Biochemistry of Metals/Metalloids Toward Remediation Process

Magdalena Victoria Monferrán and Daniel Alberto Wunderlin

Abstract The use of phytoremediation for restoration or amelioration of soils polluted with heavy metals is a promising technique for the near future. Generally, if a plant can accumulate more than 1000 mg kg^{-1} (or 1000 ppm) of Cu, Co, Cr, Ni, or Pb, or more than 10,000 mg kg^{-1} (or 10,000 ppm) of Mn or Zn, it is defined as a hyperaccumulator. The discovery and elucidation of the mechanism used by hyperaccumulators to take up, translocate, and fix heavy metals in aerial parts is of great interest to understand the role of such plants in biorestoration. The performance of plants for heavy metals phytoremediation depends on several factors, starting from the bioavailability of metals in the soil, which is conditioned by cation exchange capacity, soil pH and organic matter content, the oxidation state of the metal, presence of both natural and synthetic chelants, interaction with soil microorganisms, etc. The mechanism used by roots to uptake bioavailable metals from the soil is also crucial for the success of bioaccumulation, in addition to mechanisms used to translocate metals from root to shoots after absorption. Significant progress in understanding the mechanisms governing metal hyperaccumulation has been made in the last decade through comparative physiological, genomic, and proteomic studies of hyperaccumulators and related non-hyperaccumulator plants. Parts of these studies are discussed here. Finally, the efficiency in detoxification and sequestration is a key property of hyperaccumulators, allowing them to concentrate huge amounts of heavy metals in aerial organs without apparent phytotoxic effect. This exceptionally high heavy metal accumulation becomes even more surprising considering that it mainly occurs in leaves where photosynthesis, essential for plant survival, is accomplished, and that the photosynthetic apparatus is a major target for most of these contaminants. Although extensive laboratory studies on phytoextraction by plants have been reported, less research has been dedicated to evaluate the performance in field

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studies, which seems necessary to both validate laboratory studies and fully demonstrate the usefulness of this technique for site restoration.

Keywords Heavy metals · Phytoextraction · Soil restoration · Hyperaccumulators · Translocation · Fixation · Detoxification

Contents

1 Introduction

Soil is one of the key elements for all terrestrial ecosystems, providing nutrients for plants as well as degradation and transfer of biomass. Heavy metal contamination of soils has become a serious problem in both industrial and intensive agricultural areas, affecting crop yield and quality, soil biomass and fertility, and leads to the bioaccumulation of metals in plants, which can pose serious risks for humans through the ingestion of heavy metals bioaccumulated through the food chain (Gupta and Gupta 1998; Gratao et al. 2005; Rajkumar et al. 2009; Peralta-Videa et al. 2009). This is mainly due to the pollution of agricultural soils by increasing dependence on chemical fertilizers, which has imposed a long-term risk on environmental health (McLaughlin et al. 1999; Wong et al. 2002). Industrialized countries have regulated the emission of toxic substances. Conversely, many developing countries present rapid industrial development, which is sometimes associated with population increase coupled with lack of pollution control, causing huge increase in heavy metal contamination of agricultural soils (Ji et al. 2000).

Numerous efficient soil cleanup techniques are available, but most of them are costly, labor-intensive, and cause soil disturbances, having limited acceptability among the communities. Conventional remediation methods involve pneumatic fracturing, solidification/stabilization, vitrification, excavation, and removal of contaminated soil layer, physical stabilization or washing of contaminated soils with strong acids or chelating agents, in addition to alternative methods like chemical stabilization, i.e., metal immobilization by using different amendments (Steele and Pichtel 1998; Khan et al. 2004; Bhargava et al. 2012; Addy et al. 2012). Common methods for the immobilization of metals in soils are to apply lime, phosphates, organic matter residues, and other natural or synthetic additives, like zeolites, beringite, and hydrous oxides of Al, Fe, and Mn and also vermiculite (Vangronsveld et al. 1990; Gworek 1992; Khattak and Page 1992; Bolan and Duraisamy 2003; Malandrino et al. 2011).

1.1 Plants as Accumulators of Metals

Plants that take up heavy metals from the soil offer an alternative and less expensive method to strip heavy metals directly from the soil. The use of such plants to remediate soils and water contaminated with pollutants, a technique known as phytoremediation, is emerging as a new tool for in situ remediation (Yang et al. 2005). Phytoremediation takes advantage of the fact that a living plant acts as a solar-driven pump, which can extract and concentrate certain heavy metals from the environment (Raskin et al. 1997). This remediation method maintains the biological properties and physical structure of the soil. The technique is environmentally friendly, potentially cheap, visually unobtrusive, and offers the possibility of biorecovery of the heavy metals (Yang et al. 2005). That is because phytoremediation has gained the increasing attention of scientists, remediation engineers, and environmental professionals in governments and industries.

Phytoremediation technologies include phytoextraction, phytostabilization, phytovolatilization, phytofiltration, and phytodegradation (Fulekar et al. 2009; Marques et al. 2009). Phytoextraction refers to the uptake of contaminants from soil or water by plant roots and their translocation into the shoot, or any other harvestable plant part, to remove contaminants and promote long-term cleanup of soil or wastewater (Sas-Nowosielska et al. 2008). In this approach, plants capable of accumulating heavy metals are grown on contaminated sites and the metal-rich aboveground biomass is harvested on maturity. As a result, a fraction of the soil contaminant is removed. The success of phytoextraction depends on factors like metal availability for uptake as well as plant ability to absorb and accumulate metals in its aerial parts (Fig. 1). As per the economic feasibility, the harvested biomass is usually incinerated or composted and rarely recycled for reuse (Prasad and Freitas 2003).

Plants ideal for phytoextraction should possess multiple traits like ability to grow outside their area of collection, fast growth, high biomass, easy harvesting, and accumulation of a range of heavy metals in their harvestable parts (Jabeen et al. 2009; Seth 2012).

Plants take up essential and non-essential elements from soils in response to concentration gradients induced by selective uptake of ions by roots, or by diffusion of elements in the soil. The level of accumulation of elements differs

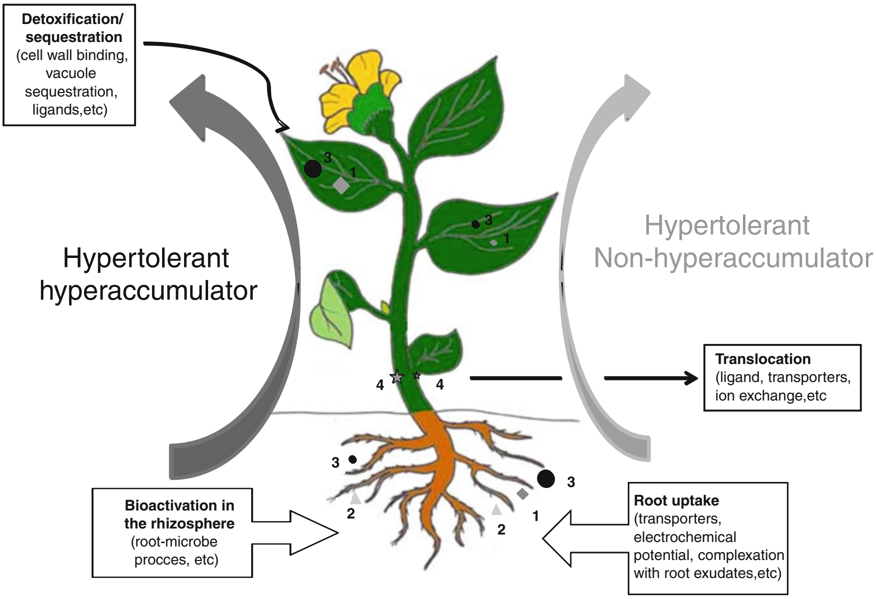

Fig. 1 Major processes proposed to be involved in metal hypertolerance and heavy metal distribution in an excluder non-hyperaccumulator (*right*) and a hyperaccumulator (*left*). (1) Heavy metal binding to the cell walls and/or cell exudates, (2) root uptake, (3) chelation in the cytosol and/or sequestration in vacuoles, (4) root-to-shoot translocation. Different symbols indicate the plant organ in which the different mechanisms occur and the spot sizes of their levels. (*CEC* cation exchange capacity)

between and within species (Huang and Cunningham 1996; McGrath and Zhao 2003). Baker (1981) suggested that plants could be classified into three categories: (1) excluders: those that grow in metal-contaminated soil and maintain the shoot concentration at low level up to a critical soil value above which relatively unrestricted root-to-shoot transport results, (2) Hyperaccumulators: those that concentrate metals in the aerial part, and (3) indicators: where uptake and transport of metals to the shoot are regulated so that internal concentration reflects external levels, at least until toxicity occurs (Fig. 1).

1.2 Hyperaccumulator Plants

The term "hyperaccumulator" was coined by Brooks et al. (1977) for plants that, in a different way from the excluder plants, actively take up large amounts of one or more heavy metals from the soil. Moreover, heavy metals are not retained in the roots but translocated to the shoot and accumulated in aboveground organs, especially leaves, at concentrations 100- to 1000-fold higher than those found in non-hyperaccumulating species. They show no symptoms of phytotoxicity (Rascioa 2011; Reeves

2006). Although a distinct feature, hyperaccumulation also relies on hypertolerance, an essential key property allowing plants to avoid heavy metal poisoning, to which hyperaccumulator plants are as sensitive as non-hyperaccumulators (Chaney et al. 1997).

Usually if a plant can accumulate more than 1000 mg kg^{-1} (or 1000 ppm) of Cu, Co, Cr, Ni, or Pb, or more than 10,000 mg kg^{-1} (or 10,000 ppm) of Mn or Zn, it is defined as a hyperaccumulator (Wu et al. 2010). The hyperaccumulator species are distributed in a wide range of vaguely related families, occurring on metal-rich soils in both tropical and temperate zones. Hyperaccumulators are found from South Africa, New Caledonia, Latin America to North America, and Europe (Baker and Brooks 1989). Initially the term hyperaccumulator referred to plants able to accumulate more than 1 mg g^{-1} Ni (dry weight) in the shoot, an exceptionally high heavy metal concentration, considering that in vegetative organs of most plants Ni toxicity starts from 10 to 15 g g^{-1}. Threshold values were successively provided to define the hyperaccumulation of other heavy metals, based on their specific phytotoxicity. According to such criterion, hyperaccumulators are plants that concentrate >10 mg g^{-1} (1 %) Mn or Zn, >1 mg g^{-1} (0.1 %) As, Co, Cr, Cu, Ni, Pb, Sb, Se, or Tl, and >0.1 mg g^{-1} (0.01 %) Cd in aerial organs of such plants, without evidences of phytotoxic damage (Verbruggen et al. 2009). Ni is hyperaccumulated by a great number of taxa (more than 75 %), reaching the highest concentration in plants. Conversely, a low number of hyperaccumulators has been found for Cd.

