Tolerance and prospection of phytoremediator woody species of Cd, Pb, Cu and Cr

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High concentrations of Cd, Pb, Cu and Cr can cause harmful effects to the environment. These highly toxic pollutants constitute a risk for aquatic and terrestrial life. They are associated with diverse bioavailable geochemical fractions, like the water-soluble fraction and the exchangeable fraction, and non-available fractions like those associated with the crystalline net of clays and silica minerals. Depending upon their chemical and physical properties we can distinguish different mechanisms of metal toxicity in plants, such as production of reactive oxygen species from auto-oxidation, blocking and/or displacement of essential functional groups or metallic ions of biomolecules, changes in the permeability of cellular membranes, reactions of sulphydryl groups with cations, affinity for reactions with phosphate groups and active groups of ADP or ATP, substitution of essential ions, induction of chromosomal anomalies and decrease of the cellular division rate. However, some plant species have developed tolerance or resistance to these metals naturally. Such evolution of ecotypes is a classic example of local adaptation and microevolution, restricted to species with appropriate genetic variability. Phytoremediator woody species, with (i) high biomass production, (ii) a deep root system, (iii) high growth rate, (iv) high capacity to grow in impoverished soils, and (v) high capacity to allocate metals in the trunk, can be an alternative for the recovery of degraded soils due to excess of metallic elements. Phytoremediation using woody species presents advantageous characteristics as an economic and ecologically viable system, making it an appropriate, practical and successful technology.

Key-words: decontamination, heavy metals, hyperaccumulation, phytodegradation, phytostabilization, phytotoxicity

Tolerância e prospecção de espécies lenhosas fitorremediadoras de Cd, Pb, Cu e Cr: Altas concentrações de Cd, Pb, Cu e Cr podem provocar efeitos danosos ao ambiente. Esses poluentes altamente tóxicos constituem um risco para a vida aquática e terrestre. Encontram-se associados às diversas frações geoquímicas biodisponíveis, a exemplo da fração solúvel em água e da fração trocável, e as não disponíveis como a fração associada à rede cristalina de argilas e de minerais silicatados. Conforme as suas propriedades químicas e físicas podem-se distinguir mecanismos diferentes de toxicidade de metais em plantas, tais como produção de espécies reativas de o*xigênio* pela auto-oxidação, bloqueio e, ou, deslocamento de grupos funcionais ou de íons metálicos essenciais de biomoléculas, mudanças na permeabilidade de membranas celulares, reações de grupos sulfidrílicos com cátions, afinidade para reações com grupos fosfatos e grupos ativos de ADP ou ATP, substituição de íons essenciais, indução de anomalias cromossomais e decréscimo da taxa de divisão celular. Várias espécies vegetais desenvolveram, naturalmente, tolerância ou resistência a esses metais. Essa evolução de ecótipos é exemplo clássico de adaptação local e de microevolução, restrita às espécies com variabilidade genética apropriada. Espécies lenhosas fitorremediadoras, com (i) elevada produção de biomassa, (ii) sistema radicular profundo, (iii) altas taxas de crescimento, (iv) capacidade de crescer em solos pobres em nutrientes e (v) alta capacidade de concentrar metais no tronco podem ser alternativas importantes para a descontaminação de solos com excesso de elementos metálicos. A fitorremediação apresenta características vantajosas, como sistema econômico e ecologicamente viável, que a torna uma tecnologia apropriada, prática e bem sucedida como opção para remediação.

Palavras-chave: descontaminação, fitodegradação, fito-estabilização, fitotoxicidade, hiperacumulação, metais pesados

INTRODUCTION

Heavy metals are natural constituent of the lithosphere, whose geochemical cycles and biochemical balances have been drastically altered by human activity (Sebastiani et al., 2004). Pollution due to heavy metals place human health at risk (Il'yasova and Schwartz, 2005) and it is responsible for several environmental problems, including the decrease of microbial activity, soil fertility and crop yields (Yang et al., 2005). The annual toxicity of all the mobilized metals exceeds the total combined toxicity of all radioactive and organic residues generated in the same period (Nriagu and Pacyna, 1988). Pollution by heavy metals normally coincides with the increase in industrialization of a given region and becomes more severe when there are neither controls nor adequate environmental norms (Pilon-Smits, 2005).

Discharges of industrial residues that contain Cd, Pb, Cu and Cr constitute a potential risk to aquatic life, animals and humans, due to the contamination of air, soil sediments, vegetation and water (Yang et al., 2005). These metallic elements, either isolated or in a group, are commonly used in industrial processes of diverse sectors like paper and cellulose, petrochemicals, chemical products, fertilizers, oil refining, steel production, non-iron metals, spare parts of vehicles, plain glass and cement, textile and leather products and the manufacture of other devices (Sanitá di Toppi and Gabrielli, 1999).

The accumulation of heavy metals in vascular plants provokes significant biochemical and physiological responses, modifying several metabolic processes (Macfarlane et al., 2003). Reduction in the net photosynthetic rate due to toxicity of these metals causes decreases in growth and in productivity (Van Assche and Clijsters, 1983). These metals affect the electron carriers and their flow from H_2O to the reaction center of photosystem 2 (PS-2) (Prasad et al., 1991). Consequently, this inhibition of the primary reactions decreases the energy level of the cells (ATP pool). The decrease in the concentration of total soluble carbohydrates can be attributed, in certain cases, to the reduction of photosynthetic activity and formation of chlorophyll caused by the accumulation of metals (Abdul Razak, 1985). A significant decline in protein concentration and enzyme activities suggests a possible interference of metallic ions in the synthesis of plant proteins (Schützendübel and Polle, 2002).

