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Review

MOLECULAR RESPONSES OF PLANTS TO ENVIRONMENTAL HEAVY METAL CONTAMINATION: LEAD AND THE USE OF SUNFLOWER IN PHYTOREMEDIATION

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Summary: Plants are susceptible to heavy metal toxicity and in order to avoid its detrimental effects they respond in a variety of ways. The toxic effect depends on the type of metal, ion concentration, plant species, and stage of plant growth. The present review describes recent advances in understanding the genetic and molecular basis of heavy metal tolerance in plants with special reference to heavy metal accumulating plants. The specifics of Pb tolerance, uptake and accumulation in plants are described. The phytoextraction of Pb by *Helianthus annuus* and other accumulating species is compared. Some approaches to improve the phytoextraction capability of sunflower plants are discussed.

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1. Introduction

The chemical composition of plants usually reflects the chemical composition of the soil they grow in. In some plants however, the contents of some trace elements far exceeds their concentration in the soil. Such plants are termed hyperaccumulators. For the various elements there are different thresholds above which the plant is regarded as a hyperaccumulator. For Co, Cu, Cr, Pb and Ni the contents should be more than 1 mg/g dry weight and for Mn and Zn – more than 10 mg/g dry weight (Baker and Brooks, 1989). A general feature of the hyperaccumulating plants is that they grow in areas with mineralized soil. They are extremely tolerant to the metal in the soil, which for other plants might be at phytotoxic concentrations. Such soils may mirror the geochemistry of the region or may result from human activity. These heavily polluted areas are often populated by endemic hyperaccumulators, specific for the region. Mostly these plants are "strict metallophytes" as they are restricted to soils rich in heavy metals. Some hyperaccumulators however are "facultative metallophytes" as they may populate equally well contaminated and

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uncontaminated soils (Pollard et al., 2002).

Tolerance to environmental contaminants has been evolved in a number of plant species. Tolerant plants are often excluders, limiting the entry, and in particular root-to-shoot translocation of pollutants. The hyperaccumulators, which combine extremely high tolerance with the accumulation of the toxic metal in overground parts represent a small number among all tolerant plants (for reviews see Verbruggen et al., 2009; Rascio and Navari-Izzo, 2011).

The question why hyperaccumulation of heavy metals occurs and how it evolved is difficult to answer. Two main hypotheses are proposed and they do not necessarily exclude each other. One is that hypeaccumulation increases the tolerance to metals and in heavy metal rich environment gives the plant selective advantage. The other is that the accumulated toxic metals serve as a defense against pathogens and herbivores (Pollard, 2000). It should be noted however that studies show that the genes involved in accumulation and tolerance are not species-specific or novel, but are rather non-accumulator genes, only differently expressed and regulated (Rascio and Navari-Izzo, 2011).

Recently, the accumulation of heavy metals by plants causes an increasing interest due to the possibility of plants to be used for phytoremediation. Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. Plants can be used for pollutant stabilization by limiting the mobility and bioavailability of pollutants in soils. May be most promising is the process of phytoextraction. The hyperaccumulator is planted in the polluted soil, the overground plant parts are harvested and after few generations the pollutants are removed from the soil. If the concentration of the heavy metal is high, the harvested material may be reused (phytomining). Rhizofiltration is the use of water plant roots to remove pollutants from waters. For organic xenobiotics phytodegradation or phytovolatilization is possible. For some metals (Hg) phytovolatilization (removal in the air) is also possible (Pilon-Smits and Pilon, 2002; Sarma, 2011; Ali et al., 2013; Paz-Ferreiro et al., 2014).

Phytoextraction is based on the use of pollutant-accumulating plants for removal of pollutants from soil by concentrating them in the harvestable parts (Salt et al., 1998; Vassilev et al., 2004). It should be noted that two types of phytoaccumulation are feasible. One is the so called natural phytoextraction: the plant takes up the substances from the soil unassisted. The other is the assisted (induced) phytoextraction: a helator solution or other agent is introduced in the soil, so metal solubility is increased, and plants can absorb the harmful agents more easily.

In the following section we describe recent advances in understanding the genetic and molecular basis of metal tolerance in plants. Special attention is paid to heavy metal accumulators and to the genes involved in tolerance, detoxification and accumulation.

2. Molecular and genetic requirements for phytoextraction

The ideal plant for phytoextraction should grow rapidly, produce a high amount of biomass, and be able to tolerate and accumulate high concentrations of metals (Vassilev et al., 2004; Pilon-Smits, 2005). To date, more than 500 species (0.2% of angiosperms) known as metal hyperaccumulators belonging to 101 plant families have been identified (Kramer, 2010). Physiological studies have paved the way for understanding metal accumulation mechanisms, including enhanced metal uptake, increased xylem loading and increased detoxification in the shoot. Molecular and genetic analyses were performed primarily on *Arabidopsis* species, Brassicaceae, as *Arabidopsis thaliana* is a model plant with its complete genome sequenced. Much research was performed also on *Thlaspi caerulescens*, Brassicaceae, facultative metallophyte, hyperaccumulator of several heavy metals (Milner and Kochian, 2008). Comparative studies on hyperaccumulators and related non-accumulating non-tolerant species have identified a large array of genes that are constitutively expressed (that is, in the absence of excess of metal ions) (Weber et al., 2006; Talke et al., 2006). These molecular insights for the moment are restricted to species related to *A. thaliana*, but the ever-growing availability of full-genome sequences will allow the inclusion of species from other plant families. By now it is obvious that phytoextraction of heavy metals from soil and water can take place only if the metal accumulator plants and the metals themselves possess some basic properties.

2.1. Permeability of the root cell membranes to heavy metal ions

2.1.1. The metal should be in a dissolved state in order to be available to plant roots. Heavy metal accessibility and mobility in the root rhizosphere is dependent both on root exudates and the coexisting symbiotic microorganisms. The essential role of the roots has been illustrated by many experiments. In the hyperaccumulator *Thlaspi caerulescens* roots in a heterogeneous soil environment do not grow equally well in all directions. Their growth is more intense in patches rich in zinc (ZnO) or cadmium (CdS). This ensures maximal metal phytoextraction by the roots; as a consequence the shoots contain more poisonous metals. It seems that at least some plants have acquired phytoextraction abilities as a result of the selective advantage of metal accumulation, e.g. as a defense against herbivores (Whiting et al., 2000).

Rhizobacterial populations are known to affect heavy metals mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, and redox changes (Abou-Shanab et al., 2003).

