase.

Metal chelation outside the cells

The polysaccharides secreted by some species allow them to adsorb toxic metals of the medium and prevent them to enter inside the cells. In lower photosynthetic organisms, this excretion falls in stress response mechanisms as it is amplified by the metal pollution. This has been shown with cyanobacteria (*Microcystis aeruginosa*: Parker *et al.* 2000; *Spirulina* sp.: Hernandez and Olguin 2002), microalgae (*Chlamydomonas reinhardtii*: Macfie and Wellbourn 2000, Pistocchi *et al.* 2000), or seaweeds (*Sargassum fluitans:* Fourest and Volesky 1997).

For higher plants, the phenomenon is less clear. A variety of organic substances is released either actively or passively by plant roots, the main components being reducing sugars, amino acids, phenolics, and organic acids (Schmidt 1999). Root exudates affect the availability of micronutrients directly by acidification of the rhizosphere, chelation, and/or reduction of some elements. In Al-tolerant plants, Al stimulates secretion of phosphate, organic acids, and mucilage from root apices that prevent the metal absorption (Archambault *et al.* 1996, Pellet *et al.* 1996, Jorge and Arruda 1997, Ma *et al.* 2001, Kochian *et al.* 2002).

Metal income inside the cells and membrane crossing

Most of metals enter a cell as cationic elements (*e.g.* Zn^{2+}), whereas others cross the plasma membrane as anionic group (*e.g.* AsO_4^{3-}) or included in small organic compounds (*e.g.* CH_3Hg^+). These species cross membranes by simple diffusion, but also *via* transporters. Different families have been reviewed by Williams *et al.* (2000) and Mendoza-Cózalt and Moreno-Sánchez (2005): the CPx-type ATPases which pump essential and non-essential metals across the plasma membrane, the cation/H^+ antiporters, the Nramp family (natural resistance associated macrophage proteins) implicated in the transport of divalent metal ions, the CDF family (cation diffusion facilitors) involved in Zn^{2+}, Co^{2+}, Mn^{2+}, and Cd^{2+} transport, and the ZIP family (Fe^{2+}, Mn^{2+}, Cd^{2+}, and Zn^{2+}-transporters). These transporters are partly found in organelle membranes (vacuole: Dietz *et al.* 1998, chloroplast envelope: Ferro *et al.* 2003, Mendoza-Cózalt and Moreno-Sánchez 2005).

Excessive HM contents induce a sharp increase in plasmalemma permeability and hence ionic imbalance, loss of turgor, and subsequent breakdown of cell metabolism (Cu^{2+}: Sicko-Goad 1982). When toxic metals and beneficial elements enter the cell together, the beneficial part may be too weak because of competition (Ernst *et al.* 2000, Parker *et al.* 2000, Shukla *et al.* 2003, Vaillant *et al.* 2004, Mendoza-Cózalt and Moreno-Sánchez 2005). It happens actually for Fe (Wallace *et al.* 1992, Sárvári *et al.* 1999). Pätsikkä *et al.* (2002) indicated, for example, that when Cu is given in excess in a growth medium, it causes Fe deficiency because both metals have partially common pathways. However, in some cases the beneficial element is an antidote against the toxic element when it is given in excess (see the last section).

Metal toxicity inside the cells

When the income of elements into the cell overrates it needs, the residual metals may interfere with the usual cellular metabolism and become toxic. To overcome the negative effects of metals, cells have developed several avoidance mechanisms such as metal exclusion or complexation, translocation, and cellular compartmentation (reviewed in Benemann 1991, Vangronsveld and Clijsters 1994, Sanità di Toppi and Gabbrielli 1999, Bertrand *et al.* 2001a). At high concentrations, avoidance is insufficient; free radicals are then produced, imposing oxidative stress (De Vos and Schat 1991, Luna *et al.* 1994, Stohs and Bagchi 1995, Olmos *et al.* 2003). Disturbances have been detected everywhere in the cell. Literature has reported many interactions of metals with bio-molecules: by association to organic acids or phosphate anions, by blocking essential groups (depletion of sulfhydryls), and displacing essential metal ions (Ochiai 1987, Stohs and Bagchi 1995, Rauser 1999). The consequences are lipid peroxidation, imbalance in ionic transport and homeostasis, high ATP content, inhibition of enzymes (antioxidant enzymes, ATPases), and DNA damage (Luo *et al.* 1996, Demidchik *et al.* 1997, Navari-Izzo and Quartacci 2001, Päivöke 2003).

The degree of toxicity is difficult to predict because the excess of several metals can be treated differently inside the cell according to the biological species (Rauser 1999, Kochian *et al.* 2002, Souza and Rauser 2003, Vaillant *et al.* 2004) or the genotypes of the same species (Wu *et al.* 2003). Nevertheless, similar defence-related secondary metabolites have been detected after various stresses (Mithöfer *et al.* 2004).

Metal disturbance for chloroplasts and photosynthetic activities

Chloroplast density: Leaf chlorosis in plants grown on metal-polluted soil can be due to a low chloroplast density caused by a reduction in the number of chloroplasts per cell and a change in cell size, suggesting that the excess of metal interferes with chloroplast replication and cell division (Cd: Baryla *et al.* 2001).

Accumulation of heavy metals in chloroplasts: Ferro *et al.* (2003) identified more than 100 proteins in the chloroplast envelope membranes. Among them is a metal transporter, a potential Cd/Zn transporting ATPase (AHM1), indicating a possibility for these metals to enter and accumulate. Despite of severe destructions that toxic metals provoke in chloroplasts, they do not accumulate in these organelles (Cu : Quartacci *et al.* 2000, Pätsikkä *et al.* 2002; Cd: Baryla *et al.* 2001, Carrier *et al.* 2003); they are rather found in abundance in the apoplast and the vacuole. This conclusion, valuable at least for higher plants, contrasts with the results of Nagel *et al.* (1996) and Mendoza-Cózalt and Moreno-Sánchez (2005) who found 50–60 % Cd in the chloroplast of a cell-wall deficient *Chlamydomonas reinhardtii* and the plant-like vacuole deficient *Euglena gracilis*, respectively. Both results indicate that even if chloroplasts have the capability to accumulate HMs, they do not represent the target organelle for these when the plant cell has a cell-wall and a vacuole. It also means that toxic metals are actively directed to the vacuole (Sanità di Toppi and Gabbrielli 1999, Navari-Izzo and Quartacci 2001).