The evolution in nature of plant ecotypes tolerant to metals is a classic example of local adaptation and microevolution restricted to species with appropriate genetic variability (Lindegaard and Barker, 1997). According to Patra et al. (2004), plants that grow in environments contaminated with traces of metals show strategies of escape or tolerance to metal toxicity that have been selected during evolution. Several plant species have developed tolerance to metals in a relatively short period of about thirty years (Hall, 2002).

Technologies exist that can eliminate and/or reduce the presence of these heavy metals in industrial effluents, such as precipitation and coprecipitation, electroplating and eletrocoagulation, cementation, membrane separation, solvent extraction, ionic exchange, adsorption and biosorption (Ahluwalia and Goyal, 2007). However, these techniques are expensive and cause great environmental impact. In recent years there has been a great interest for phytoremediation of metallic pollutants in the soil, in view of the high cost of other methods of soil remediation (Glass, 2000). Also, there is the need to use a sustainable process with low effective cost and ecologically viable that does not involve soil removal and/or deposition nor destroys the biological and functional integrity of the soil (Dickinson and Pulford, 2005; Pilon-Smits, 2005).

The main objectives of this review are to describe the tolerance of plants to Cd, Pb, Cu and Cr metals, to demonstrate the importance of prospection of woody species as hyperaccumulators of these elements and to show the potential use of these species in the process of phytoremediation of soils with high indices of contamination.

NATURAL AND ANTHROPOGENIC FACTORS AFFECTING THE BIOAVAILABILITY OF METALS

High concentrations of Cd, Pb, Cu and Cr originating from mining (Prasad and Freitas, 2003) or by anthropogenic actions such as discharges of toxic residues in rivers, lakes, maritime coast and in the air, industrial activities, farm use of fertilizers and pesticides, incineration of urban and industrial residues, among others sources, have been causing harmful effects to the environment (Ahluwalia and Goyal, 2007). These highly toxic pollutants constitute a risk for aquatic and terrestrial life, especially plants, animals and humans.

Cadmium, Pb, Cr and Cu are present in the soils as free metallic ions, metallic soluble complexes (sequestered by ligands), metallic exchangeable ions, organic linked metals, precipitated or insoluble compounds such as oxides, carbonates and hydroxides, or can constitute part of the structure of silicated materials (indigenous soil content) (Leyval et al., 1997). Several studies have demonstrated that pH is one of the most important factors in the control of the concentration of these metals in the soil solution (King, 1988). Elliott et al. (1986) proposed that under acidic conditions the phenomenon of adsorption is more important in the control of metal bioavailability, while precipitation reactions and complexation have greater influence under neutral and alkaline conditions.

As a general rule, the formation of complexes is favored at pH values next to neutrality, because, under acid conditions the ligands are protonated, whereas under alkaline conditions the metals can precipitate in the form of hydroxides (McCarthy and Perdue, 1991). However, metals have different soil behaviors. According to Hsu (1989), cations with lower affinity for oxygen (O_2), e.g. Cd, can release H^+ from the water molecule or from OH^-, as well as from the surface of oxides only at higher pH values, originating complexes of the Al-OH-M type. For those with high (O_2) affinity like Cr, Cu and Pb, the H^+ can be liberated at even low pH values forming complexes of the Al-O-M type. Under conditions of higher acidity, the H^+ cannot be liberated, which limits the specific adsorption.

Heavy metals react with diffusing ligands, macromolecules and ligands present in membranes, which sometimes confer them bioaccumulator properties, biomagnification in the food chain, persistence and disturbances in the metabolic processes of living creatures (Fernandes and Enriques, 1991). Bioaccumulation and biomagnification are responsible for transforming concentrations considered normal into toxic concentrations for different species of biota and man (Tavares and Carvalho, 1992). Once the heavy metal is mobilized in the environment, its total amount remains the same, whatever its form, whether ion, complex or precipitate.

The interactions that occur in the soil-plant system are of a complex nature. The assimilation of trace elements by plants varies greatly as a function of soil conditions. High metal concentrations in the soil do not always indicate correspondingly high levels of these metals in the plants; this depends on several factors, such as pH, cation exchange capacity, organic matter, humidity and others (Albasel and Cottenie, 1985). Toxic metallic ions penetrate cells using the same absorption processes of essential micronutrient ions. The quantity absorbed by the plant depends on the concentration and speciation of the metal in the soil solution, together with its successive movement from the soil to the root surface and from the root to the aerial part (Patra et al., 2004).

The translocation of these metallic ions to the aerial part depends on the plant species, the metal involved and the environmental conditions (Liu et al., 2007). Their genotoxic effects depend on the oxidation state of the metal, their concentration and the duration of exposition, and these are more pronounced at high concentrations and after a long exposition time (Cosio et al., 2005). Cytological analyses demonstrate numerous aberrations of chromosomes in meristematic root tissue of seedlings developed from seeds collected from trees in the polluted area, including chromosome bridges and stickiness, laggards, retarded and forward chromosomes, and their fragments (Prus-Glowacki et al., 2006).

EFFECT OF METALS ON NUTRITION AND METABOLISM OF PLANTS

Cadmium: In plants, Cd triggers a sequence of reactions leading to: (i) inhibition (Schützendübel and Polle, 2002) or growth reduction of the aerial part and the root system (Mendelssohn et al., 2001); (ii) induction of phytochelatin production (Cobbett and Goldsbrough, 2002); (iii) interference in chlorophyll biosynthesis and activity of specific enzymes, such as peroxidase, ascorbate peroxidase, catalase, glutathione synthetase, glutathione reductase, dehydroascorbate reductase, superoxide dismutase, guaiacol peroxidase, mono-dehydroascorbate reductase (Vassilev et al., 2002); (iv) induction of apoptotic bodies and oligonucleosomal DNA fragments (Souza, 2007); (v) induction of oxidative stress (Souza, 2007); (vi) damage to chloroplasts

(Vollenweider et al., 2006); (vii) reduction of transpiration and photosynthetic rates (Sanitá di Toppi and Gabbrielli, 1999); (viii) induction of premature leaf senescence and chlorosis (Souza, 2007); and (ix) stimulation of secondary metabolism, lignification and, finally, cellular death (Schützendübel and Polle, 2002).