2.1.2. The nature of the heavy metal is important as the plant to some extent might consider the metal as an essential trace element. The question whether the plants are genetically able to recognize different heavy metals has no single answer. It is reported that the accumulation of certain metals, such as Zn, Fe, and Cd by *Thlaspi caerulescens* is partially regulated by common genetic determinants. However, research on the hyperaccumulator *Arabidopsis halleri* indicates the existence of different determinants responsible for the accumulation of Zn and the accumulation of Cd. The determinants in the expression of tolerance to Zn and accumulation of Zn also appear to be different (Verbruggen et al., 2009). Special features in the case of Pb phytoextraction are discussed in section 3.

2.1.3. The root cell membranes must be permeable to heavy metal ion or to metal chelates

A number of authors have shown that the metal present in root epidermal cells enhances the expression of genes encoding the proteins of the family of ZIP (zinc/iron - regulated transporter proteins). It is known that these proteins form a transmembrane ionic channel that transports Zn^{2+} and Fe^{2+} into the cytoplasm (Milner et al., 2013). Microarray (cDNA-chip) analyses in hyperaccumulators show increased expression of the most prominent members of the ZIP family (Weber et al., 2004). However, their role in the accumulation of other metal ions (e.g. Pb^{2+}) is to be established.

2.2. The plant should be able to translocate metals between organs, tissue cells and organelles, accomplishing transmembrane metal transport

Efficient translocation of metal ions to the shoot requires a transmembrane passage and active loading into the xylem (Verbruggen et al., 2009). The need of ligands for all trace metals to be present into the xylem is controversial. Research showed that most metals in the xylem sap of the hyperaccumulators *T. caerulescens* (Salt et al. 1999) and *A. halleri* (Ueno et al., 2008) were present mainly in a free hydrated ionic form. Availability of trace metals for translocation to the shoot implies limited sequestration in the vacuoles of root cells (Fig. 1). Several authors observed much lower accumulation of heavy metals in root vacuoles, as well as a faster release of these metals from the root vacuoles in the hyperaccumulating ecotype compared with the nonaccumulating ecotype (Lasat et al., 1996, 1998; Yang et al., 2006a,b; Xing et al., 2008). Several types of transporters are involved in this translocation.

2.2.1. P-type ATPase-HMA (Heavy Metal transporting ATPases)

ATPases are a large group of enzymatic proteins that carry the transmembrane transport of metal ions against their electrochemical gradient, using the energy of ATP hydrolysis to ADP. HMAs cluster into two subclasses: those transporting monovalent cations (Cu/Ag group) and those transporting divalent cations (Zn/Co/Cd/Pb) (Arguello et al., 2007). *HMA4* was the first gene encoding a plant P1B-type ATPase of the divalent transport group to be cloned and characterized in *A. thaliana* (Mills et al., 2003), and later in *Thlaspi caerulescens* (Papoyan and Kochian, 2004) . Comparative molecular studies in accumulators and hyperaccumulators showed that these genes were expressed in cells of stelar parenchyma (Fig. 1), surrounding the vascular tissues (xylem and phloem), where HMAs are involved in the metal xylem loading (detoxification of roots), and translocation of these metals from root to shoot (Clemens, 2006; van de Mortel et al., 2006; Talke et al., 2006). The central role of HMAs in tolerance, translocation and accumulation of metals in plants makes it the main but not the only candidate for being a major determinant of „heavy metals accumulation" phenotype. The accumulating plants with a lowered expression of HMA4 translocated less metal from root to shoot (metal accumulation was shown in the pericycle

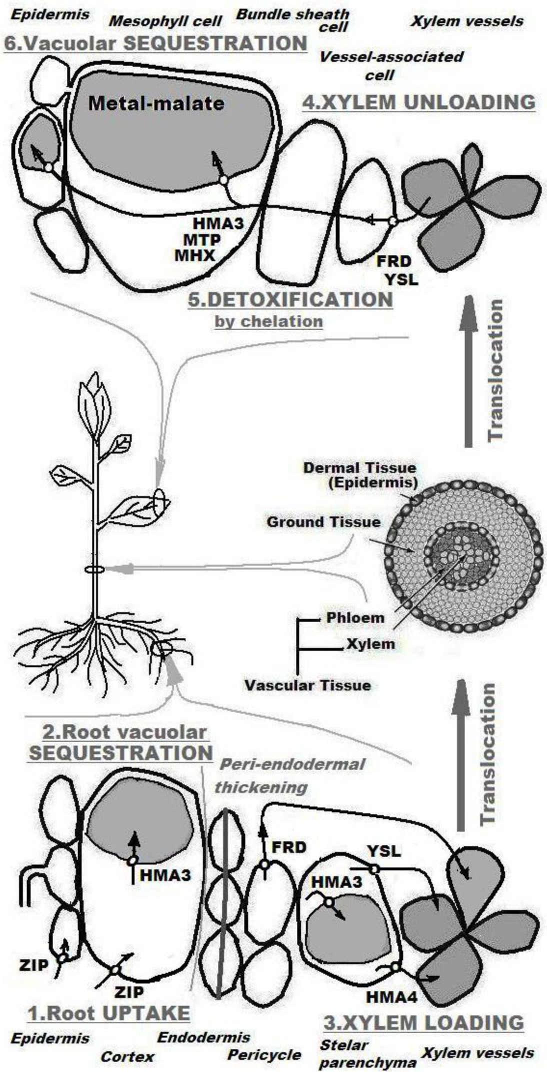

Figure 1. Schematic overview of the mechanisms employed to accumulate heavy metals. 1.) Transport systems for metals in roots and root hairs require the activity of members the Zinc/Iron regulated metal transporter Proteins (ZIP family). 2.) Xylem loading takes place when sequestration into the vacuoles of the root cortex is minimal. For some heavy metals (Pb) vacuolar sequestration in cortex and/or stelar parenchyma root cells takes place through the activity of the Heavy Metal transporting ATPase HMA3. 3.) Active xylem loading depends on reduced vacuolar root sequestration and enhanced activity of HMA4. Important part is played by FRD and YSL – proteins active in xylem loading/unloading of metal–ligand complexes. 4.) In leaves metal is unloaded from xylem into vessel-associated cells by YSL and FRD proteins. 5.) Detoxification in the cytoplasm is operated by chelation of the heavy metals. Possible ligands are histidine and nicotianamine. 6.) Vacuolar sequestration is the main pathway of detoxification of most heavy metals, as they are stored mainly in vacuoles of mesophyll and/or epidermal cells through the activity of MTP (Metal Transporter Proteins), HMA3 and possibly MHX (Metal2+/H+ eXchanger). In the vacuoles large pool of organic acids (citrate, malate) favors the formation of metal-malate/citrate complexes.

cells, Fig. 1) and were more sensitive to metal treatments. Interestingly, when expressed in nonaccumulating plants under the activity of its own promoter, HMA4 increased metal sensitivity because of the absence of a detoxification mechanism with sufficient capacity to cope with the strongly enhanced metal accumulation in the leaves (Hanikenne et al., 2008).