Molecular components and enzymatic activities

Pigments: Chorosis indicates a low Chl density (Conway 1978, Chettri *et al.* 1998, Aravind and Prasad 2004) that can be the result of a slowed down accumulation of the green pigment in case of metal pollution. The origin can be a Fe deficiency (Abadia *et al.* 1989, Fodor *et al.* 1995) or an inhibition by the toxic metal of a key step (Baryla *et al.* 2001, Schoefs and Franck 2003, Myśliwa-Kurdziel and Strzałka 2005, Schoefs and Bertrand 2005). For instance, Cd inhibits Chl biosynthesis through δ-aminolevulinic acid dehydratase (Myśliwa-Kurdziel and Strzałka 2002), photoactive protochlorophyllide (Pchlide) and NADPH:Pchlide oxidoreductase (Myśliwa-Kurdziel *et al.* 2003, Schoefs and Bertrand 2005) by its interference with the sulfhydryl site (Prasad and Strzałka 1999). Photoactive Pchlide is also degraded by Cd, whereas Cr(VI) oxidizes NADPH (Myśliwa-Kurdziel and Strzałka 2005).

Cu inhibits pigment accumulation and retards Chl integration into the photosystems (Caspi *et al.* 1999). Cu treatment also results in a larger light-harvesting antenna. Moreover, Pätsikkä *et al.* (2002) demonstrated that Cu^{2+} predisposes leaves to photoinhibition (high photosensitivity of PS2) through reduction of Chl concentration. However, the loss of Chl and sensitivity to photoinhibition could be overcome by adding excess Fe together with excess Cu to the growth medium, as Cu out-competes Fe in Fe uptake.

Cd, Cu, Hg, Ni, Pb, or Zn can replace Mg within Chl (*e.g.* Küpper *et al.* 1996, Souza and Rauser 2003, Solymosi *et al.* 2004), leading, consequently, to a breakdown in photosynthesis. In case of metal pollution, the change in the ratio of Chl *a/b* suggests that the metal differentially affects the light-harvesting complex (LHC2 of PS2), where Chl *b* is located, rather than the Chl *a* reaction centres (Clijsters and Van Assche 1985, Wisniewski and Dickinson 2003, Aravind and Prasad 2004).

Carotenoids are also sensitive to metal oxidative stress (Cu: Backor and Vaczi 2001; Co: Tewari *et al.* 2002, As: Mascher *et al.* 2002; Cd: Aravind and Prasad 2004). β-carotene is the most resistant (Wisniewski and Dickinson 2003). However other authors, after various stress experiments, observed an increased amount of carotenoids (Kobayashi *et al.* 1997, Malaga *et al.* 1997, Mallick and Rai 1999, Mallick 2004). The carotenoid accumulation is often regarded as one of the mechanisms to counteract stress in organisms (Mallick and Mohn 2000). The xanthophyll cycle—a mechanism that protects the photosynthetic apparatus—shows a clear decline in metal stress studies (Bertrand *et al.* 2001b, Wisniewski and Dickinson 2003). Fig. 1 indicates the possible inhibition of the epoxidase by Cd.

Beside photosynthetic pigments which appear especially sensitive to toxic metals, anthocyanins could play a role in the sequestration of metals, as shown at least for Mo in the vacuole of *Brassica* species (Hale *et al.* 2001).

Lipids: If metal-resistant strains show low hydrogen peroxide and lipid peroxidation contents under pollution, large amounts of fatty acid hydroperoxides can be formed within the chloroplast envelope of metal-sensitive organisms. These strong oxidizing molecules—when not too much abundant—can be metabolized in the stroma through the ascorbate-glutathione cycle (Navari-Izzo and Quartacci 2001, Mullineaux and Karpinski 2002), while the lipophilic α-tocopherol and carotenoids fulfil antioxidant action in thylakoid membranes. When harmful chemical species are persistent, they succeed in modifying the composition of thylakoid membranes (Fig. 1; Mohanty and Mohanty 1988, Wu *et al.* 2003) and the electron transport; *e.g.* Cd, affects the lipid structure around PS2, and especially the light harvesting Chl *a/b* protein complex (Krupa *et al.* 1987, Prasad 1995).

Proteins and enzymatic activities: Non-redox metals may oxidize sulfhydryl groups of proteins or peptides. This change lowers in the same proportions the pool of reducing agents such as GSH (Fig. 1, *a*), and it may modify the spatial conformation of enzymes, preventing optimal activity (Meharg 1994, Demidchik *et al.* 1997, Romero-Puertas *et al.* 2002). Concerning the polypeptide electrophoretic patterns of the thylakoid membranes, no striking difference could be observed between a Cd^{2+}-tolerant mutant and its Cd^{2+}-sensitive parent strain (Voigt and Nagel 2002).

Ferro *et al.* (2003) identified in *Arabidopsis* chloroplast envelope proteins belonging to the defence system against AOS: superoxide dismutase (SOD), ascorbate peroxidase (AP), phospholipid hydroperoxide glutathione peroxidase (PHGP), and thioredoxin M-type 1 (THM1). Most of these enzymes are soluble and act in the vicinity of the inner membrane. Fig. 1 illustrates the enzymatic defence system acting against AOS inside chloroplasts. Under severe metal pollution, not only excessive oxidation occurs, but the efficiency of anti-oxidative defences greatly reduces in non-resistant plants: Cu and Cd affect SOD and AP (Fig. 1), guaiacol peroxidase, glutathione reductase (GR), and catalase (Pätsikkä *et al.* 2002, Cho and Sohn 2004, Sobkowiak *et al.* 2004): at moderate pollution, the enzymatic activities appear stimulated, while they seem inhibited at higher concentrations. This inhibition is weak in resistant organisms, explaining probably the essential of their tolerance (Wu *et al.* 2003). Actually, it is not known whether the AOS are quickly eliminated thanks to enhanced expression of the genes controlling the biosynthesis of antioxidant enzymes and/or to increased activation of existing enzyme pools.