The time that plants use to diminish the amount of heavy metals in contaminated soils depends on the biomass production and on their bioconcentration factor (BCF), which is the ratio of metal concentration in the shoot tissue to the soil (McGrath and Zhao 2003). BCF is determined by the capacity of the roots to take up metals and their ability to accumulate, store, and detoxify metals while maintaining metabolism, growth, and biomass production (Gleba et al. 1999; Guerinot and Salt 2001; Clemens et al. 2002). With the exception of hyperaccumulators, most plants have metal bioconcentration factors lower than 1, which means that it takes longer than a human lifespan to reduce soil contamination by 50 % (Peuke and Rennenberg 2005). Hyperaccumulators have a bioconcentration factor greater than 1, sometimes reaching values as high as 50–100.

1.3 High Biomass Crops

For successful and economically feasible phytoextraction, it is necessary to use plants with BCF of 20 (or more) and a biomass production of 10 t ha^{-1}; or plants with a BCF of 10 and a biomass production of 20 t ha^{-1} (Peuke and Rennenberg 2005). The rate of phytoextraction is directly proportional to plant growth rate and the total amount of metal phytoextracted is correlated to the plant biomass, which makes the process of phytoextraction very slow (Shah and Nongkynrih 2007). This triggers the need for identification of fast growing and strongly metal-accumulating genotypes, namely biotechnological modifications, whose description is out of the scope of this chapter.

Nowadays, the use of high-biomass crops for phytoextraction of metals is attracting huge attention (Doty 2008; Capuana 2011). Fast growing trees are ideal low-cost candidates for phytoextraction due to their extensive root systems, high rates of water uptake and transpiration, rapid growth, large biomass production, and easy harvesting with subsequent growth of new plant generations without disturbance of the site (Peuke and Rennenberg 2005). Several tree species are currently under study for use in the phytoremediation of metal contaminated soils (Pulford and Watson 2003; Rosselli et al. 2003; Meers et al. 2007; Unterbrunner et al. 2007; Brunner et al. 2008; Domínguez et al. 2008). Plants ideal for phytoextraction should possess multiple traits like the ability to grow outside their area of collection, fast growth, high biomass, easy harvesting, and accumulation of a range of heavy metals in their harvestable parts (Jabeen et al. 2009; Seth 2012).

The relationship between metal hyperaccumulation and tolerance is still a subject of debate. Scientific reports range from no correlation between hyperaccumulators and the degree of tolerance to metals (Baker et al. 1994) to strong association between them (Chaney et al. 1997). There is a growing consensus that plants must also tolerate the metals that they accumulate; thus, being able to cope with high concentrations of metals in their tissues (Fig. 1).

There is also a general agreement that metal hyperaccumulation is an evolutionary adaptation by specialized plants to live in habitats that are naturally rich in specific minerals, giving them the ability to tolerate high levels of metals, in addition to protection against herbivores or pathogens, drought tolerance, and allelopathy (Boyd and Martens 1992; Macnair 1993). The hypothesis of protection against pathogens and herbivores is considered the most accepted (Boyd and Martens 1992; Huitson and Macnair 2003; Boyd 2007; Noret et al. 2007; Galeas et al. 2008). However, the mechanisms of metal uptake, tolerance to high metal concentrations, and the exact roles that high level of elements play in the survival of hyperaccumulators remains controversial.

The degree of hyperaccumulation of one or more heavy metals can vary significantly from species to species, even in diverse populations and ecotypes of the same species (Deng et al. 2007). However, hyperaccumulation depends on three basic characteristics distinguishing hyperaccumulators from related non-hyperaccumulator taxa:

- A high bioconcentration factor (BCF);
- A faster and effective root-to-shoot translocation of metals;
- A much greater ability to detoxify and sequester huge amounts of heavy metals in the leaves.

Significant progress in understanding the mechanisms governing metal hyperaccumulation has been made in the last decade through comparative physiological, genomic, and proteomic studies of hyperaccumulators and related non-hyperaccumulator plants. Part of these studies will be discussed in the following sections.

2 Factors Affecting Metal Uptake by Plants

The uptake of heavy metals by plants depends on several factors some, of which are discussed below.

Several edaphic factors like sorptive capacity of the soil, heavy metal content, cation exchange capacity (CEC), soil pH, and organic matter content affect metal hyperaccumulation in plants (Alloway 1995; Tiller et al. 1995; Cheng 2003; Chaney et al. 2007).

Soil is a complex heterogeneous medium, which consists of solid phases containing minerals, organic matter, and fluid phases (soil water and soil air), which interact allowing the entering of ions into the soil system (Alloway 1995). The ability of soils to adsorb metals from aqueous solution has decisive consequences for both agricultural issues such as soil fertility but is also associated with environmental questions such as soil pollution, remediation, and waste deposition. Heavy metals are the most toxic inorganic pollutants occurring in soils and can be of natural or anthropogenic origin (Siegel 2002). Some of them are toxic, even if their concentration is very low. Moreover, heavy metals toxicity increases with their accumulation in water, soils, and interfaces (sediments, etc.). Adsorption is the main process responsible for accumulation of heavy metals into soils and sediments (Bradl 2004).

The most important interfaces involved in the adsorption of heavy metal adsorption to soils are inorganic colloids such as clays, metal oxides and hydroxides (Bradl 2004), metal carbonates and phosphates. Also, organic colloidal matter arising from detritus and living organisms such as algae and bacteria provide interfaces for heavy metal adsorption (Kerndorf and Schnitzer 1980; Lion et al. 1982; Fein et al. 1999; Fein and Delea 1999). The adsorption of heavy metals onto the soil matrix regulates their further release, forming solutions that are also influenced by inorganic and organic ligands, some of them natural, such as humic and fulvic acids (Schlautmann and Morgan 1994; Duker et al. 1995; Zachara et al. 1994), but also man-supplied such as NTA, EDTA, polyphosphates, and others (Bowers and Huang 1986; Zachara et al. 1995a, b; Szecsody et al. 1994), which can be found in contaminated soils and wastewater. The most important parameters controlling heavy metal adsorption and their distribution between soil and water are soil type, metal speciation, metal concentration, soil pH, solid/solution mass ratio, and contact time (Cavallaro and McBride 1980; Stahl and James 1991; Martinez and Motto 2000; Temminghoff et al. 1997; Kent et al. 2000). In general, greater metal retention and lower solubility occurs at high soil pH (Teminghoff et al. 1994, 1995; Semu et al. 1987; Barrow and Cox 1992; Yin et al. 1996). The above-mentioned processes affect not only the adsorption (Bradl 2004) but also the release of metals from contaminated soils and should be considered for remediation techniques.

Generally, only a fraction of soil metal is readily available (bioavailable) for plant uptake since the bulk of soil metals is commonly found as insoluble compounds unavailable for transport into roots (Lasat 2002). Cations of heavy metals are often bound to soil particles because of soil CEC. The CEC is a measure of the soil's capacity to exchange ions. The negative charges are supplied by clay and organic matter of the soil. The binding affinity of cations reduces cation movement in vascular plants. Thus, the higher the CEC of the soil, the greater the sorption and immobilization of the metals.

Metal solubility and availability are dependent on soil characteristics and are strongly influenced by soil pH, which is considered as the major factor influencing the availability of elements in the soil for plant uptake. Plants absorb mineral elements in ionic form in solution, the presence of these forms being strongly influenced by matrix pH (Dzantor and Beauchamp 2002). A lower soil pH increases the concentration of heavy metals in the solution by decreasing their adsorption. In soil, the solution concentrations of metal contaminants tend to increase with decreasing pH, mainly because of their displacement from exchangeable sites on solid surfaces by increasing the activity of hydrogen ions as there is a decrease in pH. This can increase the availability of the contaminant for plant uptake, but can also result in concentrations of elements at levels that are toxic to the plant. Many metal cations like Cd, Cu, Hg, Pb, and Zn are reported to be more soluble and available in the soil solution at low pH (below 5.5) (Blaylock and Huang 2000). The increased availability of metals at low pH has led phytoextraction researchers to study the incorporation of acidifiers (NH_4+ containing fertilizers, organic and inorganic acids, and elemental S) into metal contaminated soils to improve the success of phytoextraction. However, in spite of the promise of some acidifying agents, little research has been carried out on this subject, which needs further investigation.

The organic content of the soil also has a strong bearing on the extent of phytoextraction of heavy metals. The addition of peat and manure is reported to increase Cu, Zn, and Ni accumulation in wheat (Narwal and Singh 1998). Peat and manure are heterogeneous substances that can concurrently exert mobilizing and stabilizing effects (Schmidt 2003). Acid peat reduces soil pH, which increases concentration of soluble metals in the soil. It also increases the CEC of soils, provides sorption sites, reduces metal mobility, and promotes higher binding affinity (Schmidt 2003).

The oxidation state of a metal contaminant also determines its solubility and relative availability for uptake by plant systems. In general, the oxidized forms of most common metal contaminants are less soluble and consequently less available for plant uptake, chromium being the exception. Synthetic chelating agents have been used to increase the uptake and translocation of metals, affording a high removal rate (Lasat 2000). Synthetic chelating agents such as EDTA can effectively increase the solubility of metal contaminants in soils (Ebbs et al. 1998). However, the use of synthetic chelating agents often leads to poor biodegradability and decreased plant growth and biomass production (Grcman et al. 2001; Blaylock et al. 1997). There are also some concerns on the mobility of chelated metals by leaching from polluted soil (Grcman et al. 2001). Recently, the use of natural low molecular weight organic acids (NLMWOA) is emerging as an alternative to synthetic chelating agents for the remediation of heavy metals. It is well known that exudation of NLMWOA by roots plays a significant role in heavy metal solubility (Krishnamurti et al. 1998; Nigam et al. 2001) and increased root growth (Uren and Reisenamer 1998). Some studies have shown that the application of NLMWOA has positive effects on the phytoextraction of heavy metals from soil (Krishnamurti et al. 1998; Nigam et al. 2001). However, higher concentrations of NLMWOA lead to decreased biomass, while lower concentrations of NLMWOA result in poor phytoextraction (Long et al. 2002). In addition, the effectiveness of NLMWOA is dependent on the species of plant being used (Long et al. 2002).

Since plant-associated microbes possess the capability of plant growth promotion and/or metal mobilization/immobilization, there has been increasing interest in the possibility of manipulating plant–microbe interactions in metal contaminated soils (Aafi et al. 2012; Azcón et al. 2010; Braud et al. 2009; Dimkpa et al. 2008, 2009a, b; Hrynkiewicz et al. 2012; Kuffner et al. 2010; Luo et al. 2011, 2012; Maria et al. 2011; Mastretta et al. 2009; Orłowska et al. 2011; Sheng et al. 2008a, b). Microbial metabolites/processes promote plant growth and metal mobilization/immobilization in vitro, but are unable to confer beneficial traits on their host in metal contaminated soils. Although promising results have been reported under laboratory conditions, showing that inoculation of beneficial microbes, particularly plant growth promoting bacteria and/or mycorrhizae, may stimulate heavy phytoextraction or phytostabilization, only a few studies have demonstrated the effectiveness of the microbial assisted heavy metal phytoremediation in field conditions (Brunetti et al. 2011; Juwarkar and Jambhulkar 2008; Rajkumar et al. 2012; Wu et al. 2011; Yang et al. 2012).

