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RESEARCH ARTICLE

Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology

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The importance of biodiversity (below and above ground) is increasingly considered for the cleanup of the metal contaminated and polluted ecosystems. This subject is emerging as a cutting edge area of research gaining commercial significance in the contemporary field of environmental biotechnology. Several microbes, including mycorrhizal and non-mycorrhizal fungi, agricultural and vegetable crops, ornamentals, and wild metal hyperaccumulating plants are being tested both in lab and field conditions for decontaminating the metalliferous substrates in the environment. As on todate about 400 plants that hyperaccumulate metals are reported. The families dominating these members are Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae. Brassicaceae had the largest number of taxa viz. 11 genera and 87 species. Different genera of Brassicaceae are known to accumulate metals. Ni hyperaccumulation is reported in 7 genera and 72 species and Zn in 3 genera and 20 species. *Thlaspi* species are known to hyperaccumulate more than one metal *i.e. T. caerulescence* = Cd, Ni. Pb, and Zn; *T. goesingense* = Ni and Zn and *T. ochroleucum* = Ni and Zn and *T. rotundifolium* = Ni, Pb and Zn. Plants that hyperaccumulate metals have tremendous potential for application in remediation of metals in the environment. Significant progress in phytoremediation has been made with metals and radionuclides. This process involves rising of plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal. Most researchers believe that plants for phytoremediation should accumulate metals only in the roots. Several aquatic species have the ability to remove heavy metals from water, viz., water hyacinth (*Eichhornia crassipes* (Mart.) Solms); pennywort (*Hydrocotyle umbellata* L.) and duckweed (*Lemna minor* L.). The roots of Indian mustard are effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn and sunflower removes Pb, U, ^{137}Cs, and ^{90}Sr from hydroponic solutions. Aquatic plants in freshwater, marine and estuarine systems act as receptacle for several metals. Hyperaccumulators accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil, as long as the metal in question is present. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix. In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. If metal availability in the soil is not adequate for sufficient plant uptake, chelates or acidifying agents would be applied to liberate them into the soil solution. Use of soil amendments such as synthetics (ammonium thiocyanate) and natural zeolites have yielded promising results. Synthetic cross-linked polyacrylates, hydrogels have protected plant roots from heavy metals toxicity and prevented the entry of toxic metals into roots. After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting the permanent removal of metals from the site. Soil metals should also be bioavailable, or subject to absorption by plant roots. Chemicals that are

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suggested for this purpose include various acidifying agents, fertilizer salts and chelating materials. The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil. Chelates are used to enhance the phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb, and Zn Researchers initially applied hyperaccumulators to clean metal polluted soils. Several researchers have screened fast-growing, high-biomass-accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots. Genes responsible for metal hyperaccumulation in plant tissues have been identified and cloned. Glutathione and organic acids metabolism plays a key role in metal tolerance in plants. Glutathione is ubiquitous component cells from bacteria to plants and animals. In phytoremediation of metals in the environment, organic acids play a major role in metal tolerance. Organic acids acids form complexes with metals, a process of metal detoxification. Genetic strategies and transgenic plant and microbe production and field trials will fetch phytoremediaition field applications.The importance of biodiversity and biotechnology to remediate potentially toxic metals are discussed in this paper. Brassicaceae amenable to biotechnological improvement and phytoremediation hype are highlighted.

Metals, radionuclides and other inorganic contaminants are among the most prevalent forms of environmental contaminants, and their remediation in soils and sediments is rather a difficult task (Cunningham et al. 1997). Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludges to land, and industrial manufacturing (Raskin et al. 1994; Cunningham et al. 1997; Blaylock and Huang, 2000). Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1979) and poses major environmental and human health problems worldwide (Ensley, 2000). Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation (Cunningham and Ow, 1996; NRC, 1997). Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment (NRC, 1999). The various conventional remediation technologies that are used to clean heavy metal polluted environments are soil *in situ* vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification and stabilization electrokinetic systems. Each of the conventional remediation technology has specific benefits and limitations (EPA, 1997; MADEP, 1993).