Cadmium seems to be the most toxic heavy metal to plants. In some species, it can promote decreases up to 50% in dry matter production. Furthermore, it is easily absorbed and translocated to different plant parts (Souza, 2007). Different plant species show a highly variable capacity to accumulate Cd in relation to the concentration found in the substrate in which they grow (Vassilev et al., 2002). Even among cultivars of the same species a wide variation in Cd absorption and translocation can occur (Sanitá di Toppi and Gabbrielli, 1999). When absorbed, it binds to the cell wall constituents and to other macromolecules in the cell interior (Vassilev et al., 2002).

Cadmium concentration in plant tissues increases with the increment of its concentration in nutrient solutions and with time of exposure (Souza, 2007), the concentration in the roots being higher than in the aerial part. According to Souza (2007) the increase in Cd concentration in the roots is not due to the increase in the absorption of this element, but due to the concomitant decrease in dry matter accumulation. Although there is a high Cd concentration in roots it is also found in leaves and stems, demonstrating that this metallic element is not totally immobilized in the root portion, but translocated to the aerial part (Unterbrunner et al., 2007).

It is well known that Cd can induce essential nutrient deficiency and even to decrease concentrations of many macronutrients in plants (Siedleska, 1995). Cadmium can influence the absorption of Cu, Fe, Zn and Mn through competition for sites or processes shared by these cations (Sanitá di Toppi and Gabbrielli, 1999). Chlorosis, one of the characteristics of Fe deficiency, is one of the symptoms of Cd toxicity caused by competition of both elements for the same absorption site in the plasma membrane (Sanitá di Toppi and Gabbrielli, 1999). Chlorosis at high Cd concentrations probably is associated with the decrease in Fe translocation to leaves (Wong et al., 1984). On the other hand, Root et al. (1975) suggested that chlorosis induced by Cd may be due to alterations in the Fe/Zn ratio, rather than to Fe deficiency, since plants treated with Cd showed a greater concentration of this micronutrient. The Cd effects on Fe and Zn absorption have presented conflicting results. Thus, depending on the species, the presence of Cd in the growth media can increase (Wong et al., 1984), decrease (Gussarsson, 1994) or not affect (Souza, 2007) the absorption of Fe in plants.

Lead: Plants absorb and accumulate Pb in roots, stems, leaves, root nodules, seeds, etc. Uptake of Pb in plants is regulated by pH, particle size and cation exchange capacity of the soils as well as by exsudation and other physico-chemical parameters (Sharma and Dubey, 2005). The increase depends on the increment of exogenous Pb levels (Patra et al., 2004). Most of the Pb absorbed by plants accumulates in roots, and only a small fraction is translocated to the aerial part (Patra et al., 2004). The retention of Pb in the roots is due to binding to ion exchange sites and extracellular precipitation, mainly in the form of Pb carbonates, with both these mechanisms occurring in the cell wall (Jarvis and Leung, 2002). However, Pb does not always penetrate the root endoderm and enter the stele. Here, the endoderm acts as a barrier to Pb absorption and penetration to the interior of the stele and its transport to the aerial plant part (Weis and Weis, 2004).

Once absorbed by plants, Pb causes multiple indirect and direct effects on growth and metabolism (Sharma and Dubey, 2005). Its effects depend on the concentration, salt type, pH and plant species involved. Lead effects are more pronounced at high concentrations and duration of exposition. This metal, in some cases, is able to stimulate metabolic processes when at low concentrations (Patra et al., 2004). However, excess Pb: (i) affects the germination of seeds (Fargasova, 1994); (ii) causes stunted growth, chlorosis and blackening of the root system (Sharma and Dubey, 2005); (iii) promotes reductions in stomatal conductance and stomata size (although it may bring about increases in stomatal number) (Xiong, 1997); (iv) reduces the activity of some enzymes (Patra et al., 2004); (v) inhibits photosynthesis due to disturbances in electron transfer reactions (Sharma and Dubey, 2005); (vi) reduces the respiration rate (Romanowska et al., 2002); and (vii) upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability (Sharma and Dubey, 2005).

The visible symptoms of Pb toxicity include chlorotic spots and necrotic lesions at the leaf surface, growth retardation (Patra et al., 2004) and leaf senescence – promoted by reduction of chlorophyll, DNA, RNA, protein and dry biomass, decreases in the activity ratio of acid:alkaline pyrophosphatases and a decline in the activities of protease and RNase (Patra et al., 2004). Chlorosis and necrosis could be due to disruption of thylakoid and stromal membranes, resulting in a decrease in photosynthesis and, consequently, reduction in the availability of photosynthates for biomass accumulation (Sharma and Dubey, 2005). Oxidative stress induced by Pb can generate great amounts of reactive oxygen species, such as superoxide, hydroxide, peroxide and the oxygen singlet (Sharma and Dubey, 2005), that involve all areas of aerobic metabolism and are usually also associated with membrane damage and lipid peroxidation (Smirnoff, 1995).

The effectiveness of Pb in displacing some cationic metals from roots is known, which suggests that Pb could play a role in destabilization of physiological barriers for the movement of solutes in the roots and thereby limit the availability of nutrients to plants (Sharma and Dubey, 2005). Several studies have shown that the presence of Pb, Cd, Zn and Cu in the substrate can decrease the absorption and transport of macronutrients in plants (Godbold and Kettner, 1991). Macronutrient deficiencies in plants are often a manifestation of toxic effects due to heavy metals (Siedleska, 1995). Some macroelements, including Ca, Mg and P play a protective role against the toxic effects of heavy metals (Rashid and Popovic, 1990). Lead competes with Ca for the same coupling site in the cell (Godbold and Kettner, 1991). Moreover, Pb can be transported through Ca channels to the symplast (Tomsig and Suszkiw, 1991).