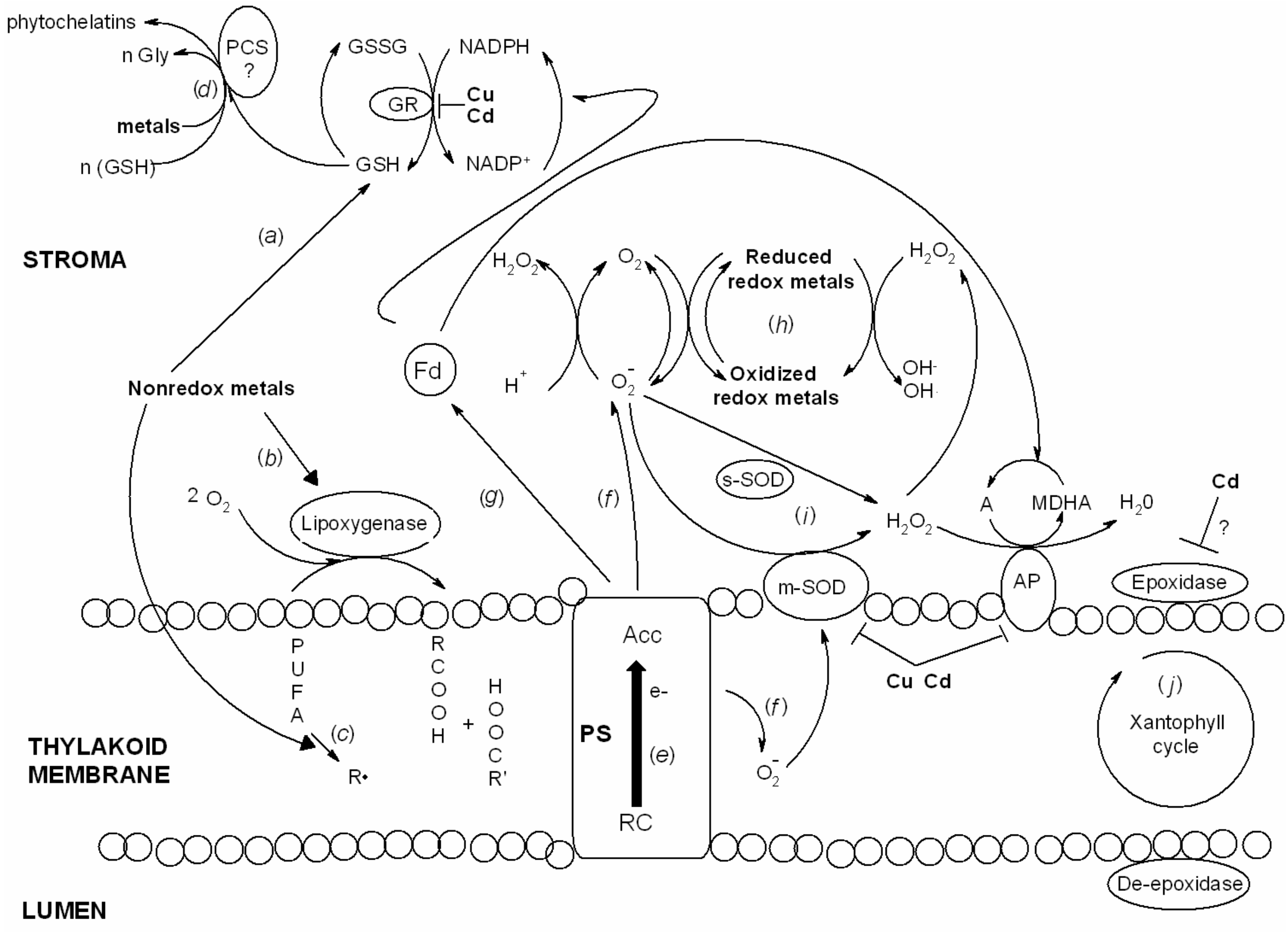

Fig. 1. Oxidative stress and defence reactions occurring in the chloroplast when excess of metal is present: (*a*) depletion of sulfhydryl groups by non-redox metals on reduced glutathione (GSH) regenerated by the glutathione reductase (GR); (*b*) activation of the lipoxygenase by non-redox metals; (*c*) peroxidation of polyunsaturated fatty acids (PUFA) in membranous phospholipids; (*d*) chelation of metals by phytochelatins either imported from cytoplasm or synthesized through the activity of a putative phytochelatin synthetase (PCS) not found yet in chloroplasts; (*e*) electron transfer from the reaction centre (RC) to the acceptor (Acc) of photosystems 1 or 2; (*f*) univalent oxygen reduction by PS2; (*g*) electron transfer through ferredoxin (Fd) and NADP$^+$ (specific to PS1); (*h*) Fenton and Haber-Weiss reactions: one electron oxido-reductions performed by redox metals leading to hydroxyl radicals (OH$^\cdot$); (*i*) spontaneous and/or SOD catalysed disproportionation of superoxide (O_2^-). Superoxide radicals generated by PS1 and PS2 are dismutated by thylakoid-bound m-SOD and hydrogen peroxide formed (H_2O_2) is reduced to water by the thylakoid-bound ascorbate peroxidase (AP). Monodehydroascorbate radical (MDHA) is reduced back to ascorbate (A) by the photoreduced ferredoxin (Fd). The O_2^- and H_2O_2 escaped from thylakoid system are scavenged by a stromal s-SOD and by a glutathione-ascorbate cycle initiated by stromal AP (Navari-Izzo and Quartacci 2001); (*j*) xanthophyll cycle preventing superoxide formation at high irradiance. Epoxidation is inhibited by Cd in diatoms (Bertrand *et al.* 2001). →: activation by metal(s); ─┤: inhibition by metal(s).

Organelle ultrastructure

HM ions directly affect the structure of the thylakoid membranes through peroxidation (Fig. 1, *c*) and oxidative stress and lead to disorganization (Ouzounidou *et al.* 1992, Luna *et al.* 1994, Gallego *et al.* 1996, Stoyanova and Tchakalova 1999, Pätsikkä *et al.* 2002). Generally, Cd induces distortion of the chloroplast ultrastructure in higher plants (bush bean: Barceló *et al.* 1988, tomato: Baszyński *et al.* 1980, but not oilseed rape: Carrier *et al.* 2003).

Photosynthetic activities

Although higher plants concentrate HMs in their roots, some harmful elements reach chlorophyllous cells. Photosynthesis is very sensitive to HMs (*e.g.* Clijsters and van Assche 1985, Yruela *et al.* 1992, Subrahmanyam and Rathore 2000), especially in unicellular organisms (Voigt *et al.* 1998, Lu *et al.* 2000). It can be visualized by the lower Chl fluorescence emitted from chloroplasts (Küpper *et al.* 1998). The most documented impact of HMs on chloroplasts is certainly that induced by Cd (Sanità di Toppi and Gabbrielli 1999). Inhibition of photosynthesis by sub-lethal Cd^{2+} concentrations has been described for various species, from cyanobacteria to higher plants (Malik *et al.* 1992, De Filippis and Ziegler 1993, Vassilev *et al.* 1995, Voigt *et al.* 1998, Baryla *et al.* 2001, Shukla *et al.* 2003).