Another heavy metal ATPase, HMA3 is localized to the vacuolar membrane and plays a role in detoxifying metals through vacuolar sequestration to roots and shoots (Fig. 1) (van de Mortel et al., 2006; Takahashi et al., 2012).

Transcriptomic studies in hyperaccumulators have also revealed a higher expression of genes encoding metal ligands or metal-ligand complex transporters. Some of these transporters also seem to play a role in trace metal translocation.

2.2.2. MATE (Multi-drug And Toxic compound Extrusion or Efflux membrane proteins)

MATE is a large family of transmembrane proteins, responsible for extrusion (discharge) of many drugs and toxic organic compounds through the membranes. Some members of the family were shown to function as drug/cation antiporters, removing toxic compounds and secondary metabolites from the cytosol by exporting them out from the cell or isolating them by accumulation into vacuoles as citrates (Delhaize et al ., 2007). Some authors have shown that overexpressed gene FRD3 in *T. caerulescens* and *A. halleri* (compared to its low expression in *A. thaliana*) is responsible for translocation of metals and their accumulation as citrates in the vascular tissue of roots (Durrett et al., 2007; van de Mortel et al., 2006; Talke et al., 2006).

2.2.3. OPT (OligoPeptide Transporters)

OPT is a superfamily of oligopeptide transporters including the Yellow - Stripe 1 - Like (YSL) subfamily. Some YSL transporters are involved in the loading and unloading of nicotianamine-metal chelates from the vascular tissues (Fig. 1). There is evidence for a role of YSL transporters in metal hyperaccumulation of *T. caerulescens*, especially for *TcYSL3* and *TcYSL7*, which are expressed in xylem parenchyma and phloem (Fig. 1) (Gendre et al., 2007; Haydon and Cobbett, 2007). Furthermore, TcYSL3 was shown to transport nickel - nicotianamine chelates (Gendre et al., 2007).

2.2.4. CDF (Cation Diffusion Facilitators in plants, also called Metal Transporter Proteins (MTPs)

This family contains members involved in vacuolar sequestration of metals (Van der Zaal et al., 1999; Migocka et al., 2014). In hyperaccumulators *MTPs* are highly expressed in both roots and shoots and may also play a role in vacuolar sequestration (Assuncao et al., 2001; Persans et al., 2001). However, a more recent study revealed that MTP1 seems to be localized at the plasma membrane, where it could mediate metal efflux from the cytoplasm (Kim et al., 2004). Other CDF members (MPT11) were proposed to be involved in the metal transport not only from cytoplasm to organelles or apoplasm, but also from the cytoplasm to the endoplasmic reticulum (Golgi). This ends up with exocytosis and removal of the metal from the cell (Peiter et al., 2007).

2.2.5. CaCA (Ca^{2+}/Cation Antiporter superfamily)

In the Ca^{2+}/Cation Antiporter superfamily, MHX is a vacuolar $Metal^{2+}$/H+ eXchanger (Shaul et al., 1999). The MHX protein is present in the leaves of *A. halleri* at much higher concentrations than in *A. thaliana* and was therefore proposed to play a role in metal vacuolar storage. Since transcript levels were similar in both species, a difference in post-transcriptional regulation was postulated (Elbaz et al., 2006).

Members of other CaCA subfamilies may also play a role in metal detoxification. CAX is the acronym for CAtion eXchanger (Shigaki and Hirschi, 2006). There is experimental evidence that cation/proton antiporters can transport metals into the vacuoles (Korenkov et al., 2007). Some of the *CAX* homologs are highly expressed or differentially expressed in response to metals in hyperaccumulators (Becher et al., 2004; Weber et al., 2006; Craciun et al., 2006; van de Mortel et al., 2006).

2.2.6. ABC (ATP-Binding Cassette)

The superfamily of ABC transporters is involved in many physiological processes. In higher plants ABC proteins constitute a large family grouped phylogenetically into eight clusters, subfamilies from ABCA to ABCI (ABCH is not found in plants). ABC transporters shuttle substrates as diverse as lipids, phytohormones, carboxylates, heavy metals (ABCC for the most part), chlorophyll catabolites and xenobiotic conjugates across a variety of biological membranes (Kretzschmar et al., 2011). Some ABC members are involved in the transport of chelated heavy metals or organic acids, which are required for the transport of heavy metals and metalloids. There is strong evidence for the role of the ABC transporters in trace metal homeostasis (Song et al., 2003; Hanikenne et al., 2005; Kim et al., 2006; Mendoza-Cozatl et al., 2010) and they may be expected to contribute to metal and metalloid hyperaccumulation, and in particular to vacuolar sequestration (Song et al., 2010; Briat, 2010; LeBlanc et al., 2013).

2.3. The plant should prevent any damage caused by metal transfer, accumulation or storage.

All this implies effective chelation. Chelation is the process of metal complexation with organic compounds (ligands). It is assumed that in the plant most metals are associated with ligands, such as organic acids, amino acids, peptides and proteins. The result is the elimination of the toxic effects of the metal and facilitating its mobility within the plant. The metal-chelator transport also enhances the capacity for metal storage in root and leaf vacuoles. Transcriptomic studies have also revealed an expression of genes encoding enzymes, which synthesize metal ligands as well as transporters of metal-ligand complexes (transporters were reviewed in the previous sections). Therefore, the plant should be able to chelate metals. Very often chelation of metals is observed in the rhizosphere before absorption by the roots. Some molecular genetic aspects of metal detoxification by chelating ligands have been clarified. However, it is not fully explained how the various chelating agents are involved in the various stages of metal transport or metal storage in the plant. Furthermore, the nature of the metal-chelators in a plant species may differ, depending on plant location and plant age (Salt et al., 1999).

2.3.1. Histidine

Histidine is considered as the most important free amino acid involved in the accumulation of metals (Callahan et al., 2006). It forms a stable complex with metals. In the roots and the xylem of hyperaccumulators the concentration of histidine is higher than in non-hyperaccumulators. Histidine, added exogenously to nonhyperaccumulators increases the metal tolerance and the concentration of histidine-metal complexes in the xylem. In agreement with this observation, the metal treatment of hyperaccumulators leads to stronger expression of ATP - phosphoribosyltransferase (the first enzyme of histidine biosynthetic pathway) compared to nonhyperaccumulators (Ingle et al., 2005). However, transgenic lines of *Arabidopsis thaliana* overproducing histidine displayed elevated metal tolerance and increased free histidine pool, but did not exhibit increased metal concentrations in the xylem sap or in the leaves (Wycisk et al., 2004). This observation suggests that additional factors are required for the metal xylem loading, translocation to vegetal organs and intracellular compartments.