Copper: The low soil mobility of Cu, due to its strong linking to organic and inorganic colloids, promotes its accumulation to toxic levels towards plants (Kalbitz and Wenrich, 1998). Of the total soil content of Cu, only a very small amount is available in the soluble form. However, due to the long exposure time enhancing absorption, considerable amounts are accumulated in plants.

Most of the Cu absorbed by the plant is retained in the roots (Wisniewski and Dickinson, 2003). Although it is an essential plant nutrient, when absorbed in large amounts it can be responsible for several types of damage at the morphological, ultrastructural and biochemical levels (Dučić and Polle, 2005). Copper when in excess induces oxidative stress (Yruela, 2005), leads to the formation of phytochelatin (Inouhe, 2005), binds to some ligands (Cu chaperones, metallothioneins, phytochelatins), and is sequestered in vacuoles (Clemens, 2001). Furthermore, free Cu ions are capable of irreversible binding to SH groups involved in the catalytic action or structural integrity of proteins (Van Assche and Clijsters, 1990).

Copper can cause disorders in plant growth and development by adversely affecting important physiological process in plants. Plants grown under high levels of Cu normally show reduced biomass and chlorotic symptoms (Yruela, 2005). Excess of Cu affects the aerial part as well as root growth, inhibiting cellular elongation due to the increase in plasmalemma permeability and cell wall lignification (Arduini et al., 1995). Inhibition of root growth is recognized as one of the most conspicuous symptoms of Cu toxicity (Kukkola et al., 2000), in which lateral development and elongation are more sensitive than root initiation (Kahle, 1993).

At the cellular level, Cu plays an essential role in signaling of transcription and protein trafficking machinery, oxidative phosphorylation and iron mobilization (Yruela, 2005). Copper excess also interferes in the activity of several enzymes (Lombardi and Sebastiani, 2005) and in some aspects associated with photosynthesis, pigment synthesis, fatty acid and protein metabolism, respiration, N fixation processes and membrane integrity, among others (Dučić and Polle, 2005). Some chloroplast proteins and the enzymes glutamine synthase (GS) and ferredoxin-dependent glutamate synthase (Fd-GOGAT), involved in the assimilation of NH_4^+, are very susceptible to heavy metal toxicity, especially Fd-GOGAT to Cu excess (Demirevska-Kepova et al., 2004). Probably its most important effect is associated with the impairment of the photosynthetic electron transport system, promoting the production of radicals that initiate the reactions of the peroxidative chain, involving membrane lipids (Dučić and Polle, 2005). In addition, Cu excess can cause alterations in the source: sink relationship, diminishing the requirements for photosynthetic products and for products that regulate Calvin cycle enzymes (Maksymiec, 1997).

The general symptoms of senescence induced by Cu include leaf fall, intervenal chlorosis and alteration of leaf pigmentation (Wisniewski and Dickinson, 2003). It was proposed that Cu interferes with the biosynthesis of the photosynthetic machinery modifying the pigment and protein composition of photosynthetic membranes (Lidon and Henriques, 1991). One of the causes of chlorosis can be attributed to low Fe availability, since Cu reduces Fe translocation to the aerial part, even at slightly higher Cu contents than normally found in plant tissue (Wallace and Cha, 1989), and can promote the generation of several harmful free radicals, such as reactive oxygen species and peroxidative compounds (Shainberg et al., 2001). Leaf chlorosis and the subsequent photosynthetic decrease are also factors that contribute to the retardation of normal plant growth.

Changes in chlorophyll and carotenoid concentrations in Cu-treated plants are frequently reported (Yruela, 2005). A lower content of chlorophyll and alterations of chloroplast structure and thylakoid membrane composition were found in leaves of plants grown under high levels of Cu (Quartacci et al., 2000). Moreover, Cu can also substitute Mg in the chlorophyll molecule present in the antenna complex and reaction centers, damaging their structure and function (Liu et al., 2004). On the other hand, the relative increase in the chlorophyll *a:b* ratio suggests that Cu differentially affects the light collector complex of PSII, where chlorophyll *b* is located, rather than chlorophyll *a* of the reaction center (Wisniewski and Dickinson, 2003). A small increase in the β-carotene content, induced by Cu, can be due to its resistance to degradation, since it is a relatively stable pigment with a protective role (Wisniewski and Dickinson, 2003).

Chromium: This metal has not been recognized as an essential element for plant growth, however, some stimulant effects have been reported (Samantaray et al., 1998), with no specific mechanism for its absorption (Shanker et al., 2005). In some cases, plant growth is stimulated at low Cr concentrations; however, at high concentrations it shows a definitive retarding effect (Samantaray et al., 1998). Chromium toxicity affects the length of the primary roots and promotes changes in the architecture of the entire root system (Samantaray et al., 1996). The inhibitory effect of Cr on root growth (Barbosa et al., 2007) and its toxic effects on cell division result from the fixation of Cr^{3+} by plant tissue and disturbances of the osmotic relations that promote restrictions to Ca^{2+} transport through the plasma membrane to the cytoplasm (Liu et al., 1992).