Photosynthetic light reactions: PS2 is especially affected by HMs (Tůmová and Sofrová 2002). The disorganization of the chloroplast structure leads to the inactivation of oxygen-evolving centres (PS2 donor side: Van Duijvendijk-Matteoli and Desmet 1975, Voigt and Nagel 2002) and impaired electron transport (Atal *et al.* 1991, Sanità di Toppi *et al.* 2003, Vaillant *et al.* 2004). To compensate the PS2 deficiency, higher photo-fluence rates are required according to Voigt and Nagel (2002). In the meantime, the collapse of anti-oxidative defence due to metal pollution might also sensitize PS2 to photoinhibition (Pätsikkä *et al.* 2002).

Other authors have also observed PS2 disturbances in various metal-polluted photosynthetic organisms. The substitution of Mn by Zn on the site of photolysis inhibits oxygen emission (Ralph and Burchett 1998). Lu *et al.* (2000) have found that Hg induces a significant increase in the proportion of the Q_B-non-reducing PS2 reaction centres of the cyanobacterium *Spirulina platensis*. The increase of Hg content led to a decrease in the maximal efficiency of PS2 photochemistry, the efficiency of excitation energy capture by the open PS2 reaction centres, and the quantum yield of PS2 electron transport. Hg also induced a decrease in the coefficients of photochemical and non-photochemical quenching. In Mn-treated ricebean, Subrahmanyam and Rathore (2000) observed a reduction in photochemical quenching (q_P) and excitation capture efficiency of open PS2 (F_v'/F_m') with a concomitant increase in non-photochemical quenching (q_N). The reader is referred to the article of Roháček and Barták (1999) for detailed definition of the above parameters.

Essentially because of PS2 disfunctioning, photophosphorylation rates decrease in the presence of Cd, without evidence for a direct inhibition by the metal of the ATPsynthase/ATPase (Teige *et al.* 1990, Voigt *et al.* 1998). However, Mg^{2+} and Mn^{2+} are characterized as activators of chloroplast ATPase (Berger and Girault 2001).

Photosynthetic dark reactions: Inhibition of carbon metabolism due to metal pollution has been reported in non metal-tolerant plants (Stiborová *et al.* 1987, Malik *et al.* 1992, De Filippis and Ziegler 1993, Voigt and Nagel 2002). CO_2 uptake can be reduced because of a reduced number of stomata in Cd-polluted plants (Baryla *et al.* 2001) or because of stomata closing (Sanità di Toppi and Gabbrielli 1999, Vaillant *et al.* 2004). In ryegrass, Zn treatment inhibits RuBPCO carboxylase capacity, whereas its unmodified oxygenase capacity protects the chloroplast (Monnet *et al.* 2001). Subrahmanyam and Rathore (2000) indicated that a reduced demand for ATP and NADPH in the Calvin cycle causes a down-regulation of PS2 photochemistry.

Weakening of metal toxicity

We reviewed the defence mechanisms that the cell can deploy to cope with toxic metals. Most of experiments have been performed using only one metal in excess for a determined duration. It is thus difficult to predict the modification of the whole metabolism when more than one metal is in excess in the environment, and when the time of contamination is variable. However, some articles relate the combined action of two or more metals in excess and the role of metal polypeptide chelators (metallothioneins and phytochelatins).

Beneficial effect of combined metals

Some authors have observed the beneficial effect of an essential metal when given with a non-essential one. For instance, Aravind and Prasad (2004) demonstrated that when *Ceratophyllum demersum*, a freshwater macrophyte, is treated with Cd along with Zn, this metal protects chloroplasts and associated photochemical functions. Since both Cd and Zn belong to group II of transition elements with similar electronic configuration and valency, they have similar geochemical and environmental properties (Siedlecka 1995). Zn is an essential element required by many key enzymes, and plays a significant structural role as stabilizer of proteins, membrane and DNA-binding proteins (Zn-fingers). It probably maintains Chl synthesis through sulfhydryl group protection, a function primarily associated with Zn (Cakmak 2000). Zn always prefers binding to the –SH groups of the protein moiety and protects them from thiol oxidation and disulfide formation (Chvapil 1973). Zn antagonizes Cd toxicity by counteracting the inhibition of photosynthetic pigment synthesis by maintaining the contents of pigments.

Detoxification of metals by phytochelatins

PCs are cytosolic peptides synthesized from glutathione and capable to chelate metals *via* thiol groups (Fig. 1, *d*; Rauser 1999). Their synthesis is induced by metal excess, Cd being the best inducer just before Zn (Souza and

Rauser 2003). These molecules have been found in all autotrophic plants analyzed so far (reviewed in Gekeler *et al.* 1989, Cobbett 2000). They participate in homeostasis (Zenk 1996, Rauser 1999), and their accumulation has been interpreted as evidence for sequestration and detoxification of metals, including elements other than Cd or Zn (*e.g*. As: Mascher *et al.* 2002). Once formed, PCs act as shuttles for HMs to the vacuole (Vögeli-Lange and Wagner 1990, Davies 1991, Vazquez *et al*. 1992, Brune *et al.* 1994, Zenk 1996, Sanità di Toppi and Gabbrielli 1999, Cobbett 2000). Unusually, PC have also been found in chloroplasts of a cell-wall deficient *Chlamydomonas reinhardtii* and the plant-like vacuole deficient *Euglena gracilis*, binding the Cd measured in chloroplasts (Nagel *et al.* 1996, Mendoza-Cózalt and Moreno-Sánchez 2005).

Metallothioneins (MTs) are also cysteine-rich metal chelators present in plants, but as their synthesis is not necessarily induced by HMs, they have no recognized essential role in metal detoxification (Sanità di Toppi and Gabbrielli 1999). No MT has been detected in chloroplasts; however, the fact that they have been found in cyanobacteria indicates that MT genes should have migrated to the nucleus, according to the endosymbiotic evolution theory (Kroth 2002).