3 Mechanism of Glutathione-Mediated Metal Stress Tolerant in Plants

GSH plays a fundamental role in many cellular detoxification processes of xenobiotics and heavy metals. GSH does this by prior activation and conjugation with such compounds (Alfenito et al. 1998). The conjugation of GSH with such molecules is governed by glutathione S transferase (Edwards and Dixon 2005). The conjugates are subsequently transported to the vacuole and protects plant cell from their harmful effects (Yazaki 2006). One protective role of GSH in plants during heavy metal stress exposure is the quenching of ROS. Second, GSH acts as a precursor for the synthesis of phytochelatins (PCs). PCs are a set of novel heavy metal-binding peptides. These were first isolated from cell suspension cultures of a higher plant after exposure to Cd (Grill et al. 1985). Since then, PCs have been found in some eukaryotes, including higher plants (Grill et al. 1988; Gekeler et al. 1989). PCs are synthesized inductively by exposure to not only Cd, but also by other heavy metals such as Hg, Cu, Zn, Pb, and Ni. During the exposure of plants to such metals, PCs are synthesized from GSH, catalyzed by phytochelatin synthase (PCS). Thereafter, numerous physiological studies have indicated their role in heavy metal detoxification as well as in the maintenance of ionic homeostasis (Zenk 1996; Hirata et al. 2005).

4 Metals Uptake by Plants

Comparative studies have revealed that the enhanced Zn uptake into *T. caerulescens* and *A. halleri* roots, in comparison to congener non-hyperaccumulator species, can be attributed to the constitutive overexpression of some genes belonging to the Zinc-regulated transporter Iron-regulated transporter Proteins (ZIP) family, coding for plasma membrane located cation transporters (Assuncão et al. 2001) (Fig. 2).

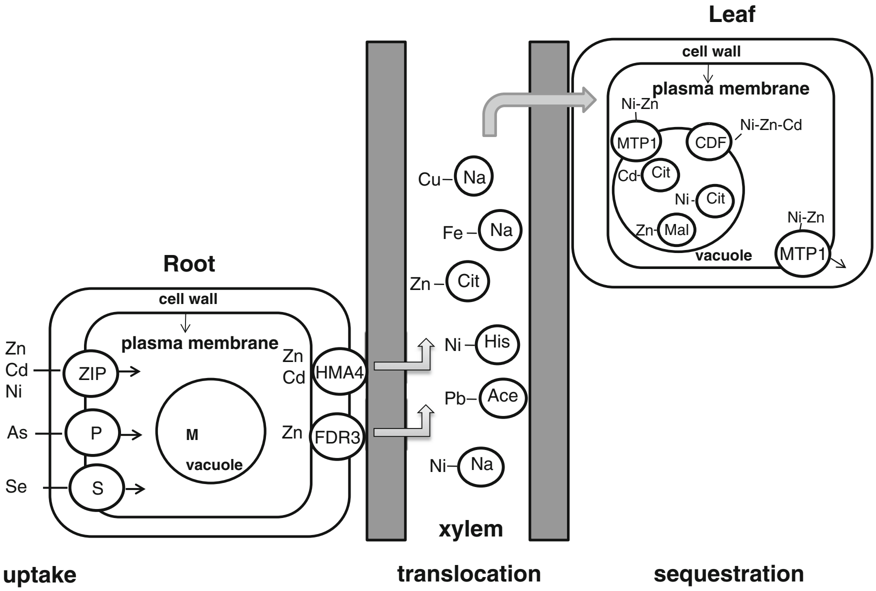

Fig. 2 Schematic picture showing transport systems constitutively overexpressed and/or with enhanced affinity to metals, which are thought to be involved in uptake, root-to shoot translocation, and heavy metal sequestration traits of hyperaccumulator plants. *Ace* acetate; *CDF* cation diffusion facilitators; *Cit* citrate; *FDR3* a member of the multidrug and toxin efflux family; *His* histidine; *HMA* heavy metal transporting ATPases; *M* metals; *Mal* malate; *MTP1* Metal transporter proteins; *NA* nicotinamine; *P* phosphate; *S* sulfate transporters; *ZIP* zinc-regulated transporter iron-regulated transporter proteins). (Modified from Rascio and Navari-Izzo 2011)

In non-hyperaccumulating plants, the expression of these ZIP genes (ZTN1 and ZTN2 in *T. caerulescens* and ZIP 6 and ZIP 9 in *A. Halleri*) is Zn-regulated (Assuncão et al. 2010), occurring at detectable levels only under Zn deficiency, while hyperaccumulators is irrespective of Zn supply still persisting at high Zn availability (Assuncão et al. 2001; Weber et al. 2004).

The electrochemical potential gradient of the plasma membrane in the root cells of plants drives Cd and other cations into the root cells (Blaylock and Huang 2000; Huang et al. 1992; Wang et al. 1994). However, external factors such as Fe concentration can reduce the uptake of Cd. For instance, in *Hordeum vulgare* (barley), Fe concentrations of 0–10 M reduced the Cd uptake (Sharma et al. 2004a, b). In *Thlaspi caerulescens* Ganges ecotype, Fe deficiency upregulates the expression of genes encoding for Fe(II) uptake, which promotes the uptake of Cd (Lombi et al. 2002). Also, in *Arabidopsis halleri* the transfer of Cd from the growing medium to the root xylem is partially shared with Zn and/or Fe transport (Ueno et al. 2008). In maize plants (*Zea mays*) the exposure to Cd enhances the release of the phytosiderophore 2-deoxymugineic acid from the roots under Fe deficiency conditions, which chelates Cd. This weak complex enables the uptake of Cd and Fe. On the other hand, it seems that in *Lactuca sativa*, an Mn-enriched medium promotes the uptake of Cd, 64 % of which is accumulated in the cell walls and potentially translocated to the consumers (Ramos et al. 2002).

The decreasing uptake of Cd by roots, supplied with increasing Zn concentration, found in the Cd/Zn hyperaccumulator *A. halleri* and in most ecotypes of *T. caerulescens*, clearly demonstrates that Cd influx is largely due to Zn transporters, with a strong preference for Zn over Cd ((Zhao et al. 2002). Surprisingly, in plants of the Ganges ecotype of *T. caerulescens*, which exhibit an exceptionally high ability to hyperaccumulate Cd in aerial tissues, Cd uptake is not inhibited by Zn, thus suggesting the presence in root cells of a specific and efficient independent Cd transport system (Lombi et al. 2001). The supposed existence of a transporter specific to this metal, regarded as unessential, raises the question as to whether Cd might play some physiological roles in that *T. caerulescens* accession. In shoots of the Ganges plants a positive correlation between Cd concentration and carbonic anhydrase activity has been found (Liu et al. 2007). The only physiological function of this heavy metal had previously been noticed in the marine diatom *Thalassiosira weissflogii*, owing to its finding in the active metal-binding site of a peculiar Cd-containing carbonic anhydrase (Lane et al. 2005).

Chromium enters plants by reduction and/or complexation with root exudates, such as organic acids, which increase the solubility and mobility of Cr through the root xylem (Bluskov et al. 2005). Both Cr(VI) and Cr(III) enter into the root cells by the symplast pathway, where Cr(VI) is reduced and accumulated in the cortex (Shanker et al. 2005). Though Cr is poorly translocated to aerial parts, it is mobilized and accumulated inside tissues depending on its chemical form (James and Barlett 1983). Hexavalent Cr damages root membranes because it has high oxidation power. In addition, it has been reported that Cr(VI) reduces the uptake of the essential elements Fe, K, Mg, Mn, P, and Ca (Gardea-Torresdey et al. 2005). Because of their similarity in ionic radius, Cr(III) replaces Fe(III) in heme proteins, decreasing their activity (Pandey and Sharma 2003). Zayed and Terry (2003) reported that Cr enters to plants as Cr(III) by a passive mechanism, while Cr(VI) uptake is inhibited by SO_4^{2-} and Ca^{2+} ions. Kim et al. (2006) suggested that Cr(VI) enters *Nicotiana tabacum* by sulfate or phosphate transport system, or by an active mechanism, being retained in the vacuoles by the cell wall. At neutral pH, Cr(VI) compounds are tetrahedral and are transported across cell membranes through similar tetrahedral ion channels, while Cr(III) is octahedral and transported through diffusion across membranes (Cohen et al. 2006). Montes-Holguin et al. (2006) reported that *Convolvulus arvensis* plants, treated with potassium dichromate or chromium nitrate, had different percents of chromium compounds in leaves, similar to chromium acetate, chromium phosphate, and chromium nitrate. The highest percent of chromium nitrate-type compounds suggests, due to the high solubility of chromium nitrate, that plants with high capability of chromium accumulation can contribute Cr to the food chain as trivalent Cr.

As Pb is not an essential element, plants do not have channels for Pb uptake. Instead, this element is bound to carboxylic groups of mucilage uronic acids on root surfaces (Morel et al. 1986; Sharma and Dubey 2005), but it is still unknown how this element goes into the root tissue. Although some plants species tolerate Pb through complexation and inactivation (*Allium cepa*, *H. vulgare,* and *Z. mays*), other species show toxicity (*Brassica napus* and *Phaseolus vulgaris*), because Pb obstructs some metabolic pathways (Wierzbicka 1999).

Several studies have shown that most of the absorbed Pb remains in roots, which makes the root the first barrier for the Pb translocation to the aboveground plant parts (Blaylock and Huang 2000).

Specific transporters for Ni hyperaccumulation have not yet been recognized. However, the preference of Zn over Ni by some Zn/Ni hyperaccumulators, supplied with the same concentration of both heavy metals, strongly suggests that a Zn transport system might also be employed for Ni entrance into roots (Assuncão et al. 2008). Substantial evidence exists that As can enter plant roots as arsenate, via transporters of the chemical analog phosphate (Meharg and Whitaker 2002) (Fig. 2). In root cells of the As hyperaccumulator *Pteris vittata*, plasma membranes have a higher density of phosphate/arsenate transporters than non-hyperaccumulator *P. tremula*, probably due to constitutive gene overexpression (Caille et al. 2005). Furthermore, the enhanced As uptake by the hyperaccumulating fern depends on the higher affinity for arsenate by the phosphate/arsenate transport systems (Poynton et al. 2004) as well as on the plant's ability to increase as bioavailability in the rhizosphere by reducing pH via root exudation of large amounts of dissolved organic carbon (Gonzaga et al. 2009). The pH decrease, in fact, enhances the water soluble As that can be taken up by the roots (Gonzaga et al. 2009; Fitz and Wenzel 2002).