2.3.2. Nicotianamine

The synthesis of nicotianamine from S-adenosylmethionine by the enzyme nicotianamine synthase is observed in all plants. Nicotianamine forms stable complexes with most metal ions, so its role seems to be in the transportation of complexed trace elements throughout the plant (Stephan and Scholz, 1993). In transgenic, nontolerant species it has been shown that overexpression of nicotianamine synthase results in improved metal accumulation and increased metal tolerance (Douchkov et al., 2005; Kim et al., 2005; Pianelli et al., 2005). However, more recent results obtained with the hyperaccumulator *Thlaspi* support participation of nicotianamine in hyperaccumulation of Ni. In spite of that increased tolerance to Zn was not observed (Callahan et al., 2007).

2.3.3. Metallothioneins

Metallothioneins are a family of cysteine-rich proteins with relatively low-molecular weight (500 to 15 000 Da). They exhibit metal affinity, binding their cysteine residues to the physiologically essential Zn, Cu, Se and others, as well as to xenobiotics such as Cd, Hg, Ag, and As (Krezel and Maret, 2007). Induced expression of metallothionein genes is higher in hyperaccumulating species, depending on the parameters of metal exposure. These proteins appear to be involved in both transport and accumulation of metals, as well as in the control of oxidative stress exerted by binding harmful oxidant radicals to their cysteine amino acid residues (Guo et al., 2008).

2.3.4. Organic acids (citrate, malate)

Because of the low association constants of organic acids with metals, Callahan et al. (2006) argued against a role of organic acids in the hyperaccumulation mechanism, in spite of their constitutively elevated concentrations in hyperaccumulators (Montargès-Pelletier et al., 2008). Thus, their role may be limited to vacuolar sequestration, where formation of metal-organic acid complexes is favored by the acidic environment of the vacuole (Haydon and Cobbett, 2007).

2.3.5. Glutathione (GSH)

Glutathione is a tripeptide (Glu-Cys-Gly), which is a major cellular antioxidant. It can form complexes with several metals and is the precursor of phytochelatins. Increased glutathione synthesis observed in hyperaccumulators is driven by constitutive activation of the sulfur assimilation pathway, in particular by the enhanced activity of mitochondrial serine acetyltransferase (SATm). This increases foliar and root GSH concentrations, which appears to provide protection against the oxidative damage by the metal (Freeman et al., 2004).

2.3.6. Phytochelatins (PCs)

Phytochelatins, general formula (Glu-Cys) $_n$Gly, where n = 2-11, are synthesized enzymatically from glutathione in the presence of certain metals and metalloids and are ubiquitous in plants (Clemens, 2006). Although PCs do have a role in basal metal detoxification, they do not seem to be involved in each metal hypertolerance (Ebbs et al., 2002; Schat et al., 2002; Hernandez-Allica et al., 2006). In hyperaccumulators, just as in nonhyperaccumulators, PCs are mainly induced in the roots, as their formation is catalysed by constitutively present phytochelatin synthase (PCS) (EC 2.3.2.15) in the presence of metal excess (Vatamaniuk et al., 2000). Metal ions can form complexes with PCs, and then be transported from the cytosol into the vacuoles with the help of ATP-binding cassette-type transporters (Salt and Rauser, 1995; Sun et al., 2007).

In some species increased expression of phytochelatin synthase (AtPCS1) leads to depletion of the main cellular antioxidant glutathione; this affects the balance between the biosynthesis of metal-phytochelatin complexes and their transport to vacuoles (or degradation). As a consequence, the sensitivity to metals is increased and their accumulation is in shoots, rather than in roots (Lee et al., 2003; Wojas et al., 2008; Wang et al., 2012). Metal, which is normally a very effective inducer of PC synthesis in other species, induces only minor PC concentrations in the roots of the hyperaccumulating *Pteris vittata*, where this function is performed primarily by glutathione (Zhao et al., 2003). These results suggest that PCs are not essential for the hyperaccumulation phenotype. However, recently it was reported that overexpression of AtPCS2 resulted in constitutive phytochelatin accumulation (even in the absence of metal excess). This resulted in partial rescue of the metal hypersensitivity in case of AtPCS1- deficiency and contributed to metal accumulation (Kühnlenz et al., 2014).

2.4. Strategies against oxidative stress

It is well documented that environmental contaminants such as metals Mn, Cu, Pb, Co, Cd; metalloids (As); nonmetals (Se) and alike, lead to the overproduction of reactive oxygen species (ROS) such as superoxide radicals (radical O^{2-}) and hydrogen peroxide (H_2O_2) in plant cells. These may cause unspecific oxidation of proteins and membrane lipids or DNA injury. To protect cells and tissues from dysfunction, plants have developed various strategies (Gill and

Tuteja, 2010). These strategies include the over expression of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), and glutathione-S- transferase (GST). In addition, non-enzymatic antioxidants with low molecular weight are synthesized, such as proline, cysteine, ascorbic acid, glutathione, which can reduce oxidative stress by scavenging ROS. Responses to metal toxicity involving these enzymes and non-enzymatic antioxidants depend on the plant species, type of tissue, and metal.

3. Phytoextraction of lead

Lead (Pb) has no biological function and it is toxic to living organisms even at low concentrations. In plants Pb uptake and translocation occurs, causing toxic effects resulting in a decrease of biomass production. It is well documented that most of Pb taken up by plants is restricted to roots and very small amount is transported to shoots. However, plant leaves show obvious symptoms of Pb toxicity through reduction in their number, size (leaf length and width), fresh and dry weights, thickness of leaf blade, cuticle, spongy and palisade mesophyll cells, changes in shape, number and size of stomata, as well as changes in photosynthetic pigments (Islam et al., 2007, 2008). At a cellular level, Pb induces accumulation of reactive oxygen species (ROS), as a result of imbalanced ROS production and ROS scavenging processes by imposing oxidative stress. Lead toxicity affects various processes in plants, such as germination and growth of plants, mineral nutrition, photosynthesis and respiration as well as cell division (Pourrut et al., 2011).

To limit the detrimental impact of Pb on plants, efficient strategies like phytoremediation are required (Gupta et al., 2013). But compared to Zn and Cd, very little is known about the molecular mechanisms of acquisition, transport, and accumulation of Pb. This is due, on the one hand, to the characteristics of the Pb ions, which precipitate with some components of the culture media, thus making it difficult to study its bioavailability to plant roots. On the other hand, there is no model plant for studying the mechanisms of tolerance to this metal. Among the 500 species known as metal hyperaccumulator and hypertolerant plants, there are few reliable reports on Pb hyperaccumulating species under natural conditions. The claims are doubtful as the methods employed rarely take into account the possibility of external contamination of stem and leaves by Pb-rich dust (van der Ent et al., 2013). Although Pb is not an essential element, some plant species proliferate in Pb-contaminated area and accumulate it in different parts. They have developed a wide range of tolerance mechanisms that are activated in response to Pb exposure. The defense mechanisms may include the following steps: the path to the roots may be blocked by physical barriers (callose, Fe/Mn plaques, cell wall). At high concentrations this barrier is not efficient and the flux of Pb ions enters the cell through the plasma membrane using Ca ion transporters. In cytoplasm Pb is chelated with PCs. The complex formed is then sequestered in the vacuoles. In hyperaccumulator plants, Pb can be transported via the apoplast to aerial parts (Fig. 1).