All compartments of the biosphere are polluted by a variety of inorganic and organic pollutants as a result of anthropogenic activities and alter the normal biogeochemical cycling. A variety of biological resources have been employed widely both in developed and developing nations for cleanup of the metal polluted sites. These technologies have gained considerable momentum in the last one decade and currently in the process of commercialization (Comis, 1995; Salt et al. 1995a; Comis, 1996; Salt et al. 1998; Vangronsveld and Cunningham, 1998; Glass, 1999; Prasad and Freitas, 1999; Alcantara et al. 2000; Ernst, 2000; Glass, 2000a; Glass, 2000b; Raskin and Ensley, 2000; Watanabe, 1997; Hamlin, 2002; Prasad, 2003). The Unites States of America's Environmental Protection Agency´s remediation program included phytoremediation of metals and radionuclides as a thurst area upto 25% during the year 2000 (Figure 1). Plants that hyper accumulate metals have tremendous potential for application in remediation of metals in the environment. This approach is emerging as an innovative tool with greater potential for achieving sustainable development and also to decontaminate metal polluted air, soil, water and for other environmental restoration applications through rhizosphere biotechnology (Desouza et al. 1999; Wenzel et al. 1999) (Figure 2). Metal hyperaccumulating plants are thus not only useful in phytoremediation, but also play a significant role in biogeochemical prospecting, and have implications on human health through food chain and possibly exhibit elemental allelopathy (metallic compounds leached through plant parts of the hyperaccumulator would supress the growth of other plants growing in the neighbourhood) and resistance against fungal pathogens (Figure 3) (Boyd et al. 1994). In order to be realistic about the phytoremediation, focussed studies on factors regulating phytoremediation are necessary (Figure 4).

The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Cunningham et al. 1997; Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Ensley, 2000). Although the term, phytoremediation, is a relatively recent invention, its an age old practice (Cunningham et al. 1997; Brooks, 1998a). Research using semi-aquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era (Timofeev-Resovsky et al. 1962; Salt et al. 1995a). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Reeves and Brooks, 1983; Baker and Brooks, 1989; Baker et al. 1991; Entry et al. 1999). Chaney, 1983 was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal-

polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Cunningham et al. 1995; Comis, 1996; Ebbs et al. 1997). By definition, a hyperaccumulator must accumulate at least 100 mg g^{-1} (0.01% dry wt.), Cd, As and some other trace metals, 1000 mg g^{-1} (0.1 dry wt.) Co, Cu, Cr, Ni and Pb and 10,000 mg g^{-1} (1 % dry wt.) Mn and Ni (Reeves and Baker, 2000; Wantanabe, 1997).

Phytoremediation consists of four different plant-based technologies each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. These include: rhizofiltration, which involves the use of plants to clean various aquatic environments; phytostabilization, where plants are used to stabilize rather than clean contaminated soil; phytovolatilization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants absorb metals from soil and translocate them to the harvestable shoots where they accumulate. Although plants show some ability to reduce the hazards of organic pollutants (Cunningham et al. 1995; Gordon et al. 1997; Carman et al. 1998), the greatest progress in phytoremediation has been made with metals (Salt et al. 1995a; Watanabe, 1997; Blaylock and Huang, 2000). Phytoremediative technologies which are soil-focused are suitable for large areas that have been contaminated with low to moderate levels of contaminants. Sites which are heavily contaminated cannot be cleaned through phytoremediative means because the harsh conditions will not support plant growth. The depth of soil which can be cleaned or stabilized is restricted to the root zone of the plants being used. Depending on the plant, this depth can range from a few inches to several meters (Schnoor et al. 1995). Phytoremediation should be viewed as a long-term remediation solution because many cropping cycles may be needed over several years to reduce metals to acceptable regulatory levels. This new remediation technology is competitive, and may be superior to existing conventional technologies at sites where phytoremediation is applicable.

Rhizofiltration

Metal pollutants in industrial-process water and in groundwater are most commonly removed by precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge (Ensley, 2000). A promising alternative to this conventional clean-up method is rhizofiltration, a phytoremediative technique designed for the removal of metals in aquatic environments. The process involves raising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov et al. 1995; Salt et al. 1995a; Flathman and Lanza, 1998; Zhu et al. 1999b). Root exudates and changes in rhizosphere pH also may cause metals to precipitate onto root surfaces. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza, 1998; Zhu et al. 1999b). Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Dushenkov et al. 1995; Salt et al. 1995a; Flathman and Lanza, 1998). Dushenkov et al. 1995 explains that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. In contrast, Zhu et al. 1999b suggest that the efficiency of the process can be increased by using plants which have a heightened ability to absorb and translocate metals within the plant. Despite this difference in opinion, it is apparent that proper plant selection is the key to ensuring the success of rhizofiltration as a water cleanup strategy.