The chemical similarity between sulfate and selenate accounts for the root uptake of Se in this form through high-affinity sulfate transporters (Fig. 2), whose activity is regulated by the S status of the plant (Hirai et al. 2003). In Se hyperaccumulators, such as *Astragalus bisulcatus* (Fabaceae) and *Stanleya pinnata* (Brassicaceae), the

Se/S ratios in shoots are much higher than in non-hyperaccumulator related species. This supports the idea of a role in this increased Se uptake of one or more sulfate transporters, which may have acquired a Se-specificity, becoming independent of the plant S status (Galeas et al. 2007).

5 Root-to-Shoot Translocation

Enhanced metal xylem loading, and translocation to the shoots, is a next key physiological step in the metal hyperaccumulation trait that accounts for the increased metal flow toward the shoot, where metals are detoxified and stored.

Different from non-hyperaccumulator plants, which retain in root cells most of the heavy metal taken up from the soil, detoxifying them by chelation in the cytoplasm or storing them into vacuoles, hyperaccumulators rapidly and efficiently translocate these elements to the shoot via the xylem. This involves, of course, the heavy metal availability for xylem loading, which derives from a low sequestration into and a ready efflux out of the vacuoles, plausibly due to specific features of root cell tonoplast (Lasat et al. 2000). Once in the xylem, metals are generally chelated to organic acids such as histidine (Krämer et al. 1996), nicotianamine (NA), citrate, and malate or oxalate (Senden et al. 1995), although also free Zn^{2+} has been found in xylem sap of *T. caerulescens* (Salt et al. 1999) (Fig. 2). Histidine has generally been reported as the ligand involved in the long distance root-to-shoot transport of Ni through xylem, such as in the Ni hyperaccumulator *Alyssum lesbiacum* (Krämer et al. 1996), but probably also in *T. caerulescens* (Morel et al. 2009). Krämer et al. (1996) showed that exogenously applied Ni, to enhance the Ni content of *A. lesbiacum* plants, increased free histidine levels, while external application of histidine to non-accumulator *A. montanum* plants greatly enhanced root elongation and plant biomass (Ni tolerance), and Ni influx through the xylem. Citrate has been shown to be transported into the xylem by FRD3, a member of the MATE family. This protein is essential for efficient iron translocation via vascular tissues (Durrett et al. 2007). Citrate is probably also involved in Zn translocation as FRD3 is much higher expressed in roots of *T. caerulescens* than those of *A. thaliana* (van de Mortel et al. 2006). This could be a side effect though, of a high Zn uptake compromising Fe uptake.

Fe and a few divalent metal ions like Zn, Ni, and Cu are also chelated and transported in plants by NA (Ling et al. 1999; Pich et al. 2001; Takahashi et al. 2003). NA is synthesized by trimerization of S-adenosylmethionine by the enzyme nicotianamine synthetase (NAS) (Shojima et al. 1990). All four NAS genes are highly expressed in *T. caerulescens* compared to *A. thaliana* (van de Mortel et al. 2006), and often show a different pattern of expression, indicating their involvement in the hyperaccumulation of Zn, Cd, and/or Ni. This could be direct or indirect. In the chloronerva mutant of tomato, which is impaired in NA biosynthesis (Ling et al. 1999), Fe, Zn, and Mn xylem transport are not, or hardly, affected compared to wild type. Cu xylem transport, however, was strongly reduced and Cu accumulated in the roots (Pich and Scholz 1996). NA is also important for Ni transport in *T. caerulescens*. Ouerdane et al. (2006) identified Ni–NA complexes in Ni-exposed *T. caerulescens* plants. They did not quantify Ni–His complexes, so it is not clear what the relative contributions of Ni–His and Ni–NA are to Ni root-to-shoot translocation, but both appear to be important.

A large body of evidence indicates that fast and efficient root-to-shoot translocation of large amounts of heavy metals in hyperaccumulator plants relies on enhanced xylem loading by a constitutive overexpression of genes coding for transport systems common to non-hyperaccumulators. The P1B-type ATPases, a class of proteins, also named Heavy Metal transporting ATPases (HMAs), are of particular importance. They operate in heavy metal transport and play a role in metal homeostasis and tolerance (Axelsen and Palmgren 1998). Genes encoding bivalent cation transporters belonging to HMAs (among which HMA4) are overexpressed in roots and shoots of Zn/Cd hyperaccumulators *T. caerulescens* and *A. halleri* (Talke et al. 2006; Papoyan and Kochian 2004; Hanikenne et al. 2008). Moreover, the HMA4 expression is upregulated when these plants are exposed to high levels of Cd and Zn, whereas it is downregulated in non-hyperaccumulator relatives (Papoyan and Kochian 2004). The overexpression of HMA4 supports a role of the HMA4 protein (which belongs to the Zn/Co/Cd/Pb HMA subclass and is localized at xylem parenchyma plasma membranes) in Cd and Zn efflux from the root symplasm into the xylem vessels, necessary for shoot hyperaccumulation. In fact, the increased expression of HMA4 enhances the expression of genes belonging to the ZIP family, implicated in heavy metal uptake. This strongly suggests that the root-to-shoot translocation acts as a driving force of the hyperaccumulation, by creating a permanent metal deficiency response in roots (Hanikenne et al. 2008).

As(III) and/or biotransformed to less toxic organic compounds such as DMA, MMA, or as inorganic As(III) complexed with thiol groups. For instance, Indian mustard store As in roots complexed with thiolate ligands (Pickering et al. 2000). In addition, the majority of plants are able to synthesize enough arsenate reductase, which reduces most of the As(V) to As(III) (Dhankher et al. 2006). Lopez et al. (2008) used X-ray absorption spectroscopy and liquid chromatography-mass spectrometry to study the speciation of As in Honey mesquite (Prosopis species). These researchers treated the plants with 50 mg As(V) L^{-1} but the analyses showed both species (As(III) and As(V)) in roots; while As(III) was found to be the predominant species in stems, demonstrating that mesquite reduced As(V) to As(III). Moreover, when plants were exposed to As(III), a percentage of the As was oxidized to As(V) in the soil matrix and was then absorbed by the roots. Arsenic was mobilized within plant tissues in the soluble fraction and chelated with cyclohexylenedinitrotetraacetic acid, nitrilo triacetic acid, or As-sulfur complexes.

In wheat, Cd is stored in root cell vacuoles by phytochelatins that are presumed to influence the symplastic radial Cd movement (Stolt et al. 2003; Cobbett 2000). Concerning Cd transport inside plants, Diatloff et al. (2006) reported for the first time that a low affinity cation transporter (LCT1), responsible for Ca transport in wheat, is also responsible for Cd transport in the yeast *Pichia pastoris*. It is likely that this transporter is also involved in Cd transport in many plants. It has also been determined that the gene ZntA, which in Arabidopsis is localized in the plasma membrane, participates in the transport of Cd from the roots to the shoots (Lee et al. 2003). Engineered crop plants bearing the ZntA gene would potentially be capable of growing in Cd impacted lands without incurring the risk of transporting Cd to the food chain. However, it seems that in *Z. mays* the entrance of Cd into the root symplast is unregulated, but its translocation toward the shoots is controlled and restricted to some extent by unknown factors (Perriguey et al. 2008). *S. kali*, a potential Cd hyperaccumulator handles Cd through the production of low molecular weight thiols (LMWT), mainly in roots and stems (de la Rosa et al. 2004). However, these same researchers have proposed that LMWT are at most contributing to half of the total-Cd binding in leaves (de la Rosa et al. 2005). In rice, the concentration of Cd in grains is governed somewhat by its uptake and transport from roots to shoots, and to a greater extent, by the transport of Cd from shoots to grain. In a study performed in China by Liu et al. (2007), it was found that about 0.73 % of the total Cd taken up by six rice cultivars was transferred to the grain. This represents an average of 1.02 mg kg^{-1}, which is 100 times higher than the concentration allowed by the European Union for Cd concentration in rice grain (Olsson et al. 2005).

In the roots, most of the Pb is bound to ion exchangeable sites in the cell walls and extracellular precipitation as phosphate and carbonate (Blaylock and Huang 2000; Sharma and Dubey 2005). The unbound Pb is moved through Ca channels, accumulating near the endodermis (Huang and Cunningham 1996; Antosiewicz 2005). Previous experimental results suggest that at low concentration, the Casparian strip of the endodermis is a partial barrier for Pb movement into the central cylinder tissue (Seregin et al. 2004). Varga et al. (1997) found that, in roots of wheat, Pb is fixed to the cell wall but it can be removed as a complex using citric acid. However, Marmiroli et al. (2005) reported that in European walnut (*Juglans regia*) Pb is retained in the lignocellulosic structure of roots. As described by Cobbett (2000), Pb, like other toxic elements, is complexed by the cysteine-rich low molecular weight polypeptides, widely known as phytochelatins. However, in *Sesbania drummondii*, Pb is transported to stems and leaves in structures similar to Pb-acetate, Pb-nitrate, and Pb-sulfide (Sharma et al. 2004a, b) (Fig. 2). In addition, Lopez et al. (2007, 2009) have reported the formation of different Pb complexes in stems and leaves of alfalfa.

6 Detoxification/Sequestration

Great efficiency in detoxification and sequestration is a key property of hyperaccumulators which allows them to concentrate huge amounts of heavy metals in aboveground organs without suffering any phytotoxic effect. This exceptionally high heavy metal accumulation becomes even more astonishing bearing in mind that it principally occurs in leaves where photosynthesis, essential for plant survival, is accomplished, and that the photosynthetic apparatus is a major target for most of these contaminants. The preferential heavy metal detoxification/sequestration does occur in locations, such as epidermis (Freeman et al. 2006), trichomes (Kupper et al. 2000), and even cuticle (Robinson et al. 2003), where they do least damage to the photosynthetic machinery. In many cases, heavy metals are also excluded from both subsidiary and guard cells of stomata (Frey et al. 2000; Cosio et al. 2005).

This may preserve the functional stomatal cells from metal phytotoxic effects. The detoxifying/sequestering mechanisms in aerial organs of hyperaccumulators consist mainly in heavy metal complexation with ligands and/or in their removal from metabolically active cytoplasm by moving them into inactive compartments, mainly vacuoles and cell walls (Fig. 2). Comparative transcriptome analyses between hyperaccumulator and related non-hyperaccumulator species have demonstrated that also the sequestration trait relies, at least in part, on constitutive overexpression of genes that, in this case, encode proteins operating in heavy metal transfer across the tonoplast and/or plasma membrane and involved in excluding them from cytoplasm. Cation Diffusion Facilitator (CDF) family members, also named Metal Transporter Proteins (MTPs), which mediate bivalent cation efflux from the cytosol, are important candidates. MTP1, a gene encoding a protein localized at tonoplast, is highly overexpressed in leaves of Zn/Ni hyperaccumulators (Kim et al. 2004; Gustin et al. 2009). It has been suggested that MTP1, besides the role in Zn tolerance, may also play a role in enhancing Zn accumulation.