3.1. Pb availability and defense against lead stress

Presumably the phytoavailability of Pb is restricted by the strong complexes of Pb within solid soil fractions, but it is affected by the presence of other heavy metals (Fahr et al., 2013). The authors reported that Pb availability was reduced when it was supplied in combination with five heavy metals: Cd, Zn, Cr, Cu, and Ni. When Pb was supplied alone or in ternary combination (with Zn and Cu), its availability increased due to the antagonistic interaction between Cu and Zn, which made Pb more available for plant uptake.

Rhizosphere organisms also affect metals availability. Some rhizobacteria can exude a number of substances, such as antibiotics (including the antifungals), phosphate solubilizers, organic acids, iron-chelating agents, called siderophores, which increase bioavailability and facilitate root absorption of heavy metals (Salt et al., 1995; Fahr, 2013). Abou-Shanab et al. (2005) investigated the correlation between Pb-metal resistance and metal mobilization abilities of rhizobacteria under lead stress. The highest incidence of the biochemical activity of isolates and lead resistance was recorded for phosphate solubilizers - 68.2%, siderophore producers - 61.6%, and acid producers - 42.9%. This implies that phosphate solubilization is not only the mechanism adopted by bacteria towards Pb in soil, but that siderophores and acid production are also involved in mobilizing Pb.

Symbiotic fungi, the arbuscular mycorryza in particular, can directly influence Pb solubility by changing the Pb form in the rhizosphere. In the rhizosphere, in comparison to bulk soil, the amount of Pb in complex with organic substances increased. In contrast, carbonate and Fe-Mn oxides of Pb did not exhibit significant changes. These results indicate that mycorrhiza could protect its host plants from the phytotoxicity of excessive Pb by changing its form from bioavailable to non-bioavailable form. The fact that Pb accumulation in mycorrhiza infected plants was significantly lower than in non-infected plants also suggests that mycorrhiza efficiently restricts excessive Pb absorption into the host plants (Huang et al., 2005).

At Pb exposure non-tolerant plant roots rapidly respond by synthesis and deposition of callose between the plasma membrane and the cell wall (Samardakiewicz et al., 2012). After callose deposition the Pb movement is restricted. Under metal stress the synthesized callose inhibits cell-to-cell transport. This may result in the prevention of a wide incursion of Pb ions, but it may simultaneously inhibit the transport of other molecules. It seems that the formation of callose is closely related to the amount of Pb entering the cell, and subsequently the level of stress.

On root surfaces of some aquatic plants Fe precipitates (plaques) are regularly formed. These Fe-plaques may provide means of attenuation and exclusion of metals. The plaques increase the sequestration of Pb on the root surface and in the rhizosphere, providing means of exclusion of Pb and other metals (Hansel et al., 2002). In some rice cultivars Fe/Mn plaques were shown to affect the patterns of Pb uptake and accumulation. Lower concentrations of Pb were found in the root tissues of rice plants with plaques. However, the capacity of the plaques is limited as they are only efficient in relatively low or moderately Pb-contaminated soil (Liu et al., 2011). Similarly, Fe plaque and organic matrix with high Pb affinity were found in root epidermis of *Typha latifolia*. It was shown that the plaques prevent accumulation and translocation of Pb into the root (Qian et al., 2012; Feng et al., 2013).

3.2. Phytoextraction of Pb by tolerant accumulator plants

It is well established that in tolerant plants, 90% of total Pb is accumulated in roots (Kumar et al., 1995). Most Pb in roots is localized in the insoluble fraction of root cell walls, which are the first barrier against Pb. Cell walls of the roots of several species can immobilize and accumulate some or even most Pb ions. The capacity of cell walls to bind divalent metal cations mainly depends on the amount of polysaccharides containing many carboxyl groups. Recent research on the edible roots of *Raphanus sativus* (radish) has demonstrated that Pb^{2+} binds to carboxyl groups of pectin in the cell walls (Inoue et al., 2013). This indicates that the cell wall is one of the preferred and essential compartments for Pb accumulation, deposition, and sequestration.

Lead is a non-essential element, thus no specialized transport systems for Pb exist. However, Pb ions are likely to enter plant cells via the existing calcium transporters (Sunkar et al., 2000). As soon as excessive Pb ions enter the cytoplasm, an additional defense mechanism is activated, protecting cells against Pb toxicity. Electron microscopy and cytochemistry experiments with the bulbous plant *Allium sativum* (garlic) showed that in roots cysteine-rich phytochelatins (PCs) were detected 1- 2 h after Pb exposure (Jiang and Liu, 2010). This indicates that Pb ions induce the synthesis of PCs. Morphological alterations and appearance of vesicules indicate that the Pb-PC complexes move into vacuoles. Similarly, Piechalak et al. (2002) demonstrated that the synthesis of PCs takes place under the influence of Pb ions in root cells of *Phaseolus vulgaris*. The complex PC–Pb formed is then transported through the cytosol into the vacuoles. The vacuole is ultimately one of the main storage sites for metal sequestration (reviewed by Sharma and Dubey, 2005; Clemens, 2006; Fahr et al., 2013). *Brassica juncea* appears to tolerate high concentrations of Pb due to its efficient cell roots vacuolar storage mechanisms. In this species, Pb sequestration was restricted to root vacuoles. Moreover, it appears that exposure to Pb causes the production of additional vacuole specifically for Pb storage in the root tips of *B. juncea* (Meyers et al., 2008).

The transport into vacuoles was also extensively investigated. *AtHMA3*, encoding a P1B−2 ATPase, a heavy metal transporter, is localized in the vacuolar membrane of roots cells in *Arabidopsis* (Talke et al., 2006; Morel et al., 2009). This transporter is involved in the transfer of PC–Pb complexed metal from the cytoplasm to the vacuole (Morel et al., 2009).