Dushenkov and Kapulnik, 2000 describe the characteristics of the ideal plant for rhizofiltration. Plants should be able to accumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal. It is also desirable plants to produce significant amounts of root biomass or root surface area. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes* (Mart.) Solms; Kay et al. 1984; Zhu et al. 1999b), pennywort (*Hydrocotyle umbellata* L.; Dierberg et al. 1987), and duckweed (*Lemna minor* L.; Mo et al. 1989). However, these plants have limited potential for rhizofiltration, because they are not efficient at metal removal, a result of their small, slow-growing roots (Dushenkov et al. 1995). These authors also point out that the high water content of aquatic plants complicates their drying, composting, or incineration. Despite limitations, Zhu et al. 1999b indicated that water hyacinth is effective in removing trace elements in waste streams. Terrestrial plants are thought to be more suitable for rhizofiltration because they produce longer, more substantial, often fibrous root systems with large surface areas for metal sorption. Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for metal removal in water. The roots of Indian mustard are effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov et al. 1995), and sunflower removes Pb (Dushenkov et al. 1995), U (Dushenkov et al. 1997a), ^{137}Cs, and ^{90}Sr (Dushenkov et al. 1997b) from hydroponic solutions.

Rhizofiltration is a cost-competitive technology in the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb, and Zn (Kumar et al. 1995b; Ensley, 2000). The commercialization of this technology is driven by economics as well as by such technical advantages as applicability to many problem metals, ability to treat high volumes, lesser need for toxic chemicals, reduced volume of secondary waste, possibility of recycling, and the likelihood of regulatory and public acceptance (Dushenkov et al. 1995; Kumar et al. 1995b). However, the application of this plant-based technology may be more challenging and susceptible to failure than other methods of similar

cost. The production of hydroponically grown transplants and the maintenance of successful hydroponic systems in the field will require the expertise of qualified personnel, and the facilities and specialized equipment required can increase overhead costs. Perhaps the greatest benefit of this remediation method is related to positive public perception. The use of plants at a site where contamination exists conveys the idea of cleanliness and progress to the public in an area that would have normally been perceived as polluted.

Phytostabilization

Sometimes there is no immediate effort to clean metal-polluted sites, either because the responsible companies no longer exist or because the sites are not of high priority on a remediation agenda (Berti and Cunningham, 2000). The traditional means by which metal toxicity is reduced at these sites is by in-place inactivation, a remediation technique that employs the use of soil amendments to immobilize or fix metals in soil. Although metal migration is minimized, soils are often subject to erosion and still pose an exposure risk to humans and other animals. Phytostabilization, also known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion; provides hydraulic control, which suppresses the vertical migration of contaminants into groundwater; and physically and chemically immobilizes contaminants by root sorption and by chemical fixation with various soil amendments (Cunningham et al. 1995; Salt et al. 1995a; Flathman and Lanza, 1998; Berti and Cunningham, 2000; Schnoor, 2000). This technique is actually a modified version of the in-place inactivation method in which the function of plants is secondary to the role of soil amendments. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

The most comprehensive and up-to-date explanation of the phytostabilization process is offered by Berti and Cunningham, 2000. Before planting, the contaminated soil is plowed to prepare a seed bed and to incorporate lime, fertilizer, or other amendments for inactivating metal contaminants. Soil amendments should fix metals rapidly following incorporation, and the chemical alterations should be long lasting if not permanent. The most promising soil amendments are phosphate fertilizers, organic matter or bio-solids, iron or manganese oxyhydroxides, natural or artificial clay minerals, or mixtures of these amendments. Plants chosen for phytostabilization should be poor translocators of metal contaminants to aboveground plant tissues that could be consumed by humans or animals. The lack of appreciable metals in shoot tissue also eliminates the necessity of treating harvested shoot residue as hazardous waste (Flathman and Lanza, 1998). Selected plants should be easy to establish and care for, grow quickly, have dense canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth. The research of Smith and Bradshaw, 1992, led to the development of two cultivars of *Agrostis tenuis* Sibth and one of *Festuca rubra* L which are now commercially available for the phytostabilization of Pb-, Zn-, and Cu-contaminated soils. Phytostabilization is most effective at sites having fine-textured soils with high organic-matter content but is suitable for treating a wide range of sites where large areas of surface contamination exist (Cunningham et al. 1995; Berti and Cunningham, 2000). However, some highly contaminated sites are not suitable for phytostabilization, because plant growth and survival is not a possibility (Berti and Cunningham, 2000). At sites which support plant growth, site managers must be concerned with the migration of contaminated plant residue off site (Schnoor, 2000) or disease and insect problems which limit the longevity of the plants. Phytostabilization has advantages over other soil-remediation practices in that it is less expensive, less environmentally evasive, easy to implement, and offers aesthetic value (Berti and Cunningham, 2000; Schnoor, 2000). When decontamination strategies are impractical because of the size of the contaminated area or the lack of remediation funds, phytostabilization is advantageous (Berti and Cunningham, 2000). It may also serve as an interim strategy to reduce risk at sites where complications delay the selection of the most appropriate technique for the site.