The Zn transport into the vacuole, in fact, may initiate a systemic Zn deficiency response that includes the enhancement of the heavy metal uptake and translocation via the increased expression of ZIP transporters in hyperaccumulator plants (Gustin et al. 2009). MTP members also mediate the Ni vacuolar storage in *T. goesingense* shoots (Persant et al. 2001). Moreover, the finding that MTP1 is localized at both vacuolar and plasma membrane suggests that it can also operate in Zn and Ni efflux from cytoplasm to cell wall (Kim et al. 2004).

Small ligands, such as organic acids, have a major role as detoxifying factors. Such ligands may be instrumental in preventing the persistence of heavy metals as free ions in the cytoplasm and even more in enabling their entrapment in vacuoles where the metal–organic acid chelates are primarily located. Citrate, for instance, is the main ligand of Ni in leaves of *T. goesingense* (Kramer et al. 2000), while citrate and acetate bind Cd in leaves of *S. nigrum* (Sun et al. 2006). Moreover, most Zn in *A. halleri* and Cd in *T. caerulescens* are complexed with malate (Sarret et al. 2002).

Leaf cell vacuoles are the sites of sequestration of excess essential and non-essential metals (Vogeli-Lange and Wagner 1990). Küpper et al. (1999) found that the highest concentration of leaf Zn and Cd was present in leaf epidermal cells, which contained four times higher concentrations of these metals compared to mesophyll cells. The epidermis may be preferred, since most epidermis cells lack chloroplasts, which could be compromised by high metal concentrations.

Milner and Kochian (2008) suggested the role of TcZNT1 in facilitating uptake of Zn from leaf apoplast into bundle sheath and mesophyll cells in *T. caerulescens*.

Once metals have reached their target destinations, they need to be stored in vacuoles. The main candidates for this function are members of the CDF protein family. These proteins have also been established as conferring tolerance to various metals like Zn, Mn, Cd, Co, or Ni by sequestering metals in the vacuoles (Montanini et al. 2007). In *T. caerulescens*, a CDF family member TcZTP1 (similar to AtMTP1/AtZAT) (van der Zaal et al. 1999; Desbrosses-Fonrouge et al. 2005) showed constitutively high expression and was suggested to play a role in Zn tolerance (Assuncão et al. 2001). Overexpression of TcZTP1 enhances tolerance and accumulation of Zn and Cd in *A. thaliana*, accumulation of Zn, and tolerance to Zn, Cd, and Ni in *N. tabacum* (Hassan and Aarts 2011).

Recently, Guimarães et al. (2009) reported that shoots and roots have different roles in metal hyperaccumulation and hypertolerance in *T. caerulescens*. Reciprocal grafting experiments using *T. caerulescens* and the non-hyperaccumulator *T. perfoliatum* showed that in *T. caerulescens*, Zn hyperaccumulation is mainly controlled by root processes, while shoot processes control the hypertolerance to Zn. The authors advocated that shoot-governed hypertolerance would be driven mainly by MTP1 (ZTP1) (Assuncão et al. 2001; Persant et al. 2001). This supports the idea that tissue-specific expression of potential genes will be crucial in mimicking metal tolerance and hyperaccumulation traits in engineered high biomass plants useful for phytoremediation. Furthermore, one can hypothesize that since shoot tissues involved in tolerance do not seem to influence the hyperaccumulation trait, there appears to be no feedback mechanism from shoots to roots for metals that are loaded and stored in the shoot tissues. This suggests that hyperaccumulators are disturbed in the feedback signal transduction pathway indicating to roots that shoots are becoming overloaded with metals and that metal uptake needs to cease. However, Hanikenne et al. (2008) postulated that, for *A. halleri*, strong activity of HMA4 in fact depletes root cells from Zn, even at high Zn concentrations, which somehow leads them to continue Zn uptake at a high rate.

The heavy metal detoxification in hyperaccumulators, in contrast with tolerant non-hyperaccumulator plants, does not rely on high molecular mass ligands, such as phytochelatins (Schat et al. 2002; Raab et al. 2004), likely because of the excessive sulfur amounts and the prohibitive metabolic cost that a massive synthesis of this kind of chelators would require (Zaho et al. 2002).

7 Improvement for Enhanced Phytoextraction

The development of transgenic plants with increased metal uptake, accumulation, and tolerance to toxicity is now being considered a promising alternative. Genetic engineering is a technique that can be applied advantageously to the development of ideal phytoremediation plants that combine high metal accumulating capacity and high aboveground biomass yield (Kärenlampi et al. 2000).

With the use of genetic engineering, it is feasible to manipulate a plant's capacity to tolerate, accumulate, and/or metabolize pollutants, and to create an ideal plant for environmental cleanup (Bhargava et al. 2012). Many genes are reported to be involved in metal uptake, translocation, sequestration, chemical modification, and tolerance. The introduction and overexpression of the hyperaccumulating genes into a non-hyperaccumulator plant could be a possible way to enhance metal uptake, accumulation, tolerance, and detoxification process (Clemens et al. 2002). The overexpression of genes encoding the rate-limiting gene product is expected to lead a faster overall rate of the pathway and to more efficient phytoremediation (Pilon-Smits and Pilon 2002).

Several reports on transgenic plants tolerant to the presence of toxic levels of metals have appeared in recent years (Reisinger et al. 2008; Bhuiyan et al. 2011; Balestrazzi et al. 2009; Haydon and Cobbett 2007; Nagata et al. 2006). In most of these studies, the overexpression of genes encoding for the enzymes of phytochelatin synthetase, ACC deaminase, S-metabolism, glutathione, Hg^{2+}-reductase, arsenate reductase, aldolase/aldehyde reductase, enzymes of histidine biosynthesis, and metallothionein (MT)-genes have been effectively carried out (Shah and Nongkynrih 2007). The bioengineering of transporter genes to manipulate the transport of metal ions inside the cell has also been successfully exploited and a combination of these genes in rapidly growing plant species has led to promising results.

Modification or overexpression of the enzymes that are involved in the synthesis of PCs is a promising approach to enhance heavy metal tolerance and accumulation in plants and has been tried to genetically transform high biomass plants into efficient phytoremediators (Zhu et al. 1999). Several attempts have been made to increase the formation of PCs by overexpressing genes encoding enzymes that could stimulate the synthesis of cysteine and glutathione.

8 Conclusions

The use of phytoremediation for site restoration or amelioration of polluted soils is a promising technique for the near future. The discovery and elucidation of the mechanism used by hyperaccumulators is of great interest to understand the role of such plants in biorestoration. However, there is a need for testing different plants under realistic field conditions to fully evaluate their usefulness for soil restoration.

On the other hand, the use of both synthetic and natural chelating agents, microorganisms (siderophores) needs further research to improve the uptake capacity of plants, leading to increased bioavailability of heavy metals, avoiding leachates of soluble metals that could reach groundwater or non-polluted areas. There is also a need to develop methods for biorecovery of heavy metals fixed in the aerial parts of plants, in addition to the safe use of wood arising from trees used for biorestoration. All in all, phytoremediation is a promising technique that needs both field and laboratory research to fulfill requisites necessaries for safe use in restoring polluted environments.

References

Aafi NE, Brhada F, Dary M, Maltouf AF, Pajuelo E (2012) Rhizostabilization of metals in soils using *Lupinus luteus* inoculated with the metal resistant rhizobacterium *Serratia* sp. MSMC 541. Int J Phytorem 14:261–274

Addy M, Losey B, Mohseni R, Zlotnikov E, Vasiliev E (2012) Adsorption of heavy metal ions on mesoporous silica-modified montmorillonite containing a grafted chelate ligand. Appl Clay Sci 59–60:115–120

Alfenito MR, Souer E, Goodman CD, Buell R, Mol J, Koes R, Walbot V (1998) Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione-S-transferases. Plant Cell 10:1135–1149

Alloway BJ (1995) Heavy metals in soils. Blackie, Glasgow

Antosiewicz DM (2005) Study of calcium-dependent lead-tolerance on plants differing in their level of Ca-deficiency tolerance. Environ Pollut 134:23–34

Assunção AGL, Martins PDC, De Folter S, Vooijs R, Schat H, Aarts MGM (2001) Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 24:217–226

Assuncão AGL, Bleeker P, Ten Bookum WM, Vooijs R, Schat H (2008) Intraspecific variation of metal preference patterns for hyperaccumulation in *Thlaspi caerulescens*: evidence for binary metal exposures. Plant Soil 303:289–299

Assuncão AGL, Herrero E, Lin YF, Huettel B, Talukdar S, Smaczniak C, Immink R, van Eldik M, Fiers M, Schat H, Aartsa M (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc Natl Acad Sci USA 107:10296–10301

Axelsen KB, Palmgren MG (1998) Inventory of the superfamily of P-Type ion pumps in *Arabidopsis*. Plant Physiol 126:696–706

Azcón R, Perálvarez MDC, Roldán A, Barea JM (2010) Arbuscular mycorrhizal fungi, *Bacillus cereus*, and *Candida parapsilosis* from a multi-contaminated soil alleviate metal toxicity in plants. Microb Ecol 59:668–677

Baker AJM (1981) Accumulators and excluders: strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Baker AJM, Reeves RD, Hajar ASM (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. and *C. Presl* (Brassicaceae). New Phytol 127:61–68

Balestrazzi A, Botti S, Zelasco S, Biondi S, Franchin C, Calligari P, Racchi M, Turchi A, Lingua G, Berta G, Carbonera D (2009) Expression of the PsMTA1 gene in white poplar engineered with the MAT system is associated with heavy metal tolerance and protection against 8-hydroxy-20 deoxyguanosine mediated-DNA damage. Plant Cell Rep 28:1179–1192

Barrow NJ, Cox VC (1992) The effects of pH and chloride concentration on mercury sorption. II. By a soil. J Soil Sci 43:305–311

Bhargava A, Gupta VK, Singh AK, Gaur R (2012) Microbes for heavy metal remediation. In: Gaur R, Mehrotra S, Pandey RR (eds) Microbial applications. I.K. International Publisher, New Delhi

Bhuiyan MSU, Min SR, Jeong WJ, Sultana S, Choi KS, Song WY, Lee Y, Lim YP, Liu JR (2011) Overexpression of a yeast cadmium factor 1 (YCF1) enhances heavy metal tolerance and accumulation in *Brassica juncea*. Plant Cell Tiss Org Cult 105:85–91

Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean-up the environment. Wiley, New York

Blaylock M, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin E (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865