Conclusion

The statements reported in this review indicate that each defence mechanism is restricted, neither to a metal, nor to an organelle. It appears then impossible to determine which one is essential. However, the cell wall and the intracellular reducing power seem to be of considerable importance. Moreover, the conclusions characterizing metal resistant plants compared to sensitive ones are precious to understand further the stress-adapted metabolism that occurred in the former group. Although the genetic machinery of metal hyper-accumulators is not yet fully understood, such organisms are already used for phytoremediation.

References

Abadia, A., Lemoine, Y., Trémolières, A., Ambard-Bretteville, F., Rémy, R.: Iron deficiency in pea: effects on pigment, lipid and pigment-protein complex composition of thylakoids. – Plant Physiol. Biochem. **27**: 679-687, 1989.

Abd-El-Monem, M., Corradi, M.G., Gorbi, G.: Toxicity of copper and zinc to two strains of *Scenedesmus acutus* having different sensitivity to chromium. – Environ. exp. Bot. **40**: 59-66, 1998.

Aravind, P., Prasad, M.N.V.: Zn protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. – Plant Sci. **166**: 1321-1327, 2004.

Archambault, D.J., Zhang, G., Taylor, G.J.: Accumulation of Al in root mucilage of an Al-resistant and an Al-sensitive cultivar of wheat. – Plant Physiol. **112**: 1471-1478, 1996.

Atal, N., Saradhi, P.P., Mohanty, P.: Inhibition of the chloroplast photochemical reactions by treatment of wheat seedlings with low concentrations of cadmium: Analysis of electron transport activities and changes in fluorescence yield. – Plant Cell Physiol. **32**: 943-951, 1991.

Backor, M., Hudak, J., Backorova, M.: Comparison between growth responses of autotrophic and heterotrophic population of lichen photobiont *Trebouxia* (Chlorophyta) on Cu, Hg and Cd chloride treatments. – Phyton Ann. Rei bot. **A 38**: 239-250, 1998.

Backor, M., Vaczi, P.: Copper tolerance in the lichen photobiont *Trebouxia erici* (Chlorophyta). – Environ. exp. Bot. **48**: 11-20, 2001.

Barceló, J., Vázquez, M.D., Poschenrieder, C.: Structural and ultrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris* L.). – New Phytol. **108**: 37-49, 1988.

Baryla, A., Carrier, P., Franck, F., Coulomb. C., Sahut, C., Havaux, M.: Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. – Planta **212**: 696-709, 2001.

Baszyński, T., Wajda, L., Król, M., Wolińska, D., Krupa, Z., Tukendorf, A.: Photosynthetic activities of cadmium-treated tomato plants. – Physiol. Plant. **48**: 365-370, 1980.

Benemann, J.: Literature review on the use of bioaccumulation for heavy metal removal and recovery. – WSRC-TR-175. Vol. 2. Westinghouse Savannah River Co., Aiken 1991.

Berger, G., Girault, G.: Comparison of different cations (Mn^{2+}, Mg^{2+}, Ca^{2+}) on the hydrolytic activity of chloroplast ATPase. – J. Bioenerg. Biomembr. **33**: 93-98, 2001.

Bertrand, M., Guary, J.C., Schoefs, B.: How plants adapt their physiology to an excess of metal. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Physiology. Pp. 751-761. Marcel Dekker, New York 2001a.

Bertrand, M., Schoefs, B., Šiffel, P., Roháček, K., Molnar, I.: Cadmium inhibits epoxidation of diatoxanthin to diadinoxanthin in the xanthophyll cycle of the marine diatom *Phaeodactylum tricornutum*. – FEBS Lett. **508**: 153-156, 2001b.

Blamey, F.P.C., Joyce, D.C., Edwards, D.G., Asher, C.J.: Role of trichomes in sunflower tolerance to manganese toxicity. – Plant Soil **91**: 171-180, 1986.

Brune, A., Urbach, W., Dietz, K.-J.: Compartmentation and transport of zinc in barley primary leaves as basic mechanisms involved in zinc tolerance. – Plant Cell Environ. **17**: 153-162, 1994.

Cakmak, L.: Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. – New Phytol. **146**: 185-205, 2000.

Carrier, P., Baryla, A., Havaux, M.: Cadmium distribution and microlocalization in oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil. – Planta **216**: 939-950, 2003.

Caspi, V., Droppa, M., Horváth, G., Malkin, S., Marder, J.B., Raskin, V.I.: The effect of copper on chlorophyll organization during greening of barley leaves. – Photosynth. Res. **62**: 165-

174, 1999.

Chettri, M.K., Cook, C.M., Vardaka, E., Sawidis, T., Larranas, T.: The effect of Cu, Zn and Pb on the chlorophyll content of the lichen *Cladonia convoluta* and *Cladonia rangiformis*. – Environ. exp. Bot. **39**: 1-10, 1998.

Cho, U.H., Sohn, J.Y.: Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and lipid peroxidation in *Arabidopsis thatliana*. – J. Plant Biol. **47**: 262-269, 2004.

Chvapil, M.: New aspects in the biological role of zinc: a stabilizer of macromolecules and biological membranes. – Life Sci. **13**: 1041-1049, 1973.

Clijsters, H., Van Assche, F.: Inhibition of photosynthesis by heavy metals. – Photosynth. Res. **70**: 31-40, 1985.

Cobbett, C.S.: Phytochelatins and their roles in heavy metal detoxification. – Plant Physiol. **123**: 825-832, 2000.

Connolly, E.L., Guerinot, M.L.: Iron stress in plants. – Genome Biol. **3**: 1024.1-1024.4, 2002.

Conway, H.L.: Sorption of arsenic and cadmium and their effects on growth, micronutrient utilization, and photosynthetic pigment composition of *Asterionella formosa*. – J. Fish. Res. Board Can. **35**: 286-294, 1978.

De Filippis, L.F., Ziegler, H.: Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of *Euglena*. – J. Plant Physiol. **142**: 167-172, 1993.

Demidchik, V., Sololik, A., Yurin, V.: The effect of Cu^{2+} on ion transport systems of the plant plasmalemma. – Plant Physiol. **114**: 1313-1325, 1997.

De Vos, C.H.R., Schat, H.: Free radicals and heavy metal tolerance. – In: Rozema, J., Verkleij, J.A.C. (ed.): Ecological Responses to Environmental Stresses. Pp. 22-30. Kluwer Academic Publ., Dordrecht 1991.