Phytovolatilization

Some metal contaminants such as As, Hg, and Se may exist as gaseous species in environment. In recent years, researchers have searched for naturally occurring or genetically modified plants that are capable of absorbing elemental forms of these metals from the soil, biologically converting them to gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization, the most controversial of all phytoremediation technologies. Mercury and Se are toxic (Wilber, 1980; Suszcynsky and Shann, 1995), and there is doubt about whether the volatilization of these elements into the atmosphere is safe (Watanabe, 1997). Selenium phytovolatilization has been given the most attention to date (Lewis et al. 1966; Terry et al. 1992; Bañuelos et al. 1993; McGrath, 1998), because this element is a serious problem in many parts of the world where there are areas of Se-rich soil (Brooks, 1998b). However, there has been a considerable effort in recent years to insert bacterial Hg ion reductase genes into plants for the purpose of Hg phytovolatilization (Rugh et al. 1996; Heaton et al. 1998; Rugh et al. 1998; Bizily et al. 1999). Although there have been no efforts to genetically engineer plants which volatilize As, it is likely that researchers will pursue this possibility in the future. According to Brooks, 1998b, the release of volatile Se compounds from higher plants was first reported by Lewis et al. 1966. Terry et al. 1992 report that members of the Brassicaceae are capable of releasing up to 40 g Se ha-1 day –1 as various gaseous compounds.

Some aquatic plants, such as cattail (*Typha latifolia* L.), are also good for Se phytoremediation (Pilon-Smits et al. 1999a). Unlike plants that are being used for Se volatilization, those which volatilize Hg are genetically modified organisms. *Arabidopsis thaliana* L. and tobacco (*Nicotiana tabacum* L.) have been genetically modified with bacterial organomecurial lyase (*MerB*) and mercuric reductase (*MerA*) genes (Heaton et al. 1998; Rugh et al. 1998). These plants absorb elemental Hg(II) and methyl mercury (MeHg) from the soil and release volatile Hg(O) from the leaves into the atmosphere (Heaton et al. 1998). The phytovolatilization of Se and Hg into the atmosphere has several advantages. Volatile Se compounds, such as dimethylselenide, are 1/600 to 1/500 as toxic as inorganic forms of Se found in the soil (DeSouza et al. 2000). The volatilization of Se and Hg is also a permanent site solution, because the inorganic forms of these elements are removed and the gaseous species are not likely to be redeposited at or near the site (Atkinson et al. 1990; Heaton et al. 1998). Furthermore, sites that utilize this technology may not require much management after the original planting. This remediation method has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton et al. 1998; Rugh et al. 2000). Heaton et al. 1998 suggest that the addition of Hg(O) into the atmosphere would not contribute significantly to the atmospheric pool. However, those who support this technique also agree that phytovolatilization would not be wise for sites near population centers or at places with unique meteorological conditions that promote the rapid deposition of volatile compounds (Heaton et al. 1998; Rugh et al. 2000). Unlike other remediation techniques, once contaminants have been removed via volatilization, there is a loss of control over their migration to other areas. Despite the controversy surrounding phytovolatilization, this technique is a promising tool for the remediation of Se and Hg contaminated soils.

Phytoextraction

Phytoextraction is the most commonly recognized of all phytoremediation technologies, and is the focus of the research proposed in this prospectus. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific cleanup technology. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix (Kumar et al. 1995a). In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. If metal availability in the soil is not adequate for sufficient plant uptake, chelates or acidifying agents may be used to liberate them into the soil solution (Huang and Cunningham, 1996; Huang et al. 1997a; Lasat et al. 1998). After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting the permanent removal of metals from the site. As with soil excavation, the disposal of contaminated material is a concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar et al. 1995a). In some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Comis, 1996; Cunningham and Ow, 1996). Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar et al. 1995a) to acceptable levels. The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years (Kumar et al. 1995a; Blaylock and Huang, 2000). This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar et al. 1995a; Blaylock and Huang, 2000). Many factors determine the effectiveness of phytoextraction in remediating metal-polluted sites (Blaylock and Huang, 2000). The selection of a site that is conducive to this remediation technology is of primary importance. Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow for normal cultivation practices, which employ the use of agricultural equipment. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Kumar et al. 1995a; Cunningham and Ow, 1996; Blaylock et al. 1997; McGrath, 1998). It is the combination of high metal accumulation and high biomass production that results in the most metal removal. Ebbs et al. 1997 reported that *B. juncea*, while having one-third the concentration of Zn in its tissue, is more effective at Zn removal from soil than *T. caerulescens*, a known hyperaccumulator of Zn. This advantage is due primarily to the fact that *B. juncea* produces ten-times more biomass than *T. caerulescens*. Plants being considered for phytoextraction must be tolerant of the targeted metal, or metals, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant (Blaylock and Huang, 2000). Other desirable plant characteristics include the ability to tolerate difficult soil conditions (*i.e.*, soil pH, salinity, soil structure, water content), the production of a dense root system, ease of care and establishment, and few disease and insect problems. Although some plants show promise for phytoextraction, there is no plant which possesses all of these desirable