Bluskov S, Arocena JM, Omotoso OO, Young JP (2005) Uptake, distribution, and speciation of chromium in *Brassica Juncea*. Int J Phytorem 7:153–165

Bolan NS, Duraisamy VP (2003) Role of inorganic and organic soil amendments on immobilization and phytoavailability of heavy metals: a review involving specific case studies. Aust J Soil Res 41:533–555

Bowers AR, Huang CP (1986) Adsorption characteristics of metal-EDTA complexes onto hydrous oxides. J Colloid Interface Sci 110:575–590

Boyd RS, Martens SN (1992) The raison d'être for metal hyperaccumulation by plants. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept, Andover

Boyd RS (2007) The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. Plant Soil 293:153–176

Bradl HB (2004) Prediction of the adsorption equilibrium of mixtures composed of supercritical gases. J Colloid Interface Sci 277:1–18

Braud A, Jézéquel K, Bazot S, Lebeau T (2009) Enhanced phytoextraction of an agricultural Cr, Hg- and Pb-contaminated soil by bioaugmentation with siderophore producing bacteria. Chemosphere 74:280–286

Brooks RP, Lee J, Reeves RD, Jaffré T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49–57

Brunner I, Luster J, Günthardt-Goerg MS, Frey B (2008) Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environ Poll 152:559–568

Brunetti G, Farrag K, Rovira PS, Nigro F, Senesi N (2011) Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, southern Italy. Geoderma 160:517–523

Caille N, Zhao FJ, McGrath SP (2005) Comparison of root absorption, translocation and tolerance of arsenic in the hyperaccumulator *Pteris vittata* and the nonhyperaccumulator *Pteris tremula*. New Phytol 165:755–761

Capuana M (2011) Heavy metals and woody plants-biotechnologies for phytoremediation. iForest 4:7–15

Cavallaro N, McBride MB (1980) Activities of Cu^{2+} and Cd^{2+} in soil solutions as affected by pH. Soil Sci Soc Am J 44:729–732

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotech 8:279–284

Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual 36:1429–1443

Cheng S (2003) Heavy metals in plants and phytoremediation. Environ Sci Pollut Res 10:335–340

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Curr Opin Plant Biol 3:211–216

Cohen MD, Prophete C, Sisco M, Chen L, Zelikoff JT (2006) Pulmonary immunotoxic potentials of metals are governed by select physicochemical properties: chromium agents. J Inmunotoxicol 3:69–81

Cosio C, De Santis L, Frey B, Diallo S, Keller C (2005) Distribution of cadmium in leaves of *Thlaspi caerulescens*. J Exp Bot 56:565–575 de la Rosa G, Peralta-Videa JR, Montes M, Parsons JG, Gardea-Torresdey JL (2004) Cadmium uptake and translocation in tumbleweed (*Salsola kali*), a potential Cd-hyperaccumulator desert plant species: ICP/OES and XAS studies. Chemosphere 55:1159–1168

de la Rosa G, Martinez–Martinez A, Pelayo H, Peralta-Videa JR, Sanchez-Salcido B, Gardea-Torresdey JL (2005) Production of low-molecular weight thiols as a response to cadmium uptake by tumbleweed (*Salsola kali*). Plant Physiol Biochem 43:491–498

Deng DM, Shu WS, Zhang JZ, Zou HL, Lin Z, Ye ZH, Wong MH (2007) Zinc and cadmium accumulation and tolerance in populations of *Sedum alfredii*. Environ Pollut 147:381–386

Desbrosses-Fonrouge AG, Voigt K, Schroder A, Arrivault S, Thomine S, Kramer U (2005) *Arabidopsis thaliana* MTP1is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. FEBS Lett 579:4165–4174

Dhankher OP, Rosen BP, McKinney EC, Meagher RB (2006) Hyperaccumulation of arsenic in the shoots of *Arabidopsis* silenced for arsenate reductase (ACR2). Proc Natl Acad Sci USA 103:5413–5418

Diatloff E, Forde BG, Roberts SK (2006) Expression and transport characterisation of the wheat low-affinity cation transporter (LCT1) in the methylotrophic yeast *Pichia pastoris*. Bichem Biophys Res Comm 344:807–813

Dimkpa CO, Svatos A, Merten D, Büchel G, Kothe E (2008) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54:163–172

Dimkpa CO, Merten D, Svatoš A, Büchel G, Kothe E (2009a) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696

Dimkpa CO, Merten D, Svatoš A, Büchel G, Kothe E (2009b) Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol Biochem 41:154–162

Domínguez MT, Marañón T, Murillo JM, Schulin R, Robinson BH (2008) Trace element accumulation in woody plants of the Guadiamar valley, SW Spain: a large-scale phytomanagement case study. Environ Pollut 152:50–59

Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333

Duker A, Ledin S, Karlsson B (1995) Adsorption of zinc on colloidal (hydr)oxides of Si, Al and Fe in the presence of a fulvic acid Allard. Appl Geochem 10:197–205

Durrett TP, Gassmann W, Rogers EE (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiol 144:197–205

Dzantor EK, Beauchamp RG (2002) Phytoremediation, Part I: fundamental basis for the use of plants in remediation of organic and metal contamination. Environ Prac 4:77–87

Ebbs SE, Norvell WA, Kochian LV (1998) The effect of acidification and chelating agents on the solubilization of uranium from contaminated soil. J Environ Qual 27:1486–1494

Edwards R, Dixon D (2005) Plant glutathione transferases. Methods Enzymol 401:169–186

Fitz WJ, Wenzel WW (2002) Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation. J Biotechnol 99:259–278

Fein JB, Boily JF, Güclü K, Kaulbach E (1999) Experimental study of humic acid adsorption onto bacteria and Al-oxide mineral surfaces. Chem Geol 162:33–45

Fein JB, Delea D (1999) Experimental study of the effect of EDTA on Cd adsorption by *Bacillus subtilis*: a test of the chemical equilibrium approach. Chem Geol 161:375–383

Freeman JL, Zhang LH, Marcus MA, Fakra S, McGrath SP, Pilon-Smits EA (2006) Spatial imaging, speciation and quantification of Se in the hyperaccumulator plants *Astragalus bisulcatus* and *Stanleya pinnata*. Plant Physiol 142:124–134

Frey B, Keller C, Zierold K, Schulin R (2000) Distribution of Zn in functionally different leaf epidermal cells in the hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 23:675–687

Fulekar MH, Singh A, Bhaduri AM (2009) Genetic engineering strategies for enhancing phytoremediation of heavy metals. Afr J Biotechnol 8:529–535

Galeas ML, Zhang LH, Freeman JL, Wegner M, Pilon-Smits SEAH (2007) Seasonal fluctuations of selenium and sulphur accumulation in selenium hyperaccumulators and related non-accumulators. New Phytol 173:517–525

Galeas ML, Klamper EM, Bennett LE, Freeman JL, Kondratieff BC, Quinn CF, Pilon-Smits EA (2008) Selenium hyperaccumulation reduces plant arthropod loads in the field. New Phytol 177:715–724

Gardea-Torresdey JL, de la Rosa G, Peralta-Videa JR, Montes M, Cruz-Jimenez G, Cano-Aguilera I (2005) Differential uptake and transport of trivalent and hexavalent chromium by tumbleweed (*Salsola kali*). Arch Environ Cont Toxicol 48:225–432

Gekeler W, Grill E, Winnacker EL, Zenk MH (1989) Survey of the plant kingdom for the ability to bind heavy metals through phytochelatins. Z Naturf 44:361–369

Gleba D, Borisjuk NV, Borisjuk LG, Knee R, Poulev A, Skarzhinskaya M, Dushenkov S, Logendra S, Gleba YY, Raskin I (1999) Use of plant roots for phytoremediation and molecular farming. Proc Natl Acad Sci USA 96:5973–5977

Gonzaga MI, Ma LQ, Santos JA, Matias MI (2009) Rhizosphere characteristics of two arsenic hyperaccumulating Pteris ferns. Sci Total Environ 407:4711–4716

Gratao PL, Prasad MNV, Cardoso PF, Lea PJ, Azevedo RA (2005) Phytoremediation: green technology for the clean up of toxic metals in the environment. Braz J Plant Physiol 17:53–64

Grcman HS, Velikonja-Bolta D, Vodnik B, Kos DL (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant Soil 235:105–114

Grill E, Winnacker EL, Zenk MH (1985) Phytochelatins: the principal heavy-metal complexing peptides of plants. Science 230:674–676

Guerinot ML, Salt DE (2001) Fortified foods and phytoremediation: two sides of the same coin. Plant Physiol 125:164–167

Guimarães MAD, Gustin JL, Salt DE (2009) Reciprocal grafting separates the roles of the root and shoot in zinc hyperaccumulation in *Thlaspi caerulescens*. New Phytol 184:323–329

Gupta UC, Gupta SC (1998) Trace element toxicity relationships to crop production and livestock and human health: implications for management. Commun Soil Sci Plant Anal 29:1491–1522

Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn hyperaccumulating plants. Plant J 57:1116–1127

Gworek B (1992) Lead inactivation in soils by zeolites. Plant Soil 143:71–74

Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U (2008) Evolution of metal hyperaccumulation required cis regulatory changes and triplication of HMA4. Nature 453:391–395

Hassan Z, Aarts MGM (2011) Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. Environ Exp Bot 72:53–63

Haydon MJ, Cobbett CS (2007) A novel major facilitator superfamily protein at the tonoplast influences Zn tolerance and accumulation in *Arabidopsis*. Plant Physiol 143:1705–1719

Hirai MY, Fujiwara T, Awazuhara M, Kimura T, Noji1 M, Saito K (2003) Global expression profiling of sulphur-starved *Arabidopsis* by DNA microarray reveals the role of O-acetyl-L-serine as a general regulator of gene expression in response to sulphur nutrition. Plant J 33:651–663

Hirata K, Tsuji N, Miyamoto K (2005) Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. J Biosci Bioeng 100:593–599

Hrynkiewicz K, Dabrowska G, Baum C, Niedojadlo K, Leinweber P (2012) Interactive and single effects of ectomycorrhiza formation and *Bacillus cereus* on metallothionein mt1 expression and phytoextraction of Cd and Zn by willows. Water Air Soil Pollut 223:957–968

Huang JW, Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134:73–84

Huang JW, Shaff JE, Grunes DL, Kochian LV (1992) Aluminum effects on calcium fluxes at the root apex of aluminum-tolerant and aluminum-sensitive wheat cultivars. Plant Physiol 98:230–237

Huitson SB, Macnair MR (2003) Does Zn protect the Zn hyperaccumulator *Arabidopsis halleri* from herbivory by snails? New Phytol 159:453–459

Jabeen R, Ahmad A, Iqbal M (2009) Phytoremediation of heavy metals: physiological and molecular mechanisms. Bot Rev 75:339–364