Dietz, K.J., Heber, U., Mimura, T.: Modulation of the vacuolar H^+-ATPase by adenylates as basis for the transient CO_2-dependent acidification of the leaf vacuole upon illumination. – Biochim. biophys. Acta **1373**: 87-92, 1998.

Ernst, W.H.O., Neilssen, H.J.M., Ten Bookum, W.M.: Combination toxicology of metal-enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic soils. – Environ. exp. Bot. **43**: 55-71, 2000.

Ferro, M., Salvis, D., Brugière, S., Miras, S., Kowalski, S., Louwagie, M., Garin, J., Joyard, J., Rolland, N.: Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. – Mol. cell. Proteom. **2**: 325-345, 2003.

Fodor, F., Böddi, B., Sárvári, É., Záray, G., Cseh, E., Láng, F.: Correlation of iron content, spectral forms of chlorophyll and chlorophyll-proteins in iron deficient cucumber (*Cucumis sativus*). – Physiol. Plant. **93**: 750-756, 1995.

Fourest, E., Volesky, B.: Alginate properties and heavy metal biosorption by marine algae. – Appl. Biochem. Biotechnol. **61**: 33-44, 1997.

Foy, C.D., Chaney, R.L., White, M.C.: The physiology of metal toxicity in plants. – Annu. Rev. Plant Physiol. **29**: 511-566, 1978.

Gallego, S.M., Benavides, M.P., Tomaro, M.L.: Effect of heavy metal ion excess on suflower leaves evidence for involvement of oxidative stress. – Plant Sci. **121**: 151-159, 1996.

Gekeler, W., Grill, E., Winnacker, E.L., Zenk, M.H.: Survey of the plant kingdom from the ability to bind heavy metals through phytochelatins. – Z. Naturforsch. **44c**: 361-369, 1989.

Goyald, R., Seaward, M.R.D.: Metal uptake in terricolous lichens. I. Metal localization within the thallus. – New Phytol. **89**: 631-645, 1981.

Goyald, R., Seaward, M.R.D.: Metal uptake in terricolous lichens. III. Translocation in the thallus of *Peltigera canina*. – New Phytol. **90**: 85-98, 1982.

Hale, K.L., McGrath, S.P., Lombi, E., Stack, S.M., Terry, N., Pickering, I.J., George, G.N., Pilon-Smits, E.A.H.: Molybdenum sequestration in *Brassica* species. A role for anthocyanins? – Plant Physiol. **126**: 1391-1402, 2001.

Hernandez, E., Olguin, E.J.: Biosorption of heavy metals influenced by the chemical composition of *Spirulina sp. (Arthrospira)* biomass. – Environ. Technol. **23**: 1369-1377, 2002.

Jorge, R.A., Arruda, P.: Aluminium-induced organic acids exudation by roots of an aluminium-tolerant tropical maize. – Phytochemistry **45**: 675-681, 1997.

Kobayashi, J.S., Kakizono, T., Nishio, N., Nagai, S., Kurimara, Y., Tsuji, Y.: Antioxidant role of astaxanthin in the green alga *Haematococcus pluvialis*. – Appl. Microbiol. Biotechnol. **48**: 351-356, 1997.

Kochian, L.V., Pence, N.S., Letham, D.L.D., Pineros, M.A., Jurandir, V.M., Hoekenga, O.A., Garvin, D.F.: Mechanisms of metal resistance in plants: aluminium and heavy metals. – Plant Soil **247**: 109-119, 2002.

Kroth, P.G.: Protein transport into secondary plastids and the evolution of primary and secondary plastids. – Int. Rev. Cytol. **221**: 191-255, 2002.

Krupa, Z., Skórzyńska, E., Maksymiec, W., Baszyński, T.: Effect of cadmium treatment on the photosynthetic apparatus and its photochemical activities in greening radish seedlings. – Photosynthetica **21**: 156-164, 1987.

Küpper, H., Küpper, F., Spiller, M.: Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. – J. exp. Bot. **47**: 259-266, 1996.

Küpper, H., Küpper, F., Spiller, M.: *In situ* detection of heavy metal substituted chlorophylls in water plants. – Photosynth. Res. **58**: 123-133, 1998.

Lu, C.M., Chau, C.W., Zhang, J.H.: Acute toxicity of excess mercury on the photosynthetic performance of cyanobacterium, *S. platensis* – assessment by chlorophyll fluorescence analysis. – Chemosphere **41**: 191-196, 2000.

Luna, C.M., Gonzalez, C.A., Trippi, V.S.: Oxidative damage caused by an excess of copper in oat leaves. – Plant Cell Physiol. **35**: 11-15, 1994.

Luo, H., Lu, Y., Shi, X., Mao, Y., Delal, N.S.: Chromium (IV)-mediated Fenton-like reaction causes DNA damage: implication to genotoxicity of chromate. – Ann. clin. lab. Sci. **26**: 85-191, 1996.

Ma, J.F., Ryan, P.R., Delhaize, E.: Aluminium tolerance in plants and the complexing role of organic acids. – Trends Plant Sci. **6**: 273-278, 2001.

Macfie, S.M., Wellbourn, P.M.: The cell wall as a barrier to uptake of metal ions in the unicellular green alga *Chlamydomonas reinhardtii* (Chlorophyceae). – Arch. environ. Contam. Toxicol. **39**: 413-419, 2000.

Malaga, G., Calmanovici, G., Puntarulo, S.: Oxidative damage to chloroplasts from *Chlorella vulgaris* exposed to ultraviolet-*b* radiation. – Physiol. Plant. **101**: 455-462, 1997.

Malik, D., Sheoran, I.S., Singh, R.: Carbon metabolism in leaves of cadmium treated wheat seedlings. – Plant Physiol. Biochem. **30**: 223-229, 1992.

Mallick, N.: Copper-induced oxidative stress in the chlorophycean microalga *Chlorella vulgaris*: response of the antioxidant system. – J. Plant Physiol. **161**: 591-597, 2004.

Mallick, N., Mohn, F.H.: Reactive oxygen species: response of algal cells. – J. Plant Physiol. **157**: 183-193, 2000.

Mallick, N., Rai, L.C.: Responses of the antioxidant systems of the nitrogen fixing cyanobacterium *Anabaena doliolum* to copper. – J. Plant Physiol. **155**: 146-149, 1999.

Mascher, R., Lippmann, B., Holzinger, S., Bergmann, H.: Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. – Plant Sci. **163**: 961-969, 2002.