traits. Finding the perfect plant continues to be the focus of many plant-breeding and genetic-engineering research efforts.

Biodiversity prospecting for phytoremediation of metals in the environment

"Biodiversity prospecting" offers a several opportunities of which the most important is to save as much as possible of the world's immense variety of ecosystems. Biodiversity prospecting would lead to the discovery of a wild plants that could clean polluted environments of the world. This subject is at its infancy with a great hope of commercial hype. The desire to capitalize on this new ideas need to provide strong incentives for conserving nature (Myers, 1990).

Toxic trace elements are increasing in all compartments of the biosphere; including, air, water and soil, as a result of anthropogenic processes. For example, the metal concentration in river water and sediments increased several thousand fold by effluents from industrial and mining wastes (Siegel, 2002). Aquatic plants in freshwater, marine and estuarine systems act as receptacle for several metals (Crites et al. 1997; Cole, 1998; Hansen et al. 1998; Kadlec et al. 2000; Kaltsikes, 2000; Odum et al. 2000). Published literature indicate that an array of bioresources (biodiversity) have been tested in field and laboratory (Table 1). Remediation programs relying on these materials may be sucessful (Comis, 1995; Glass, 1999; Glass, 2000a; Valdes, 2002; Wise et al. 2002) (Figure 5).

The most successful monitoring methods for metals in the environment are based on bacterial heavy metal biosensors viz., a) gene based biosensors and b) protein based biosensors (Prasad, 2001a; Tsao, 2003). Mosses, liverworts and ferns are also capable of growing on metal-enriched substrates. These plants possess anatomical and physiological charcteristics enabling them to occupy unique ecological niches in natural metalliferous and man made environments. For example, groups of specialized bryophytes are found on Cu enriched substrates; so-called 'copper mosses' and come from widely separated taxonomic groups. Other bryophytes are associated with lead and zinc enriched substrates. However, the information aboutbryophytes growing on serpentine soils is rather scanty (Prasad, 2001a). Pteridophytes (ferns) are associated with serpentine substrates invarious parts of the world Brake fern, *Pteris vittata*, a fast growing plant is reported to tolerate soils contaminated with arsenic as much as 1500 p.p.m and its fronds concentrate the toxic metal to 22,630 p.p.m in 6 weeks (Ma et al. 2001b). Among angiosperms, about 400 metal hyperaccumulators have been identified which would serve as a reservoir for biotechnological application (Brooks, 1998b) (Figure 6).

Metal hyperaccumulators for phytoremediation hype

Mine reclamation and biogeochemical prospecting depends upon right selection of plant species and sampling. The selection of heavy metal tolerant species is a reliable tool to achieve success in phytoremediation. 163 plant taxa belonging to 45 families are found to be metal tolerant and are capable of growing on elevated concentrations of toxic metals (Table 2). The use of metal tolerant species and their metal indicator and accumulation is a function of immense use for biogeochemical prospecting (Brooks, 1983; Badri and Sringeul, 1994; McInnes et al. 1996).

Brassicaceae had the highest number taxa *i.e.* 11 genera and 87 species that are established for hyperaccumulation of metals (Figure 7). In Brassicace Ni hyperaccumulation is reported in 7 genera and 72 species (Reeves et al. 1996; Reeves et al. 1999), and Zn in 3 genera and 20 species (Figure 8). Different genera of Brassicaceae are known to accumulate metals (Figure 9) (Delorme et al. 2001).