Elsholtzia argyi (Lamiaceae) is a plant species from South China, which exists in two ecotypes. One ecotype is tolerant to Cu and Pb and is spread widely in the ancient Pb/Cu-mining areas (mining site ecotype - ME).The other ecotype grows on non-contaminated agricultural soils (non-mining site ecotype – NME) (Islam et al., 2008). In hydroponics with elevated Pb concentration in both ecotypes Pb concentration decreases sharply in the order root > stem > leaf. Results showed somewhat increased uptake of Pb by roots of NME plants. However, the ME plants have extraordinary capacity to transport Pb from roots to shoots. The total Pb uptake of NME plants is the same or higher, but in their shoots and leaves Pb is less. Nevertheless, these plants (NME) fail to grow longer at higher concentrations of Pb, while ME grows well at all Pb concentrations in the nutrient solution (Islam et al., 2008). In the ME ecotype of *Elsholtzia argyi*, Pb is found in fine particles dispersed throughout root cell membranes and cell wall fractions, whereas in NME ecotype roots most Pb was found as large aggregates deposited in the cell wall fractions. These differences in localization explain why the NME roots were not able to transfer Pb to above ground parts via the apoplast (Islam et al., 2007). The authors suggest that the increased tolerance to Pb of the ME ecotype is due to the better preservation of their membranes, in particular the membranes of chloroplasts.

3.3. Phytoextraction of Pb by hyperaccumulator plants

The native Chinese species *Sedum alfredii* is a Cd/Zn cohyperaccumulator (Yang et al., 2004), which also exhibits an extraordinary tolerance and considerable accumulation ability for Pb (He et al., 2002). Previous studies (Yang et al., 2006a, b) on Zn and Cd indicated that this plant possessed capacity to translocate metals from roots to shoots. However, investigations have indicated a very low mobility of Pb out of vascular bundles and Pb is largely retained in root cell walls (Liu et al., 2007; Tian et al., 2011). Similar results were obtained (Manousaki and Kalogerakis, 2009) for another hyperaccumulator of Cd - *Atriplex halimus*, in which, after combined treatment (Pb/Cd), Pb accumulation was observed mainly in roots whereas Cd accumulation – in shoots. These results indicate that hyperaccumulators of Zn and Cd have difficulty to uptake, translocate and accumulate Pb in shoots.

Phytoremediation however, would not be practical in most cases if the bulk of Pb is trapped in roots. Thus, attempts were made to assist Pb hyperacumulation in shoots by applying helators to enhance its bioavailability and transport above the ground. To achieve this Liu et al. (2007) used combined treatment of *Sedum alfredii* hydroponics with EDTA and IAA (auxin) to change the distribution of Pb in the plant. Treatment of hyperaccumulator ecotype with Pb and EDTA (0.2 mM each) increased Pb concentration in shoots. IAA (0.1 mM) additionally increased Pb in shoots two-fold. Further studies of Tian et al. (2011) showed howeve, that the efficiency of Pb uptake by *Sedum alfredii* was less when Pb was provided in Pb-EDTA form. In this case Pb is also preferentially localized in the vascular bundles. These studies show that Pb-EDTA chelates facilitate normal development of plants, but significantly decrease the amount of accumulated Pb.

Recently, Auguy et al. (2013) identified *Hirschfeldia incana*, a member of the Brassicaceae family, close relative of *Arabidopsis*, as a Pb accumulator plant. The authors demonstrated that in this species high amount of Pb was accumulated in roots. Some Pb (3%) was translocated from roots to the leaves and even to the siliques (see Table 1). The large differences of Pb concentration between root and leaves indicated the existence of an important transport restriction. However, this species appears to possess all mechanisms necessary to translocate Pb from the roots to the aerial parts and to accumulate Pb in different organs. Gene expression profiles in *H. incana* showed a significant Pb-induced up-regulation of *HMA4* (coding ATP-binding cassette transporter) and *MT2a* (coding metallothionein) in roots and aerial parts. Apparently the observed restriction of Pb translocation towards aerial parts (compared to other heavy metals) was not due to decreased expression of *HMA4*, but rather to less specific loading of this transmembrane transporter with Pb.

To sum up, the bioavailable form of Pb affects primarily the root system of plants (Figure 1). Plant roots rapidly respond either: 1/ in non-tolerant plants by the synthesis and deposition of callose, creating a barrier that stops Pb; 2/ in tolerant accumulator plants by the uptake of large amounts of Pb and its sequestration in the vacuole, which is accompanied by changes in root growth and branching pattern; 3/ in hyperaccumulators plants by translocation of an insignificant quantity to the above ground parts (Fahr et al., 2013). In general, plants may prevent the toxic effect of Pb by induction of higher levels of metal chelators - metallothioneins and phytochelatins and the increase of other organic (citrates) and inorganic (sulphides) complexes. Phytochelatin binds to Pb ions leading to sequestration of Pb ions in root and leaf vacuoles and thus serves as an important component of the Pb detoxification mechanism in plants (Gupta et al., 2013).

4. Phytoextraction of lead by sunflower (*Helianthus*).

Cultivated sunflower (*Helianthus annuus* L., family *Asteraceae*) is a high biomass-producing oil crop, presently not classified as a hyperaccumulator. It was reported however, that *Helianthus* can be successfully used to clean contaminated waters (Dushenkov et al, 1995). The possibility of hyperaccumulation of multiple metals was also investigated in hydroponic cultures (January et al., 2008). It was shown that common sunflower can accumulate significant amounts of Pb from contaminated soil, particularly when assisted by EDTA and phytohormones (Sinegani and Khalilikhah, 2008). It was also demonstrated that the use of N-fertilizers enhanced markedly the accumulation of Pb in sunflower roots and shoots (Rahman et al., 2013). In general, the exposure of sunflower to medium-term Pb treatment leads to higher concentrations of Pb in the roots than in the above-ground parts (Doncheva et al., 2013). The accumulation of Zn in sunflower organs is in reversed order, thus Zn causes more membrane damage than Pb (Kocheva et al., 2015). The explanation for the stronger damaging effect of Zn on leaf cell membranes could be found in the higher mobility of Zn ions towards the leaves in comparison to Cd and Pb (Rivelli et al., 2012).

4.1. Morphological changes after Pb treatment

Different sunflower genotypes revealed differences in their leaf anatomy after treatment with Pb. In cultivated *H.*

Table 1-1. Some plant species that tolerate and accumulate Pb.