James BR, Barlett RJ (1983) Behavior of chromium in soils VII adsorption and reduction of hexavalent forms. J Environ Qual 12:177–181

Ji GL, Wang JH, Zhang XN (2000) Environmental problems in soil and groundwater induced by acid rain and management strategies in China. In: Huang PM, Iskandar IK (eds) Soils and groundwater pollution and remediation. CRC Press, London

Juwarkar AA, Jambhulkar HP (2008) Phytoremediation of coal mine spoil dump through integrated biotechnological approach. Bioresour Technol 99:4732–4741

Kärenlampi S, Schat H, Vangronsveld J, Verkleij JAC, van der Lelie D, Mergeay M, Tervahauta AI (2000) Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. Environ Poll 7:225–231

Kerndorf H, Schnitzer M (1980) Sorption of metals on humic acid. Geochim Cosmochim Acta 44:1701–1708

Kent DB, Abrams RH, Davis JA, Coston JA, Le Blanc DR (2000) Unsaturated-zone wedge beneath a large, natural lake. Water Resour Res 36:3401–3409

Khan FI, Husain T, Hejazi R (2004) An overview and analysis of site remediation technologies. J Environ Manag 71:95–122

Khattak RA, Page AL (1992) Mechanism of manganese adsorption on soil constituents. In: Adriano DC (ed) Biogeochemistry of trace metals. Lewis Publication, Boca Raton

Kim D, Gustin JL, Lahner B, Persans MW, Baek D, Yun DJ, Salt DE (2004) The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*. Plant J 39:237–251

Kim YJ, Kim JH, Lee CE, Mok YG, Choi JS, Shin HS, Hwang S (2006) Expression of yeast transcriptional activator MSN1 promotes accumulation of chromium and sulfur by enhancing sulfate transporter level in plants. FEBS Lett 580:206–210

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638

Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. Plant Physiol 122:1343–1354

Krishnamurti GS, Cielinski RG, Huang PM, van Rees KCJ (1998) Kinetics of cadmium release from soils as influenced by organic acids: implementation in cadmium availability. J Environ Qual 26:271–277

Kuffner M, De Maria S, Puschenreiter M, Fallmann K, Wieshammer G, Gorfer M, Strauss J, Rivelli AR, Sessitsch A (2010) Culturable bacteria from Zn- and Cd accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability. J Appl Microbiol 108:1471–1484

Küpper H, Zhao FJ, McGrath SP (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305–311

Küpper H, Lombi E, Zhao FJ, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75–84

Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FM (2005) A cadmium enzyme for a marine diatom. Nature 435:42

Lasat MM, Pence NS, Garvin DF, Abbs SD, Kochian LV (2000) Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 51:71–79

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120

Lee J, Bae H, Jeong J, Lee JY, Yang YY, Huang I, Martinoia E, Lee Y (2003) Functional expression of bacterial heavy metal transporter in *Arabidopsis* enhances resistance to and decreases uptake of heavy metals. Plant Physiol 133:589–596

Ling HQ, Koch G, Baumlein H, Ganal MW (1999) Map-based cloning of chloronerva, a gene involved in iron uptake of higher plants encoding nicotianamine synthase. Proc Natl Acad Sci USA 96:7098–7103

Lion LW, Altmann RS, Leckie JO (1982) Trace-metal adsorption characteristics of estuarine particulate matter: evaluation of contributions of iron/manganese oxide and organic surface coatings. Environ Sci Technol 16:660–666

Liu J, Qian M, Cai G, Yang J, Zhu Q (2007) Uptake and translocation of Cd in different rice cultivars and the relation with Cd accumulation in rice grain. J Hazard Mater 143:443–447

Lombi E, Zhao FJ, McGrath SP, Young SD, Sacchi GA (2001) Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. New Phytol 149:53–60

Lombi E, Tearall KL, Howarth JR, Zhao FJ, Hawkesford MJ, McGrath SP (2002) Influence or iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 128:1359–1367

Long XX, Ni WZ, Ye ZQ (2002) Effect of organic acids application on Zn uptake and accumulation by two ecotypes of *Sedum alfredii* Hance. J Plant Nutr Fertil 8:467–472

Lopez ML, Peralta-Videa JR, Parsons JG, Benitez T, Gardea-Torresdey JL (2007) Gibberellic acid, kinetin, and the mixture indole–3-acetic acid–kinetin assisted with EDTA-induced lead hyperaccumulation in alfalfa plants. Environ Sci Technol 41:8165–8170

Lopez ML, Peralta-Videa JR, Parsons JG, Gardea-Torresdey JLn (2008) Concentration and biotransformation of arsenic by *Prosopis* sp. grown in soil treated with chelating agents and phytohormones. Environ Chem 5:1–12

Lopez ML, Peralta-Videa JR, Parsons JG, Gardea-Torresdey JL (2009) Effect of indole-3-acetic acid, kinetin, and ethylenediaminetetraacetic acid on plant growth and uptake and translocation of lead, micronutrients, and macronutrients in alfalfa plants. Int J Phytorem 11:131–149

Luo SL, Chen L, Chen JI, Xiao X, Xu TY, Wan Y, Rao C, Liu CB, Liu YT, Lai C, Zeng GM (2011) Analysis and characterization of cultivable heavy metal-resistant bacterial endophytes isolated from Cd-hyperaccumulator *Solanum nigrum* L. and their potential use for phytoremediation. Chemosphere 85:1130–1138

Luo S, Xu T, Chen L, Chen J, Rao C, Xiao X, Wan Y, Zeng G, Long F, Liu C, Liu Y (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. Appl Microbiol Biotechnol 93:1745–1753

Macnair MR (1993) The genetics of metal tolerance in vascular plants. New Phytol 124:541–559

Malandrino M, Abollino O, Buoso S, Giacomino A, La Gioia C, Mentasti E (2011) Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite. Chemosphere 82:169–178

Maria SD, Rivelli AR, Kuffner M, Sessitsch A, Wenzel WW, Gorfer M, Strauss J, Puschenreiter M (2011) Interactions between accumulation of trace elements and macronutrients in *Salix caprea* after inoculation with rhizosphere microorganisms. Chemosphere 84:1256–1261

Marmiroli M, Antonioli G, Maestri E, Marmiroli N (2005) Evidence of the involvement of plant ligno-cellulosic structure inthe sequestration of Pb: an X-ray spectroscopy based analysis. Environ Pollut 134:217–227

Marques APGC, Rangel AOSS, Castro PML (2009) Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. Crit Rev Environ Sci Tech 39:622–654

Martinez CE, Motto HL (2000) Solubility of lead, zinc and copper added to mineral soils. Environ Pollut 107:153–158

Mastretta C, Taghavi S, van der Lelie D, Mengoni A, Galardi F, Gonnelli C, Barac T, Boulet J, Weyens N, Vangronsveld J (2009) Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce cadmium phytotoxicity. Int J Phytorem 11:251–267

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282

McLaughlin MJ, Parker DR, Clarke JM (1999) Metals and micronutrients and food safety issues. Field Crops Res 60:143–163

Meers E, Vandecasteele B, Ruttens A, Vangronsveld J, Tack FMG (2007) Potential of five willow species (*Salix* spp.) for phytoextraction of heavy metals. Environ Exp Bot 60:57–68

Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and non-resistant plant species. New Phytol 154:29–42

Milner MJ, Kochian LV (2008) Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. Annl Bot 102:3–13

Montanini B, Blaudez D, Jeandroz S, Sanders D, Chalot M (2007) Phylogenetic and functional analysis of the cation diffusion facilitator (CDF) family: improved signature and prediction of substrate specificity. BMC Genomics 8:107

Montes-Holguin MO, Peralta-Videa JR, Meitzner G, Martinez–Martinez A, de la Rosa G, Castillo-Michel H, Gardea-Torresdey JL (2006) Biochemical and spectroscopic studies of the response of *Convolvulus arvensis* L. to chromium(III) and chromium(VI) stress. Environ Toxicol Chem 25:220–226

Morel JL, Mench M, Guckert A (1986) Measurement of Pb^{2+}, Cu^{2+} and Cd^{2+} binding with mucilage exudates frommaize (*Zea mays* L.) roots. Biol Fertil Soils 2:29–34

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P (2009) AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. Plant Physiol 149:894–904

Nagata T, Ishikawa C, Kiyono M, Pan-Hou H (2006) Accumulation of Hg in transgenic tobacco expressing bacterial polyphosphate. Biol Pharm Bull 29:2350–2353

Narwal RP, Singh BR (1998) Effect of organic materials on partitioning, extractability and plant uptake of metals in an alum shale soil. Water Air Soil Pollut 103:405–421

Nigam R, Srivastava S, Prakash S, Srivastava MM (2001) Cadmium mobilization and plant availability-the impact of organic acids commonly exuded from roots. Plant Soil 230:107–113

Noret N, Meerts P, Vanhaelen M, Dos Santos A, Escarré J (2007) Do metal-rich plants deter herbivores? a field test of the defence hypothesis. Oecologia 152:92–100

Olsson IM, Erikson J, Oborn I, Skerfving S, Oskarsson A (2005) Cadmium in food production systems: a health risk for sensitive population groups. Ambio 34:344–351

Orłowska E, Przybyłowicz W, Orlowski D, Turnau K, Mesjasz-Przybyłowicz J (2011) The effect of mycorrhiza on the growth and elemental composition of Ni-hyperaccumulating plant *Berkheya coddii* Roessler. Environ Pollut 159:3730–3738

Ouerdane L, Mari S, Czernic P, Lebrun M, Lobinski R (2006) Speciation of noncovalent nickel species in plant tissue extracts by electrospray Q-TOFMS/MS after their isolation by 2D size exclusion-hydrophilic interaction LC (SEC-HILIC) monitored by ICP-MS. J Anal Atom Spectrom 21:676–683

Pandey N, Sharma CP (2003) Chromium interference in iron nutrition and water relations of cabbage. Environ Exp Bot 49:195–200

Papoyan A, Kochian LV (2004) Identification of *Thlaspi caerulescens* genes that may be involved in heavy metal hyperaccumulation and tolerance: characterization of a novel heavy metal transporting ATPase. Plant Physiol 136:3814–3823

Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J (2009) The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. Int J Biochem Cell Biol 41:1665–1677

Perriguey J, Sterckeman T, Morel JL (2008) Effect of rhizosphere and plant related factors on the cadmium uptake by maize (*Zea mays* L.). Environ Exp Bot 63:333–341

Persant MW, Nieman K, Salt DE (2001) Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. Plant Biol 98:9995–10000

Peuke AD, Rennenberg H (2005) Phytoremediation: molecular biology, requirements for application, environmental protection, public attention and feasibility. EMBO Rep 6:497–501

Pich A, Scholz G (1996) Translocation of copper and other micronutrients in tomato plants (*Lycopersicon esculentum* Mill.): nicotianamine-stimulated copper transport in the xylem. J Exp Bot 47:41–47