Considerable progress had been achieved recently in unravelling the genetic secrets of metal-eating plants. Genes responsible for metal hyperaccumulation in plant tissues have been identified and cloned (Moffat, 1999). These finding are expected to identify new non-conventional crops, *metallocrops* that can decontaminate metals in the environment (Raskin, 1996; Ebbs et al. 1997; Ebbs and Kochian, 1998). The fundamental aspects of microbe/plant stress responses to different doses of metals coupled with break through research innovations in biotechnology would successfully provide answers as how to apply the biodiversity for advancing phytoremediation technology.

Ornamentals

Neerium oleander leaves collected from urban areas of Portugal accumulated lead upto 78 mg/g dry weight in leaves and is suitable for monitoring lead in air (Freitas et al. 1991). *Canna* x *generalis* is an important ornamental cultivated in urban landscape. Hydroponic cultures of this plant treated with lead for one month suggest that this plant is a suitable for phytoextraction of lead as the plant produces appreciable quantity of biomass (Figure 10 and Figure 11) (Trampczynska et al. 2001) (*Pelargonium*sp. "Frensham"), scented geranium was identified as one of the most efficient metal hyperaccumulator plants (Saxena et al. 1999). In a greenhouse study, young cuttings of scented geranium grown in artificial soil and fed different metal solutions, were capable of taking up large amounts of three major heavy metal contaminants (*i.e.* Pb, Cd and Ni) in a relatively short time. These plants were capable of extracting from the feeding solution and stocking in their roots amounts of lead, cadmium and nickel equivalent to 9%, 2.7% and 1.9% of their dry weight material respectively. With an average root mass of 0.5-1.0 g in dry weight, scented geranium cuttings could extract 90 mg of Pb, 27 mg of Cd and 19 mg of Ni from the feeding solution

in 14 days. If these rates of uptake could be maintained under field conditions, scented geranium should be able to cleanup heavily contaminated sites in less than 10 years (growth and uptake in nutrient solution is extremely different to that in soil, and scientific studies indicate the hydroponic culture is not indicative of a real-world situation, due to ion competition, root impedence, and the fact that plants do not grow root hairs when they are grown in solution). For example, a phytoremediation lead clean up program consisting of 16 successive croppings of scented geranium planted at a density of 100 plants m^{-2} over the summer could easily remove up to 72 g of lead m^{-2} yr^{-1}. In our estimates, scented geranium would extract 1000-5000 kg of lead per ha^{-1} yr^{-1}. Thus, these reported figures are close to Cunningham and Ow, 1996 estimations of metal removal rates of 200-1000 kg ha^{-1} yr^{-1} for plants capable of accumulating 1.0-2.0% metal. Thus, if scented geranium is planted in soil where the lead contamination is 1000 mg $kg^{-1}$of soil, which is the acceptable limit for the province of Ontario (Canada), it can clean up the soil completely in 8 years. Scented geranium also has the ability to survive on soils containing one or more metal contaminants (either individually or in combination) and on soils contaminated with a mixture of metal and hydrocarbons (up to three metal-hydrocarbon contaminated soils > 3% total hydrocarbon in combination with several metal contaminants.

Serpentinophytes as model systems for the unique feature of metal hyperaccumulation

Serpentinized rocks are distributed all over the world and and the harbours a distinct, often endemic plant community (Brooks, 1987). Serpentine soils are characterized by disproportionate amounts of magnesium (Mg) in relation to calcium (Ca) and often contain eleveated concentrations of available nickel (Ni) (Kruckeberg, 1984, Brooks, 1987). Nickel can cause toxicity in serpentine soils due to its high solubility in the soil solution. Serpentine outcrops have been referred to as barrens because they are often sparsely vegetated with extremely poor in essential nutrients and thus is of not much agricultural value. They can generally be distinguished by their gray-green or reddish rocky soils and shrubby or stunted vegetation with small leathery leaves. Serpentinophytes often experience drought, nutrient stress and excessive exposure to heavy metal and high light intensity. Finally, serpentine soils are thin. This means there is less substrate on which nutrients and water can be held and made available to plants. Deep serpentine soil occurs only in valleys, in alluvial soil, where rains wash small particles downward. Vegetation in these valleys is denser with ultramafic soils. In north-east of Portugal the serpentinized area is about 8,000 ha with characteristic geology and flora. Many of the hyperaccumulators inhabit in serpentine soils as well as in a wide range of environmental conditions. In addition to sepentinophytes, a number of wild brassicaceae are the best suitable species being hyper accumulators for phtoremediation. Serpentine habitats and species are threatened worldwide. Mainly due to habitat loss, several serpentine endemic species have become extinct or they are highly threatened. These habitats, often regional hotspots of biodiversity should be preserved and actions to preserve these unique spots should be promptly implemented (Arianoutsou et al. 1993; Harrison, 1999).