Plant species	Plant tissues of Pb accumulation	Pb content (Q) [μg.g^{-1} DW]	Translocation index (TI)* [%]	Reference
Amaranthus paniculatus	shoot	6.2 - 11.3	56.36 - 66.47	Rahman et al. (2013)
	root	4.8 - 5.7		
	whole plant	11.0 - 17.0		
Arabidopsis thaliana	leaves	700	41.16	Auguy et al. (2013)
	roots	1000		
	whole plant	1700		
Atriplex halimus L.	shoots	2.0 - 23.0	6.25 - 26.43	Manousaki and Kalogerakis (2009)
	roots	30.0 - 64.0		
	whole plant	32.0 - 87.0		
Brassica chinensis L.	in shoot tissue	5.0 - 15.0	-	Xie et al. (2006)
Brassica juncea	shoot	5.5 - 11.6	68.75 - 81.11	Rahman et al. (2013)
	root	2.5 - 2.7		
	whole plant	8.0 - 14.3		
Elsholtzia argyi	leafs	100	48.05	Peng et al. (2005)
	stems	11000		
	roots	12000		
	whole plant	23100		
Elsholtzia splendens	leafs	90	4.16	Peng et al. (2005)
	stems	300		
	roots	9000		
	whole plant	9390		
Festuca arundinacea	shoot	10 - 5500	1.9 - 70.51	Begonia et al., (2005)
	root	500 - 2300		
	whole plant	510 - 7800		
Helianthus annuus	shoot	8.5 - 15.5	62.04 - 71.75	Rahman et al. (2013)
	root	5.2 - 6.1		
	hole plant	13.7 - 21.6		
H. annuus after Pb-EDTA treat.	leafs	3700	62.75	Boonyapookana et al. (2005)
	stems	850		
	roots	2700		
	whole plant	7250		
H. annuus cv. 1114	leaves	500	10.71	Doncheva et al. (2013)
	stems	700		
	roots	10000		
	whole plant	11200		
H. annuus cv. 1114 after Pb-EDTA treat.	leaves	100	73.70	Doncheva et al. (2013)
	stems	320		
	roots	150		
	whole plant	570		

Table 1-2. Some plant species that tolerate and accumulate Pb.

Plant species	Plant tissues of Pb accumulation	Pb content (Q) [µg.g^{-1} DW]	Translocation index (TI)* [%]	Reference
H. annuus ^{x} *H. argophyllus*	leaves	230	3.85	Doncheva et al. (2013)
	stems	230		
	roots	11500		
	whole plant	11960		
H. annuus ^{x} *H. argophyllus* after Pb-EDTA treatment	leaves	70	35.21	Doncheva et al. (2013)
	stems	180		
	roots	460		
	whole plant	710		
Hirschfeldia incana	roots and shoots			Auguy et al. (2013)
	vacuolar storage			
	shoot	530 - 3580		
	root	7500 - 10700	6.60 - 25.07	
	whole plant	8030 - 14280		
Matricaria recutita L.	shoots	11	4.91	Geneva et al. (2014)
	roots	213		
	whole plant	224		
Pteris vittata L.	in fronds	680	-	Wu et al. (2007)
Salix integra	leaves	8.6 - 89.3	2.80 - 4.16	Wang et al. (2014)
	new shoots	14.6 - 49.4		
	wood	216 - 930		
	roots	8320 - 24600		
	whole plant	8559 - 25669		
Salvia officinalis L.	shoots	8.0	10.25	Stancheva et al. (2009)
	roots	70.0		
	whole plant	78.0		
Sedum alfredii	in shoots	2.3	-	
	shoots	50 - 80		Wu et al. (2007)
	roots	350 - 580	12.5 - 12.0	Liu et al. (2008)
	whole plant	400 - 660		
Sesbania drummondii	root cell walls	-	-	Sharma et al. (2004)
	roots and shoots			
	vacuoles	-	-	Ruley et al. (2006)
	in shoots	100 - 460	-	
Sesbania exaltata Raf.	shoots	100 - 270	4.16 - 5.55	Miller et al. (2011)
	roots	2300 - 4600		
	whole plant	2400 - 4870		
Sorghum bicolor L.	shoots	150 - 640	0.64 - 2.30	Xu Y. et al. (2007)
	roots	23000 - 27000		
	whole plant	23150 - 27640		
Spinacia oleracea	whole plant	990	-	Lamhamdi et al. (2013)

Table 1-3. Some plant species that tolerate and accumulate Pb.

Plant species	Plant tissues of Pb accumulation	Pb content (Q) [µg.g^{-1} DW]	Translocation index (TI)* [%]	Reference
Tribulus terrestris L.	shoots	170		Stancheva et al. (2011)
	roots	460		
	whole plant	630	27.0	
Tribulus terrestris L.	in fruits	85	53.42	Stancheva et al. (2014)
	leaves and stems	110		
	in roots	170		
	whole plant	365		
Triticum aestivum	in grain	15 - 17	3.27 - 3.85	Karak et al. (2010) Lamhamdi et al. (2013)
	in straw	25 - 30		
	whole plant	1220		
Vetiveria zizanioides L.	roots and shoots	~10	-	Paz-Alberto et al. (2007)

*The ability for Pb translocation from roots to shoots was calculated by the translocation index (TI) % = SQ / WPQ x 100, where, SQ - lead content in the shoots [µg.g^{-1} DW].
WPQ – lead content in the whole plant (µg.g^{-1} DW); DW - Dry Weight. Wherever Pb content and TI are shown only - under particular conditions or are shown ranging - under diverse conditions. For details see the relevant references.

annuus the exposure to Pb decreased the thickness of both the leaves and mesophyll (Doncheva et al., 2013). In contrast, in the hybrid line *H. annuus*×*H. argophyllus* Pb increased the thickness of the leaves and that of the mesophyll. These differences could be a mechanism to maintain a functionally active photosynthetic area thus contributing to the higher Pb tolerance of the interspecific line. This is confirmed by measurements of the photosynthetic activity (Doncheva et al., 2013). After exposure to Pb, the abaxial parts of the leaves of the sunflower plants were covered by numerous nonglandular trichomes of different length, made up of one or several cells. In addition, the base of the trichomes was swollen. Glandular trichomes were found mostly in Pb-treated plants, and they were composed of stalks and spherical heads (Doncheva et al., 2013). Trichomes have a substantial storage capacity to act as a sink during detoxification of heavy metals and xenobiotics. After glutathione conjugation and sequestration to the vacuole the trichomes may increase the heavy metal accumulation and the capacity of the plant for phytoremediation. The changes of the trichomes in the Pb-treated sunflower plants suggest increased capacity for detoxification (Doncheva et al., 2013).

Stomata play a critical role in gas exchange and water evaporation. Stomatal aperture on the abaxial side of the leaves was decreased significantly when the cultivated sunflower plants were exposed to Pb. This treatment however does not change significantly the stomata of the hybrid line. The stomata closure may be induced by increased hydrogen peroxide production resulting from unbalanced signaling pathway, which could occur in

Pb-treated plants (Doncheva et al., 2013; Pourrut et al., 2011).

Measurement of the electrolyte leakage from plant tissues is a method for evaluation of membrane integrity as affected by various types of abiotic stress, growth and developmental processes, and genotypic variation. It was shown that exposure of *Helianthus* plants to excess Pb for longer periods caused an increase in electrolyte leakage from the leaves of treated plants, but short-term Pb treatment caused only a slight increase in net effluxes from the apoplast and symplast, while the addition of EDTA induced less electrolyte leakage from these cell compartments (Doncheva et al., 2013; Kocheva et al., 2015).