Pich A, Manteuffel R, Hillmer S, Scholz G, Schmidt W (2001) Fe homeostasis in plant cells: does nicotianamine play multiple roles in the regulation of cytoplasmic Fe concentration? Planta 213:967–976

Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE (2000) Reduction and coordination of arsenic in Indian mustard. Plant Physiol 122:1171–1177

Pilon-Smits EAH, Pilon M (2002) Phytoremediation of metals using transgenic plants. Crit Rev Plant Sci 21:439–456

Poynton CY, Huang JWW, Blaylock MJ, Kochian LV, Ellass MP (2004) Mechanisms of arsenic hyperaccumulation in *Pteris* species: root as influx and translocation. Planta 219:1080–1088

Prasad MNV, Freitas H (2003) Metal hyperaccumulation in plants biodiversity prospecting for phytoremediation technology. Elect J Biotechnol 6:275–321

Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees: a review. Environ Int 29:529–540

Raab A, Feldman J, Meharg AA (2004) The nature of arsenic–phytochelatin complexes in *Holcus lanatus* and *Pteris cretica*. Plant Physiol 134:1113–1122

Rajkumar M, Prasad MNV, Freitas H, Ae N (2009) Biotechnological applications of serpentine bacteria for phytoremediation of heavy metals. Crit Rev Biotech 29:120–130

Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30:1562–1574

Ramos I, Esteban E, Lucena JJ, Garate A (2002) Cadmium uptake and subcellular distribution in plants of *Lactuca* sp. Cd–Mn interaction. Plant Sci 162:761–767

Rascioa N, Navari-Izzob F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181

Raskin I, Smith Robert D, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226

Reeves RD (2006) Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N (eds) Phytoremediation of metal-contaminated soils, NATO SCIENCE series: IV: earth and environmental sciences. Springer, New York

Reisinger S, Schiavon M, Terry N, Pilon-Smits EA (2008) Heavy metal tolerance and accumulation in Indian mustard (*Brassica juncea* L.) expressing bacterial gamma-glutamylcysteine synthetase or glutathione synthetase. Int J Hytoremed 10:440–454

Robinson BH, Lombi E, Zhao FG, McGrath SP (2003) Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*. New Phytol 158:279–285

Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272

Salt DE, Prince RC, Baker AJM, Raskin I, Pickering IJ (1999) Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy. Environ Sci Technol 33:713–717

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse A, Marcus MA, Manceau A (2002) Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. Plant Physiol 130:1815–1826

Sas-Nowosielska A, Kucharski R, Pogrzeba M, KrzyZak J, Kuperberg JM, Japenga J (2008) Phytoremediation technologies used to reduce environmental threat posed by metal contaminated soils: theory and reality. In: Barnes I, Kharytonov MM (eds) Simulation and assessment of chemical processes in a multiphase environment. Springer, New York

Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker PM (2002) The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. J Exp Bot 53:2381–2392

Schlautmann A, Morgan JJ (1994) Adsorption of aquatic humic substances on colloidal-size aluminum oxide particles: influence of solution chemistry. Geochim Cosmochim Acta 58:4293–4303

Schmidt U (2003) Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation and leaching of heavy metals. J Environ Qual 32:1939–1954

Semu E, Singh BR, Selmer-Olsen AR (1987) Adsorption of mercury compounds by tropical soils II. Effect of soil: solution ratio, ionic strength, pH, and organic matter. Water Air Soil Pollut 32:1–10

Senden MN, Van der Meer AM, Verburg TG, Wolterbeek HT (1995) Citric acid in tomato plant roots and its effect on cadmium uptake and distribution. Plant Soil 171:333–339

Seregin IV, Shpigu LK, Ivanov VB (2004) Distribution and toxic effects of cadmium and lead on maize roots. Russ J Plant Physiol 51:525–533

Seth CS (2012) A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. Bot Rev 78:32–62

Shah M, Nongkynrih K (2007) Metal hyperaccumulation and bioremediation. Biol Plant 51:618–634

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753

Sharma NC, Gardea-Torresdey JL, Parsons J, Sahi SV (2004a) Chemical speciation and cellular deposition of lead in *Sesbania drummondii*. Environ Toxicol Chem 23:2068–2073

Sharma SS, Kaul S, Metwally A, Goyal KC, Finkemeier I, Dietz KJ (2004b) Cadmium toxicity to barley (*Hordeum vulgare*) as affected by varying Fe nutritional status. Plant Sci 166:1287–1295

Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiol 17:35–52

Sheng X, He L, Wang Q, Ye H, Jiang C (2008a) Effects of inoculation of bio-surfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. J Hazard Mater 155:17–22

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008b) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156:1164–1170

Shojima S, Nishizawa NK, Fushiya S, Nozoe S, Irifune T, Mori S (1990) Biosynthesis of phytosiderophores: in vitro biosynthesis of 2 deoxymugineic acid from L-methionine and nicotianamine. Plant Physiol 93:1497–1503

Siegel FR (2002) Environmental geochemistry of potentially toxic heavy metals. Springer, Heidelberg

Stahl RS, James RR (1991) Zinc sorption by B horizon soils as a function of pH. Soil Sci Soc Am J 55:1592–1597

Steele MC, Pichtel J (1998) Ex-situ remediation of metal contaminated superfund soil using selective extractants. J Environ Eng 124:639–645

Stolt JP, Sneller FEC, Bryngelsson T, Lundborg T, Schat H (2003) Phytochelatin and cadmium accumulation in wheat. Environ Exp Bot 49:21–28

Sun R, Zhou Q, Jin C (2006) Cadmium accumulation in relation to organic acids in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator. Plant Soil 285:125–134

Szecsody E, Zachara JM, Bruckhart PL (1994) Adsorption-dissolution reactions affecting the distribution and stability of CoIIEDTA in iron oxide-coated sand. Environ Sci Technol 28:1706–1716

Takahashi M, Terada Y, Nakai I, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK (2003) Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. Plant Cell 15:1263–1280

Talke IN, Hanikenne M, Krämer U (2006) Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. Plant Physiol 142:148–167

Temminghoff EJM, Van der Zee SEATM, Keizer MG (1994) The influence of pH on the desorption and speciation of copper in a sandy soil. Soil Sci 158:398–408

Temminghoff EJM, Van der Zee SEATM, de Haan FAM (1995) Speciation and calcium competition effects on cadmium sorption by sandy soil at various pHs. Eur J Soil Sci 46:649–655

Temminghoff EJM, Van der Zee SEATM, de Haan FAM (1997) Copper mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved organic matter. Environ Sci Technol 31:1109–1115

Tiller KG, Oliver DP, Mclaughlin MJ, Merry RH, Naidu R (1995) Managing Cd contamination of agricultural land. In: Adriano DC (ed) Biogeochemistry of trace elements—2. Advances in environmental science. Science Reviews Limited, Northwood

Ueno D, Iwashita T, Zhao FJ, Ma JF (2008) Characterization of Cd translocation and identification of Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri*. Plant Cell Physiol 49:540–548

Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M (2007) Heavy metal accumulation in tree growing on contaminated sites in central Europe. Environ Pollut 148:107–114

Uren NC, Reisenaner HM (1998) The role of root exudates in nutrient acquisition. In: Tinker PB, Lanchli A (eds) Advances in plant nutrition. Praeger, New York

van de Mortel JE, Almar Villanueva L, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, Ver Loren van Themaat E, Koornneef M, Aarts MGM (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 142:1127–1147

van der Zaal BJ, Neuteboom LW, Pinas JE, Chardonnens AN, Schat H, Verkleij JAC, Hooykaas PJJ (1999) Overexpression of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiol 119:1047–1056

Vangronsveld J, Van Assche F, Clijsters H (1990) Immobilization of heavy metals in polluted soils by application of a modified alumino-silicate: biological evaluation. In: Barcelo J (ed.) Environmental contamination. In: Proceeding of 4th international conference at Barcelona. CEP Consultants, Edinburgh

Varga A, Zaray G, Fodor F, Cseh E (1997) Study of interaction of iron and lead during their uptake process in wheat roots by total-reflection X-ray fluorescence spectrometry. Spectrochim Acta B 52B:1027–1032

Verbruggen N, Hermans C, Schat H (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776

Vogeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Plant Physiol 92:1086–1093

Wang MY, Glass ADM, Shaff JE, Kochian LV (1994) Ammonium uptake by rice roots. Plant Physiol 104:899–906

Weber M, Harada E, Vess C, von Roepenack-Lahaye E, Clemens S (2004) Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* root identifies nicotinamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. Plant J 37:269–281

Wierzbicka M (1999) Comparison of lead tolerance in *Allium cepa* with other plant species. Environ Pollut 104:41–52

Wong SC, Li XD, Zhang G, Qi SH, Min YH (2002) Heavy metals in agricultural soils of the pearl river delta, south China. Environ Pollut 119:33–44

Wu SC, Wong CC, Shu WS, Khan AG, Wong MH (2011) Mycorrhizo-remediation of lead/zinc mine tailings using vetiver: a field study. Int J Phytorem 13:61–74

Wu G, Kang H, Zhang X, Shao H, Chu L, Ruan C (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 173:1–8

Yang XE, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

Yang Q, Tu S, Wang G, Liao X, Yan X (2012) Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by *Pteris vittata* L. Int J Phytorem 14:89–99

Yazaki K (2006) ABC transporters involved in the transport of plant secondary metabolites. FEBS Lett 580:1183–1191

Yin Y, Allen HE, Li Y, Huang CP, Sanders PF (1996) Adsorption of mercury(II) by soil: effects of pH, chloride, and organic matter. Environ Qual 25:837–844

Zachara JM, Resch CT, Smith SC (1994) Influence of humic substances on Co^{2+} sorption by a subsurface mineral separate and its mineralogic components. Geochim Cosmochim Acta 58:553–566

Zachara JM, Smith SC, Kuzel S (1995a) Adsorption and dissociation of Co-EDTA complexes in iron oxide-containing subsurface sands. Geochim Cosmochim Acta 59:4825–4844

Zachara JM, Gassmann PL, Smith SC, Taylor D (1995b) Oxidation and adsorption of Co(II)EDTA2-complexes in subsurface materials with iron and manganese oxide grain coatings eochim. Cosmochim Acta 59:4449–4463

Zayed AM, Terry N (2003) Chromium in the environment: factors affecting biological remediation. Plant Soil 249:139–156

Zhao FJ, Hamon RE, Lombi E, McLaughlin MJ, McGrath SP (2002) Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 53:535–543

Zenk MH (1996) Heavy metal detoxification in higher plants—a review. Gene 179:21–30

Zhu YL, Pilon-Smits EAH, Tarun AS, Weber SU, Jouanin L, Terry N (1999) Cd tolerance and accumulation in Indian mustard is enhanced by overexpressing γ-glutamylcysteine synthetase. Plant Physiol 121:1169–1177