Meharg, A.A.: Integrated tolerance mechanisms – constitutive and adaptative plant-responses to elevated metal concentrations in the environment. – Plant Cell Environ. **17**: 989-993, 1994.

Mendoza-Cózalt, D.G., Moreno-Sánchez, R.: Cd^{2+} transport and storage in the chloroplast of *Euglena gracilis*. – Biochim. biophys. Acta **1706**: 88-97, 2005.

Mithöfer, A., Schulze, B., Boland, W.: Biotic and heavy metal stress response in plants: evidence for common signals. – FEBS Lett. **566**: 1-5, 2004.

Mohanty, N., Mohanty, P.: Cation effects on primary processes of photosynthesis. – In: Singh, R., Sawhney, S.K. (ed.): Advances in Frontier Areas of Plant Biochemistry. Pp. 1-18. Prentice-Hall, New Delhi 1988.

Monnet, F., Vaillant, N., Vernay, P., Coudret, A., Sallanon, H., Hitmi, A.: Relationship between PSII activity, CO_2 fixation, and Zn, Mn and Mg contents of *Lolium perenne* under zinc stress. – J. Plant Physiol. **158**: 1137-1144, 2001.

Mullineaux, P., Karpinski, S.: Signal transduction in response to excess light: getting out of the chloroplast. – Curr. Opin. Plant Biol. **5**: 43-48, 2002.

Myśliwa-Kurdziel, M., Prasad, M.N.V., Strzałka, K.: Photosynthesis in metal stressed plants. – In: Prasad, M.N.V. (ed.): Heavy Metal Stress in Plants: from Molecules to Ecosystems. 2nd Ed. Pp. 146-181. Springer-Verlag, Heidelberg 2003.

Myśliwa-Kurdziel, M., Strzałka, K.: Influence of metals on the biosynthesis of photosynthetic pigments. – In: Prasad, M.N.V., Strzałka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 201-228. Kluwer Academic Publ., Dordrecht 2002.

Myśliwa-Kurdziel, B., Strzałka, K.: Influence of Cd(II), Cr(VI) and Fe(III) on early steps of deetiolation process in wheat: fluorescence spectral changes of protochlorophyllide and newly formed chlorophyllide. – Agr. Ecosys. Environ. **106**: 199-207, 2004.

Nagel, K., Adelmeier, U., Voigt, J.: Subcellular distribution of cadmium in the unicellular green alga *Chlamydomonas reinhardtii*. – J. Plant Physiol. **149**: 86-90, 1996.

Navari-Izzo, F., Quartacci, M.F.: Phytoremediation of metals. – Minerva Biotech. **13**: 73-83, 2001.

Ochiai, E.I.: General Principles of Biochemistry of the Elements. – Plenum Press, New York 1987.

Olmos, E., Martinez-Solano, J.R., Piqueras, A., Hellin, E.: Early steps in oxidative burst induced by cadmium in cultured tobacco cells (BY-2 line). – J. exp. Bot. **54**: 291-301, 2003.

Ouzounidou, G., Eleftheriou, E.P., Karataglis, S.: Ecophysiological and ultrastructural effects of copper in *Thlapsi ochroleucum* (*Cruciferae*). – Can. J. Bot. **70**: 947-957, 1992.

Päivöke, A.E.A.: Soil pollution alters ATP and chlorophyll contents in *Pisum sativum* seedlings. – Biol. Plant. **46**: 145-148, 2003.

Parker, D.L., Mihalick, J.E., Plude, J.L., Plude, M.J., Clark, T.P., Egan, L., Flom, J.J., Rai, L.C., Kumar, H.D.: Sorption of metals by extracellular polymers from the cyanobacterium *Microcystis aeruginosa* f. *flos-aquae* strain C3-40. – J. appl. Phycol. **12**: 219-224, 2000.

Pätsikkä, E., Kairavuo, M., Šeršen, F., Aro, E.M., Tyystijärvi, E.: Excess copper predisposes photosystem II to photoinhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll. – Plant Physiol. **129**: 1-9, 2002.

Pellet, D.M., Papernik, L.A., Kochian, L.V.: Multiple aluminium-resistance mechanisms in wheat. – Plant Physiol. **112**: 591-597, 1996.

Pistocchi, R., Mormile, A.M., Guerrini, F., Isani, G., Boni, L.: Increased production of extra- and intracellular metal-ligands in phytoplankton exposed to copper and cadmium. – J. appl. Phycol. **12**: 469-477, 2000.

Prasad, M.N.V.: Cadmium toxicity and tolerance to vascular plants. – Environ. exp. Bot. **35**: 525-545, 1995.

Prasad, M.N.V., Strzałka, K.: Impact of heavy metals on photosynthesis. – In: Prasad, M.N.V., Hagenmeyer, J. (ed.): Heavy Metal Stress in Plants – From Molecules to Ecosystems. Pp. 117-138. Springer-Verlag, Berlin 1999.

Quartacci, M.F., Pinzino, C., Sgherri, C.L.M., Dalla Vecchia, F., Navari-Izzo, F.: Growth in excess copper induces changes in the lipid composition and fluidity of PSII-enriched membranes in wheat. – Physiol. Plant. **108**: 87-93, 2000.

Ralph, P.J., Burchett, M.D.: Photosynthesis response of *Halophila ovalis* to heavy metal stress. – Environ. Pollut. **103**: 91-101, 1998.

Rauser, W.E.: Structure and function of metal chelators produced by plants. – Cell Biochem. Biophys. **31**: 1-30, 1999.

Roháček, K., Barták, M.: Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. – Photosynthetica **37**: 339-363, 1999.

Romero-Puertas, M.C., Palma, J.M., Gómez, M., Del Río, L.A., Sandalio, L.M.: Cadmium causes the oxidative modification of proteins in pea plants. – Plant Cell Environ. **25**: 677-686, 2002.

Salt, D.E., Prince, R.C., Pichering, I.J., Raskin, I.: Mechanisms of cadmium immobilization and accumulation in Indian mustard. – Plant Physiol. **109**: 427-433, 1995.

Sanità di Toppi, L., Gabbrielli, R.: Response to cadmium in higher plants. – Environ. exp. Bot. **41**: 105-130, 1999.

Sanità di Toppi, L., Gremigni, P., Pawlik-Skowronska, B., Prasad, M.N.V., Cobbett, C.S.: Responses to heavy metals in plant molecular approach. – In: Sanità di Toppi, L., Pawlik-Skowronska, B. (ed.): Abiotic Stresses in Plants. Pp. 33-156. Kluwer Academic Publ., Dordrecht 2003.