It is difficult to separate the effects of Cr^{3+} and Cr^{6+} in the plant since both can be interconverted (Shanker et al., 2005) and immobilized in the soil (Cervantes et al., 2001). Both chromate and dichromate are considered to be negatively charged and there is a limited chance of them being adsorbed by organic materials, as stressed by Panda and Choudhury (2005). According to these authors, Cr^{6+}, in contrast to Cr^{3+}, is absorbed by the plant due to its natural soil mobility. The Cr^{6+} form is a biologically toxic oxidation state and to date there is no evidence indicating any potential biological role in plants (Von Burg and Liu, 1993). The related opinions to the form in which this element is extracted and translocated in the plant are contradictory. Generally, this phenomenon is attributed to the different culture techniques, the bioavailability of Cr^{3+} and CrO_4^{2-} as a function of pH and to the concentration of other ions in the root substrata (McGrath, 1982).

The solubility of Cr^{3+} can be increased or diminished in the presence of other elements in the soil-plant system. This fact can cause interactions between Cr^{3+} and other essential elements that can have a significant effect on the concentration of nutrients and their plant distribution, as well as modifications to some physiological and morphological plant processes (Panda and Choudhury, 2005). Chromium in the soil solution is absorbed by roots through transporters used for absorption of metals essential to plant metabolism. Its toxic effects depend primarily on its speciation, which in turn determines its absorption, translocation and accumulation (Shanker et al., 2005). The mechanism of Cr^{6+} transport is active, involving transporters of essential anions like sulphate (Cervantes et al., 2001). Elements like Fe, S and P compete with Cr when they bind to the transporter (Samantaray et al., 1998).

Chromium stress can induce metabolic modifications in plants, such as alterations in photosynthesis (Barbosa et al., 2007), degradation of photosynthetic pigments and induction of oxidative stress (Panda and Choudhury, 2005). Furthermore, Cr promotes reduction of leaf area and biochemical changes responsible for the inhibition of chlorophyll synthesis (Vajpayee et al., 1999) and disorganization of the chloroplast ultrastructure (Panda and Choudhury, 2005). Chromium stress also causes leaf chlorosis and necrosis (Barbosa et al., 2007), oxidative damages in biomolecules such as lipids and proteins (Vajpayee et al., 2002), disturbances in mineral nutrition (Barbosa et al., 2007), increase in glutathione and ascorbic acid production (Shanker, 2003), alterations in the metabolic pool that intermediates the production of phytochelatins and histidine, interference in the activity of nitrate reductase (Panda and Patra, 2000), root Fe^{3+} reductase (Shanker et al., 2004), plasma membrane H^+-ATPase (Dietz et al., 2001), Na^{2+}/K^+-dependent ATPase (Pauls et al., 1980), Ca^{2+}-dependent ATPase (Serpersu et al., 1982), alkaline phosphatases (Viola et al., 1980), superoxide dismutase, catalase (Shanker et al., 2003) and peroxidase (Samantaray et al., 2001) and, eventually, plant growth reduction, hindering its development and, finally, being able to cause its death (Barbosa et al., 2007).

The effect of Cr ions in photosynthesis and in the transference of excitation energy can also be due to abnormalities in the ultrastructure of chloroplasts linked to the development of the lamellar system, with an ample thylakoidal space and few grana (Van Assche and Clijsters, 1983). The disorganization of the chloroplast ultrastructure, the inhibition of the electron transport process and the electron deviation from the electron donor site of PSI to Cr^{6+} are possible explanations for the decreased photosynthetic rates induced by Cr (Shanker et al., 2005).

Due to its structural similarity with some essential elements, Cr can affect the mineral nutrition of plants in a complex way (Shanker et al., 2005). Once accumulated and distributed within the plant, it can interact with other essential elements and significantly affect the concentration and distribution of nutrients in the plant, as well as modify its morphology and some physiological processes (Barbosa et al., 2007). Formation of complexes of Cr with organic acids can play an important role in the inhibitory and stimulatory effects of Cr in the translocation of different mineral nutrients (Panda and Choudhury, 2005). An excess of Cr interferes in the absorption of Na, Fe, Mn, Cu, N, P, K and Mg (Barbosa et al., 2007).

One of the reasons for the decrease in the absorption of some nutrients in Cr-stressed plants is the inhibition of the plasma membrane H^+ ATPase activity (Shanker, 2003). Chromium strongly inhibits the incorporation of P, K, Ca, Mg, Fe, Mn, Zn and Cu in different cellular constituent in *Coconuts nucifera* (Biddappa and Bopaiah, 1989). The inhibitory effects of Cr on plant growth are the result of specific interactions between Cr and P, Cr and Fe or Cr and Cu (Barbosa et al., 2007). Such interactions could be associated with the chemical properties of these metals, for example the charge (Cr^{3+} and Fe^{3+}) and the effective ionic radius (Cr and Cu).

Leaf chlorosis promoted by Cr^{3+} could be caused either by the inhibition of Fe absorption or the reduction of N transport (Barbosa et al., 2007). High Cr concentration can disturb the chloroplast ultrastructure thereby disturbing the photosynthetic process (Panda and Choudhury, 2005). The decrease in the chlorophyll *a:b* ratio (Shanker, 2003) induced by Cr indicates that the toxicity of Cr probably reduces the size of the peripheral parts of the antenna complex. The decrease in chlorophyll *a* can be due to destabilization and degradation of proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway can also contribute to the general reduction in chlorophyll content in the majority of Cr-stressed plants (Shanker et al., 2005).

MECHANISMS OF RESISTANCE OR TOLERANCE TO METALS IN PLANTS

Physiological and genetic factors determine which species can or cannot evolve tolerance (Baker and Proctor, 1990). Genetic evidence exists for multiple independent evolutionary origins of tolerant populations to heavy metals (Vekemans and Lefèbvre, 1997). The populations only develop tolerance for different metals when these are present at high concentrations in the soil of origin. This suggests that the genes for different types of tolerance are different and that selection acts to increase the frequency of genes that give rise to tolerance to a particular metal, present in a determined location (Macnair, 1993). There are also reports of co-tolerance, where tolerance to a metal somehow confers tolerance to other metals that are not present in toxic concentrations in the soils in which the plants are growing (Schat and Vooijs, 1997).