Edible plants and vegetables crops

The dominant leaf vegetable producing species viz. *Amaranthus spinosus*, *Alternanthera philoxeroides* and *A. sessiles* growing on the sewage sludge of Musi river located in greater Hyderabad City (close to 17º26' N latitude and 78º27' E longitude), Andhra Pradesh, India was investigated for metal accumulation. The transfer factor for metals was calculated Metal content in plant part (dry wt.)/ Metal content in soil (dry.wt). Transfer factor and metal content Cd (non-essential), Zn and Fe (essential) in plant parts of these selected species indicate their aility to bioconcentrate in their tissues (Figure 12). The concentration of these metals is invariably high in leaf tissue (Bañuelos and Meek, 1989; Prasad, 2001b).Thus, it is possible to use these species to restore the biosolid and sewage sludge contaminated sites, while exercising caution on human consumption. *Alternanthera philoxeroides* was used for removal of lead and mercury from polluted waters It is also possible to supplement the dietary requirement of human food with Zn and Fe as these being essential nutrients and the plant species are edible. However, there is a need to monitor the metal transfer factor through food chain (Bañuelos and Meek, 1989; Bañuelos et al. 1993a; Bañuelos et al. 1993b).

Natural by products as biofilters of toxic metals

In addtion to use of wild and cultivated species besides cell cultures, a wide variety of agricultural and forestry by products have been used as biosorbents of toxic metals in a bid to develop biofilters for specific applications. *i.e.:* i) Cotton – Hg; Groundnut skins – Cu; Tree Bark (Pinus, Acacia etc.) - variety of metals; Agrowaste - variery of metals; waste tea leaves - Pb, Cd, and Zn; *Pinus radiata* – U; Apple waste -Variety of metals; Cellulose - Variety of metals; Rice hulls - Variety of metals; Exhausted coffee grounds - Hg; *Pinus pinaster* bark - Zn, Cu, Pb. Saw mill dust (wood waste)– Cr; Freshwater green algae – variety of metals; Marine algae– Pb, Ni; ii) *Sphagnum* (moss peat) - Cr(VI); iii) Immobilized *Aspergillus niger, A. oryzae* - Cd, Cu, Pb, and Ni ; Olive mill waste *Olea europea* Cr, Ni, Pb, Cd, and Zn, Cu and Ni; *Streptomyces rimosus* (bacteria); *Saccharomyces cerevisiae* (yeast); *Penicillium chrysogenum* (fungi), *Fuscus* vesiculosus and Ascophyllum nodosum (marine algae) Zn, Cu andNi; *Phanerochaete chrysosporium, P. versicolar* - Pb, Ni, Cr, Cd, Cu; *Pinus radiata* – U; Immobilized *Pseudomonas putida* 5-X and *Aspergillus niger*, *Mucor rouxxi* – Cu; Actionomycetes, *Aspergillus niger, A.oryzae, Rhizopus arrhizus, R. nigricans*- Cd; *Rhizopus arrhizus* – Cr(VI), Pb; *Rhizopus nigricans, Phanarochaete chrysogenum* –Pb; *Aspergillus*

niger and *Rhizopus arrhizus* - Ni (Prasad and Freitas, 2000 and the references there in).

Acacia nilotica bark serves as an adsorbent of toxic metals. Bark (1 g) when added to 100 ml of aqueous solution containing 10 mg ml^{-1} metal solution exhibited different metal adsorption values for different metals. The order of metal adsorption being Cr > Ni > Cu > Cd> As > Pb. A similar trend of metal adsorption was observed when the bark is reused (1strecycle) Cr > Ni > Cu > Cd > Pb and also in the column-sorption. In order to verify the metal removal property of *A. nilotica* bark, toxicity bioassay with *Salix viminalis* stem cuttings in hydroponic system augmented with Cd, Cr and Pb together with *A. nilotica* bark powder was carried out. The results of toxicity bioassay confirmed the metal adsorption property of the bark powder. The functions of toxicity studies include leaf area, root length and number of new root primordia produced per stump. The leaf area, root length and number of new root primordia increased considerably in the presence of *A. nilotica* bark. The order of metal toxicity for leaf area and new root primordial is Cd > Cr > Pb. However, for root length the order of metal toxicity is Cr > Cd > Pb. The metal budgets of the leaf and root confirmed that the bark powder had adsorbed substantial amount of toxic metals and thus, alleviates the toxicity imposed by the various tested elements (Prasad et al. 2001).