Sárvári, É., Fodor, F., Cheh, E., Varga, A., Záray, G., Zolla, L.: Relationship between changes in ion content of leaves and chlorophyll-protein composition in cucumber under Cd and Pb stress. – Z. Naturforsch. **54c**: 746-753, 1999.

Schmidt, W.: Mechanisms and regulation of reduction-based iron uptake in plants. – New Phytol. **141**: 1-26, 1999.

Schoefs, B., Bertrand, M.: Chlorophyll biosynthesis – a review. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. 2nd Ed. Pp. 37-54. Taylor & Francis, Boca Raton – London – New York 2005.

Schoefs, B., Franck, F.: Protochlorophyllide reduction: Mechanisms and evolution. – Photochem. Photobiol. **78**: 543-557, 2003.

Shukla, U.C., Singh, J., Joshi, P.C., Kakkar, P.: Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat seedlings. – Biol. Trace Elem. Res. **92**: 257-273, 2003.

Sicko-Goad, L.: A morphometric analysis of algal response to low dose, short term heavy metal exposure. – Protoplasma **110**: 75-86, 1982.

Siedlecka, A.: Some aspects of interactions between heavy metals and plant mineral nutrients. – Acta Soc. Bot. Pol. **3**: 265-272, 1995.

Sobkowiak, R., Rymer, K., Rucińska, R., Deckert, J.: Cadmium-induced changes in antioxidant enzymes in suspension culture of soybean cells. – Acta biochim. pol. **51**: 219-222, 2004.

Solymosi, K., Lenti, K., Myśliwa-Kurdziel, B., Fidy, J., Strzałka, K., Böddi, B.: Depending on concentration, Hg^{2+} reacts with different components of the NADPH:protochlorophyllide oxidoreductase macrodomains. – Plant Biol. **6**: 358-363, 2004.

Souza, J.F., Rauser, W.E.: Maize and radish sequester excess cadmium and zinc in different ways. – Plant Sci. **65**: 1009-1022, 2003.

Stiborová, M., Ditrichová, M., Březinová, A.: Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley and maize seedlings. – Biol. Plant. **29**: 453-467, 1987.

Stohs, S.J., Bagchi, D.: Oxidative mechanisms in the toxicity of metal ions. – Free Rad. Biol. Med. **18**: 321-336, 1995.

Stoyanova, D., Tchakalova, T.: Cadmium induced ultrastructural changes in shoot apical meristem of *Elodea canadensis* Rich. – Photosynthetica **37**: 47-52, 1999.

Subrahmanyam, D., Rathore, V.S.: Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. – Photosynthetica **38**: 449-453, 2000.

Teige, M., Huchzermeyer, B., Schultz, G.: Inhibition of chloroplast ATPsynthase/ATPase is a primary effect of heavy metal toxicity in spinach plants. – Biochem. Physiol. Pflanzen **186**: 165-168, 1990.

Tewari, R.K., Kumar, P., Sharma, P.N., Bisht, S.S.: Modulation of oxidative stress responsive enzymes by excess cobalt. – Plant Sci. **162**: 381-388, 2002.

Tůmová, E., Sofrová, D.: Response of intact cyanobacterial cells and their photosynthetic apparatus to Cd^{2+} ion treatment. – Photosynthetica **40**: 103-108, 2002.

Vaillant, N., Monnet, F., Hitmi, A., Sallanon, H., Coudret, A.: Comparative study of responses in four *Datura* species to a zinc stress. – Chemosphere (in press), 2004.

Van Duijvendijk-Matteoli, M.A., Desmet, G.M.: On the inhibitory action of cadmium on the donor side of Photosystem II in isolated chloroplasts. – Biochim. biophys. Acta **408**: 164-168, 1975.

Vangronsveld, J., Clijsters, H.: Toxic effect of metals. – In: Farango, M.E. (ed.): Plants and the Chemical Elements. Pp. 149-177. VCH Verslagsgesellschaft, Weinheim 1994.

Vassilev, A., Iordanov, I., Chakalova, E., Derin, V.: Effect of cadmium stress on growth and photosynthesis of young barley (*Hordeum vulgare*) plants. 2. Structural and functional changes in the photosynthetic apparatus. – Bulg. J. Plant Physiol. **21**: 12-21, 1995.

Vazquez, M.D., Poschenrieder, C., Barcelo, J.: Ultrastructural effects and localization of low cadmium concentrations in bean roots. – New Phytol. **120**: 215-226, 1992.

Vazquez, M.D., Poschenrieder, C., Barcelo, J., Baker, A.J.M., Hatton, P., Cope, G.H.: Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens*. – Bot. Acta **107**: 243-250, 1994.

Vögeli-Lange, R., Wagner, G.L.: Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Implication of a transport function for cadmium-binding peptides. – Plant Physiol. **92**: 1086-1093, 1990.

Voigt, J., Nagel, K.: The donor side of photosystem II is impaired in a Cd^{2+}-tolerant mutant strain of the unicellular green alga *Chlamydomonas reinhardtii*. – J. Plant Physiol. **159**: 941-950, 2002.

Voigt, J., Nagel, K., Wrann, D.: A cadmium-tolerant *Chlamydomonas* mutant strain impaired in photosystem II activity. – J. Plant Physiol. **153**: 566-573, 1998.

Wallace, A., Wallace, G.A., Cha, J.W.: Some modifications in trace metal toxicities and deficiencies in plants resulting from interactions with other elements and chelating agents : the special case of iron. – J. Plant Nutr. **15**: 1589-1598, 1992.

Williams, L.E., Pittman, J.K., Hall, J.L.: Emerging mechanisms for heavy metal transport in plants. – Biochim. biophys. Acta **1465**: 104-126, 2000.

Wisniewski, L., Dickinson, N.M.: Toxicity of copper to *Quercus robur* (English Oak) seedlings from a copper-rich soil. – Environ. exp. Bot. **50**: 99-107, 2003.

Wu, F., Zhang, G., Dominy, P.: Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. – Environ. exp. Bot. **50**: 67-78, 2003.

Yruela, I., Montoya, G., Picorel, R.: The inhibitory mechanism of Cu(II) on the Photosystem II electron transport from higher plants. – Photosynth. Res. **33**: 227-233, 1992.

Zenk, M.H.: Heavy metal detoxification in plants – A review. – Gene **179**: 21-30, 1996.