4.2. Biochemical effects of Pb in sunflower plants

Pb induces a number of specific changes in the enzymes and other biochemical parameters in plants (Pourrut et al., 2011). Such changes were reported for *Helianthus* and it was suggested that sunflower plants may be used as bioindicators of environmental pollution with Pb (Krystofova et al., 2009).

Pb taken up by sunflower induces stress reactions, which are revealed as changes of the levels of certain molecules. Firstly, the total content of proteins slightly increases in both aerial parts and roots. Later, a strong decrease of total content of proteins in the whole plant occurs (Krystofova et al., 2009). Some enzyme activities were found to be up-regulated: alanine transaminase (ALT), aspartate transaminase (AST) and urease (Krystofova et al., 2009). In sunflower leaves treatment with Pb induced the expression of two new Mn-SOD (superoxide dismutase) isozymes (Mn-SOD I and II). Their expression could be an early defense reaction (within the first 1–8 h of treatment), aimed at the prevention of oxidative damage (Doncheva et al., 2013).

Experiments showed characteristic changes in the concentration of free cysteine taking place in the course of Pb treatment. The content of cysteine in roots is ten times higher than in shoots. As the Pb treatment progresses in time, the concentration of cysteine declines both in roots and shoots. Glutathione (GSH) and phytochelatins (PC) are important cell constituents, which help the plants to withstand oxidative stress. Both are synthesized from cysteine. Their content is the same in roots and shoots. As the time of treatment of plants with Pb increases, their concentration gets higher. Presumably, the observed reduction of cysteine content is related to its utilization for the biosynthesis of GSH and phytochelatins (Krystofova et al. 2009).

4.3. Advantages of the sunflower plants for phytoextraction of Pb

Helianthus annuus plants possess some important advantages, which make them suitable for phytoremediation. They grow well under variety of conditions, and also fast, producing high biomass, and can be easily harvested. As in most plants, Pb-treatment of *H. annuus* causes a decrease in plant growth (Pourrut et al., 2011; Doncheva et al., 2013). However, the application of N and P fertilizers enhances the growth of shoots and roots of sunflower; this, combined with EDTA facilitates the phytoremediation efficiency in Pb polluted soils (Rahman et al., 2013). High doses of fertilizers speed up growth and increase biomass production of plants that can tolerate and accumulate Pb. This is observed in plants with edible parts - radish, carrot, potato; also in shoots and roots of Indian mustard (*Brassica juncea*), *Amaranthus* (Inoue et al., 2013; Rahman et al., 2013). Comparison of all these plants shows that sunflower has the best ability to remove Pb from contaminated soil (Rahman et al., 2013), with highest translocation index (TI) 71.75% (Table 1). Boonyapookana et al. (2005) juxtapose phytoaccumulation of Pb by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). Overall, Pb accumulation potential of *H. annuus* was greater than that of *N. tabacum* and *V. zizanioides*. The highest measured Pb concentrations were found in *H. annuus* roots, stems, and leaves - 2.7; 0.85 and 3.7 mg/g dry weight, respectively; and translocation index - 62.75% (Table 1), grown in hydroponics with Pb-EDTA. The authors affirm that *H. annuus* best meets the requirements for an accumulator plant and has the potential for use in the restoration of abandoned mines and factories contaminated with Pb (Boonyapookana et al., 2005).

The use of plants for phytoextraction of heavy metals from contaminated soil is limited by the ability of the plants to grow on these soils and take up and store the target metals. However, the availability of the metals in the soil solution for plant uptake is also important.

The use of chelating agents to assist phytoextraction is subject to numerous research, which has demonstrated results varying widely depending both on the plant and the metal. Growing concern is the fate of the chelator, which may leach and stay in the soil, compromising the remediation effort (Evangelou et al., 2007). Attention has focused recently on biodegradable chelators such as the naturally occurring EDDS (ethylene diamine disuccinic acid), produced by some bacteria. Experiments were performed to investigate conditions to increase the phytoextraction potential of *Helianthus annuus* (Fässler et al., 2010). Root and shoot growth of Pb or Zn-stressed sunflower in hydroponics was most effectively increased by the addition of 0.1 nM IAA (indole-3-acetic acid, auxin); the extraction of both metals also increased by this treatment. EDDS applied alone decreased metal uptake by the plants, thus reducing metal stress and promoting plant growth. However, the combined application of IAA with EDDS significantly increased metal uptake in comparison to control plants.

Uptake of and tolerance to Pb depends on the type of the root system. In sunflower, Pb accumulation and tolerance was shown to differ between seedlings with primary roots and seedlings with adventitious roots only (in which the primary roots were cut off). Seedlings with adventitious roots accumulated less metal and were more tolerant to Pb (Strubinska and Hanaka, 2011). This suggests that adventitious roots have additional mechanisms that protect them against Pb penetration and Pb-induced oxidative stress. Although these mechanisms are still not known their investigation is promising. It should be noted that usually higher Pb accumulation is observed in roots. Roots are often the harvestable part of plants. This accumulator potential may be used in the phytoremediation process (Fahr et al., 2013). Of interest is the close sunflower relative *Helianthus tuberosus* (Jerusalem artichoke, earth apple or topinambour), which is easy to cultivate and grows up large quantities of edible root tubers.

Mutagenesis of selected high biomass plant species may also produce improved phytoremediating cultivars. Sunflowers were treated with the chemical mutagen ethyl methanesulfonate (EMS). The effect of chemical mutagenesis on metal concentration and extraction by new sunflower mutants was directly assessed on a metal-contaminated field (Nehnevajova et al., 2007). The best sunflower mutant showed a significantly enhanced metal extraction ability: 7.5 times for Cd, 9.2 times for Zn, and 8.2 times for Pb in aboveground parts, as compared to the control plants. Furthermore, the use of sunflower oil and biomass for technical purposes (lubricants, biodiesel, biogas) should produce an additional value and improve the economical balance of phytoextraction (Nehnevajova et al., 2007).

According to several authors, plants that are able to yield more than 20 t. ha^{-1} . year^{-1} and have concentration of the targeted metals of about 1% in the harvestable dry mass may be successfully used for phytoextraction. The requested dry mass potential is not a limitation for sunflower. Thus, the main attention is focused on how to achieve high shoot metal concentrations. It is considered that three key factors control shoot metal accumulation: metal solubility, metal absorption by roots, and metal translocation from roots to shoots (Vassilev et al., 2004). The complete genome sequence of *Helianthus annuus* is not known yet. Nonetheless, the use of molecular genetic approaches will contribute to better understanding the biochemical processes involved in sunflower Pb/heavy metal uptake and accumulation. As this is achieved, the way is opened, as it is for other plants (Krämer and Chardonnens, 2001), to create *Helianthus annuus* cultivars with desirable characteristics, suitable for phytoremediation of polluted land.

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