It has been observed that tolerant species possess defense mechanisms linked to cellular antioxidants and antioxidant enzymes that protect several vital physiological processes against damage resulting from reactive forms of oxygen produced by metallic stresses (Panda and Choudhury, 2005). There are reports of the hyperactivity of oxidant enzymes and the accumulation of cellular antioxidants in several plants species under Cu and Pb stress (Ali et al., 2003). Several species resistant to Cu have been found in contaminated and uncontaminated areas (Liu et al., 2004). According to De Vos et al. (1992), tolerance to Cu is related to the function of glutathione as an antioxidant substance against free radicals and hydrogen peroxide formed by Cu excess.

Tissue culture studies have demonstrated that multiple resistance to metals appeared in mature trees exposed to heavy metals in different contaminated areas (Watmough and Dickinson, 1996). Characteristics of resistance to metals can be induced in suspension cell cultures through successive exposures and gradual increases of the metal concentration in the growth media (Dickinson et al., 1992). Rooted cuttings of *Salix* sp. can be acclimated to metallic stresses in hydroponic conditions (Punshon and Dickinson, 1997). These studies have contributed to explain how the plants survive and grow in potentially toxic environments (Dickinson et al., 1992; Turner and Dickinson, 1993b).

Plant tolerance and/or resistance to metallic stress can be associated with one or more mechanisms, such as: (i) the excretion of chelating compounds that reduce the availability of the metal in the soil or water; (ii) the exclusion of the metal through selective absorption of elements; (iii) the retention of the metal in roots, preventing its translocation to the aerial part; (iv) the chelation or sequestration of heavy metals by ligands, compartmentalization, biotransformation and mechanisms of cellular repair; (v) the development of enzymes tolerant to the metal (Hall, 2002; Cobbett and Goldsbrough, 2002; Patra et al., 2004); (vi) the increase of production of intracellular compounds linked to the metal (Sharma and Dietz, 2006); (vii) the immobilization of the metal in the cellular wall (Cosio et al., 2005); (viii) homeostatic cellular mechanisms to regulate the concentration of metal ions inside the cell (Benavides et al., 2005); (ix) induction of heat-shock proteins (Heckathorn et al., 2004); (x) release of phenols from roots (Jung et al., 2003); (xi) the increase of tolerance to mineral deficiency or the decrease of nutritional requirements; (xii) the increase in absorption of certain macronutrients; and (xiii) the development of the capacity to absorb and to use minerals in the presence of heavy metals (Meda et al., 2007). As a result of these tolerance and/or resistance mechanisms (alone or in combination), some plants can grow in environments contaminated with metals where other species do not survive (Hall, 2002).

In the case of biotransformation, metal toxicity in plants can be decreased by chemical reduction of the element and/or by its incorporation into organic compounds (Salt et al., 1998). Chromium, for example, can be reduced chemically in the root system of certain plant species from Cr^{6+} to Cr^{3+}, as part of a detoxification mechanism (Shanker et al., 2005). Intraspecific and intravarietal differences exist with regard to tolerance to Cr excess that can be controlled by different genes through diverse biochemical pathways (Samantaray et al., 1998). With regard to the cellular repair mechanism, the primary component of cellular resistance to high Cu concentration seems to be intensified by the resistance of the plasma membrane during the repair of membrane damages induced by Cu, probably involving metallothioneins (Strange and Macnair, 1991).

Inside plant cells, the metals in excess, together with those not used in metabolism, need to be stored to prevent toxicity (Briat and Lebrun, 1999). Several potential storage mechanisms, at the cellular level, can be involved in the detoxification and tolerance to metal stress (Cobbett and Goldsbrough, 2002). Moreover, some plant species are capable of accumulating great amounts of metals in the aerial part, while others accumulate them in the roots (Barbosa et al., 2007). It has been verified that Cr, for example, accumulates mainly in the roots and little is carried to the aerial part (Shanker et al., 2005). Possibly, this is due to its immobilization in the root cell vacuoles, becoming less toxic (Arduini et al., 1996). This could be a natural plant response to its toxicity (Shanker et al., 2004).

Chelation of metallic ions by specific ligands of high affinity diminishes the concentration of free ions in solution. The main ligands associated with metals found in plant tissue include amino acids, oligo- and polypeptides (glutathione, phytochelatins, metallothioneins) (Patra et al., 2004), macrocyclic agents (porphyrins, cobalamines, chlorophylls), polysaccharides and glycosides (rhamnogalacturonans), nucleobases, oligo-polynucleosides and nucleotides (DNA fragments) (Lobinski and Potin-Gautier, 1998). Several of these bioligands associated with metals have been localized in the plant vascular system (Briat and Lebrun, 1999). When these systems are overloaded, defense mechanisms to oxidative stress are activated (Patra et al., 2004).

Metallothioneins and phytochelatins are two representative classes of chelant peptides of heavy metals existing in plants. Genes directly codify metallothioneins, which have low molecular weights, are rich in cysteine polypeptides and are induced by Cu (Cobbett and Goldsbrough, 2002). Phytochelatins possess low molecular weights, are enzymatically synthesized, have peptides rich in cysteine and bind to various metals including Cd, Cu (Inouhe, 2005) and Pb (Kahle, 1993) via the sulphydryl and carboxyl residues, but their biosynthesis is controlled preferentially by Cd (Inouhe, 2005). Moreover, they are associated with the intra- and extra-cellular precipitation of Pb as carbonates, sulphates and phosphates, playing an important role in the detoxification of this metal in plant tissues (Salt et al., 1998). Metallothioneins and phytochelatins have been indicated as possible Cu chelants in the cytosol (Van Hoof et al., 2001). The manipulation of phytochelatin gene expression is one of the potential mechanisms to increase the plant's capacity for phytoremediation (Cobbett and Goldsbrough, 2002).

One of the main tolerance mechanisms of the plant to Cd seems to be the intracellular complexation of this element in innocuous forms. According to Steffens (1990), phytochelatins, whose synthesis is induced by the heavy metal itself, can sequester and detoxify excess Cd ions. The accumulation of phytochelatins in plant cells exposed to Cd has been reported in diverse species (Grill et al., 1985). The compartmentalization of Cd and phytochelatins occurs at the vacuole level (Cobbett and Goldsbrough, 2002). The increase in content of thiolic and/or sulphydrylic groups in phytochelatins, responsible for the complexation of heavy metals in these peptides, is proportional to the increment of Cd absorption by plant roots (Grill et al., 1985).

The heavy metals Pb and Cd induce chromosomal aberrations and disturbed mitotic divisions in the *Pinus sylvestris* population (Prus-Glowacki et al., 2006). However, plants show considerable constitutional tolerance to Pb and, in some cases, reach levels of induced tolerance (Sharma and Dubey, 2005). High constitutional tolerance for Pb is associated with high levels of Ca in the tissue during the administration of Pb and with high tolerance to Ca deficiency (Patra et al., 2004). Besides, oxalate compounds secreted by roots can reduce Pb bioavailability (Sharma and Dubey, 2005).

PHYTOREMEDIATION

Phytoremediation can be applied to organic and inorganic pollutants present in solid, liquid and air substrata, mainly in soils contaminated with heavy metals, oil hydrocarbons, pesticides, explosives, chlorinated solvents and industrial toxic by-products (Prasad and Freitas, 2003; Pilon-Smits, 2005). Phytoremediation can be achieved through several processes, for example: (i) phytoextraction, which consists in the use of plants that accumulate pollutants, like metals or organic compounds that concentrate in the plant part that is harvested; (ii) phytodegradation that is associated with microorganisms degrading organic pollutants; (iii) rhizofiltration, whereby plants that use their roots to absorb and concentrate pollutants are used; (iv) phytostabilization, mainly of metals in waters and sewers, to diminish the bioavailability of pollutants to the environment, and phytovolatilization to volatilize pollutants (Pulford and Watson, 2003; Weis and Weis, 2004; Pilon-Smits, 2005).

Two basic strategies of phytoextraction have been developed: (i) phytoextraction assisted by synthetic chelants (ethylenodiaminotetra-acetic acid – EDTA, diethylenotriaminopenta-acetic acid – DTPA and nitrilotri-acetic acid – NTA), called induced phytoextraction (Huang et al., 1997; Salt et al., 1998; Wu et al., 1999); and (ii) continuous, long run phytoextraction (Salt et al., 1998).

Induced phytoextraction consists of two basic processes that involve metal release to the soil solution combined with metal transport, via xylem, to the aerial part of the plant to be harvested (Salt et al., 1998). This type of phytoextraction is more advanced and currently commercially implemented (Nascimento and Xing, 2006). A good example of this sort of phytoremediation is that reported for Pb in soil in which EDTA was applied (Salt et al., 1998). However, the main limitation for the use of synthetic chelants in the field, especially EDTA, resides in its low biodegradation. This results in the soil maintaining high contents of soluble metals for long periods, which increases lixiviation risks (Meers et al., 2004).

Phytoextraction is adopted for long-term remediation. The time required for extraction depends on the contamination levels, but it is usually between one and 20 years (Kumar et al., 1995). It is estimated that plants can remove from 180 to 530 kg Pb ha^{-1} $year^{-1}$ (Huang and Cunningham, 1996). After harvest, the volume of the contaminated plant material can be subsequently reduced by incineration, composting or stored as dangerous material or, if economically viable, used for the metal recovery (Gardea-Torresdey et al., 2005; Yang et al., 2005). In the case of woody species, the wood can be industrialized (Pilon-Smits, 2005).

In continuous phytoextraction, metal absorption is carried out by hyperaccumulator plants that grow in soils rich in heavy metals (Salt et al., 1998). These plants are naturally capable of accumulating metals to more than 1% of their shoot dry biomass (Huang et al., 1997). This process is based on the genetic and physiological capacities of some plants to accumulate, translocate and resist high metal concentrations. However, there are the disadvantages of low biomass production and slow growth, as well as lack of hyperaccumulator plants for the more important metallic pollutants in the environment, like Pb and Cd (Jarvis and Leung, 2002). Even so, some plant species are actually used for phytoextraction of Cd, Cu, Pb and Cr (Baker et al., 1991).

High production of biomass, a deep root system, high growth rate, capacity to grow in soils poor in nutrients and to concentrate metals, associated with the characteristics of resistance to metals, are important factors for the use of plant species in the method of soil decontamination (Pilon-Smits, 2005; Yang et al., 2005). Currently, about 400 hyperaccumulator species of metals pertaining to the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae and Euphorbiaceae families have been identified. However, most of these species shows low biomass production (Prasad and Freitas, 2003). Surprisingly, knowledge is scarce regarding the response of woody plants to toxic metal levels (Kukkola et al., 2000).

In recent years, the interest in the potential use of trees for soil cover and for phytoremediation of soil contaminated by heavy metals has increased due to the high biomass production and wide genetic variability of some species (Dickinson and Pulford, 2005; Unterbrunner et al., 2007). There is much evidence for the natural establishment of trees in contaminated soil, and that some types of trees can survive under severe adverse conditions (Turner and Dickinson, 1993a,b). Several species of *Salix* are explored in programs for the removal of soil Cd (Dickinson and Pulford, 2005; Unterbrunner et al., 2007). In these species, the Cd concentration in the aerial part tends to increase as soil Cd concentration increases (Vandecasteele et al., 2002; Unterbrunner et al., 2007).

Interest has also extended to fast growing woody species that could be used in high density cropping systems for extraction of metals from the soil through absorption and harvesting of the aerial part, using successive pruning (Pulford and Watson, 2003). Accelerated growth and regular pruning are associated with fast translocation of nutrients and, consequently, of soil heavy metals (EPA, 1999). Clones of *Populus*, resulting from crosses of *P deltoides* x *P. maximowiczii* (Erídano) and *P. deltoides* x *P. euramericana* (I-214), if cultivated at a population density of 10,000 plants ha^{-1} (short rotation forest), in soils with high heavy metal concentrations, could produce about 119 tons of stem dry biomass ha^{-1} in a cycle of 11 years (Bonari, 2001). This would correspond to 902 and 962 g ha^{-1} of Cu and 2,700 and 2,058 g ha^{-1} of Cr in the stems of I-214 and Erídano clones, respectively, according to preliminary results of absorbed heavy metals by these species obtained under greenhouse conditions (Sebastiani et al., 2004).

A drawback for the rapid selection of genotypes tolerant to heavy metals is the long growth period of the trees. Broad tree genomes and facultative tolerance, such as root redistribution in less contaminated soil zones, enable the survival of determined woody species in soils polluted by heavy metals, even with reduced growth indices (Dickinson et al., 1992). True tolerance requires the development of one or more genetically based physiological mechanisms (Dickinson et al., 1991). However, this very genetic stability of tolerance is questionable, since it can either be induced or inhibited in woody species. Therefore, the capacity of acclimation to fluctuating stress, due to pollution, becomes more important for species survival (Dickinson et al., 1991). Furthermore, other factors, such as soil fertility, can increase the resistance to the metal (Pulford et al., 2002).

The physical phytostabilization of soils contaminated by heavy metals is one of the main benefits of the use of trees in phytoremediation processes (Dickinson and Lepp, 1997). Therefore, besides the direct stabilization of the soil by roots, the vegetation cover decreases the risk of soil loss due to erosion. On the other hand, tree senescence also produces an increase in the metal levels by loss of water (Pulford and Watson, 2003). Although there are seasonal variations in metal concentration in woody plants, mainly at the foliar level (Ehlin, 1982), leaf fall adds significant amounts of organic matter to the soil surface layers, promoting nutritional cycling, soil aggregation and water retention capacity.

Avicennia marina accumulates metals in the roots in proportion to their concentration in the sediments. The accumulation in leaves varies depending on the metal (Weis and Weis, 2004). After exposure to Cu, the leaves excrete significant amounts of this metal in conjunction with the saline crystals excreted at the adaxial surface of the leaf (MacFarlane and Burchett, 2000). Similarly, *Spartina alterniflora* actively excretes metals in saline crystals released through hydathodes. The theoretical potential of Cd, Pb, Cr, and Cu exudation is of the order of 145, 260, 104 and 260 g ha^{-1} $year^{-1}$, respectively, through saline excretions (Kraus, 1988). Furthermore, leaves of *S. alterniflora* liberate from two to four times more Pb, Cu and Cr than leaves of *Phragmites australis* at the peak of the growth season (Burke et al., 2000). The difference in metal release between these two species may be due to the presence of saline glands in *S. alterniflora*, since these are absent in *P. australis* (Weis and Weis, 2004).

CONCLUDING REMARKS

Plant species show different allocation patterns for Cd, Pb, Cr and Cu, whose translocation from roots to the aerial part and their release from foliar tissue can be an important step for metal flow in ecosystems. Contamination by these metals affects growth, distribution and the biological cycle of plant species, promoted by several different toxicity mechanisms, such as alterations in (i) carbohydrate and N metabolism; (ii) the activity of certain metalloenzymes; (iii) protein synthesis; (iv) photosynthetic activity; (v) the production of oxygen reactive species by auto-oxidation; (vi) the obstruction of functional groups and the displacement of metallic ions essential to biomolecules. Furthermore, it promotes changes in (i) the permeability of cellular membranes; (ii) the reactions of sulphydrylic groups with cations; (iii) the affinity for reactions with phosphate groups and active groups of ADP or ATP; (iv) the substitution of essential ions; (v) the induction of chromosomal anomalies; and (vi) the rate of cellular division.

Plant tolerance to these metallic elements can be associated with one or more mechanisms, such as (i) excretion of chelating compounds that reduce the availability of the metal in the soil or water; (ii) metal exclusion through selective absorption of elements; (iii) retention of the metal in roots preventing its translocation to the aerial part; (iv) immobilization of the metal in the cellular wall; (v) chelation or sequestration of heavy metals by ligands; (vi) compartmentalization; (vii) biotransformation and cellular repair mechanisms; (viii) production increase of intracellular compounds that bind the metal; (ix) development of tolerant enzymes to the metal; (x) increase of tolerance to mineral deficiency; (xi) decrease of nutritional requirements; (xii) increase in absorption of certain macronutrients; and (xiii) capacity to absorb and use minerals in the presence of heavy metal.

Phytoremediator woody species, with (i) high biomass production, (ii) a deep root system, (iii) high growth rate, (iv) high capacity to grow in soils with low nutrient availability and (v) high capacity to allocate metals in the trunk, can be an alternative for the recovery of degraded soils due to excess metallic elements. Phytoremedation using woody species is ecological and economically viable due to the low cost of implantation, promoting soil stabilization that limits the propagation of metallic contaminants. This technology is emergent and in development. Regarding the phytoremediation strategy, a better understanding is necessary of the absorption, transport and tolerance of metals in woody plants, since they are of great importance for the planning of large-scale application of this technique under field conditions.

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