Quercus ilex L. phytomass from stem, leaf and root as adsorbent of chromium, nickel, copper, cadmium and lead at ambient temperature was investigated. The metal uptake capacity of the root for different metals was found to be in the order of: Ni > Cd > Pb > Cu > Cr; stem Ni > Pb> Cu > Cd > Cr and leaf Ni > Cd > Cu > Pb > Cr. The highest amount adsorbed was Ni (root > leaf > stem). Data from this laboratory demonstrated that Ni is mostly sequestered in the roots where concentrations can be as high as 7.30 nmol/g dry weight, when one year old seedlings were treated with Ni (2000 mg/l) in pot culture experiments, compared to 0.13 nmol/g dry weight, in the control. This proves that the root biomass of *Q. ilex* has the capacity for complexing Ni. Chromium exhibited the least adsorption values for all the three types of phytomass compared to other metals. The trend of adsorption of the phytomass was similar for nickel and cadmium *i.e.* root > leaf > stem. Desorption with 10 mM Na_2 EDTA was effective (55-90%). Hence, there exists the possibility of recycling the phytomass. The biosorption results of recycled phytomass suggests, that the selected adsorbents are reusable (Prasad and Freitas, 2000).

The putative role of hyperaccumulators and serpentinophytes in elemental allelopathy

Hyperaccumulators provide protection against fungal and insect attack. Recent studies suggests that Ni-hyperaccumulation has a protective function against fungal and bacterial pathogens in *Streptanthus polygaloides* and *Thlaspi montanum* (Boyd et al. 1994). An antiherbivory effect of Zn has been found in the Zn hyperaccumulator *T. caerulescens*. (Brooks, 1998b). Species of *Thlaspi* are known to hyperaccumulate more than one metal. Several examples of plants that hyperaccumulate toxic metals and their concomitant functions on herbivory and influence of leachates on surrounding flora would add new dimension to the science of allelopathy.

FUTURE DIRECTIONS

Glutathione and organic acids metabolism plays a key role in metal tolerance in plants (Arisi et al. 1997; Huang et al. 1998; Schäfer et al 1998; Zhu et al. 1999a; Arisi et al. 2000; Ma et al. 2001a).Glutathione is ubiquitous component cells from bacteria to plants and animals. In plants, it is the major low molecular mass thiol compound (28). Glutathione occurs in plants mainly as as reduced GSH (95%). Its synthesis is mediated by the enzymes glutamylcysteine synthetase (EC 6.3.2.2) and glutathione synthetase (EC 6.3.2.3). Glutathione metabolism is also connected with cysteine and sulphur metabolism in plants.Cysteine concentration limits gluthatione biosynthesis. Low-molecular thiol peptides phytochelatins (PCs) often called class III metalothioneins are synthetized in plants from glutathione induced by heavy metal ions (Mejare and Bulow, 2001; Prasad and Strzalka, 2002).

These peptides are synthetized from glutathione by means of α-glutamylcysteine transferase enzyme (EC 2.3.2.15), which is also called phytochelatin synthase (PCS) catalyzing transfer reaction of (α-Glu-Cys) group from a glutathione donor molecule to glutathione, an acceptor molecule. PCS is a cytosolic, constitutive enzyme and is activated by metal ions viz., Cd^{2+},Pb^{2+}, Ag^{1+}, Bi^{3+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, and Au^{2+}. PCs thus, synthesized chelate heavy metals and form complexes and these complexes are transported through cytosol in an ATP–dependent manner through tonoplast into vacuole. Thus the toxic metals are swept away from cytosol. Some high-molecular weight complexes (HMW) with S-2 can also be formed from these LMW complexes in vacuole (Clemens et al. 1999; Clemens, 2001).

Transgenic plants with modified genes of PCS and genes of glutathione synthesis enzymes: α-GCS and GS, and enzymes connected with sulphur metabolism, *i.e.* serine acetyl transferase, need special attention studied in order to achieve success (Liang et al. 1999; Pilon-Smits et al. 1999b). Plants under heavy metals stress produce free radicals and reactive oxygen species and have to withstand the oxidative stress before aquiring tolerance to toxic metals. Glutathione is then used for the synthesis of PCs as well as for dithiol (GSSG) production. The ascorbate-glutathione pathway is involved in plant defence against oxidative stress. Organic acids play a major role in metal tolerance (Ma et al. 2001a) by forming complexes with metals, a process of metal detoxification. Chelation of metals with excluded organic acids in the rhizosphere and rhizospheric processes is an important aspect of

investigation for remediation. These metabolic pathways underscore the physiological, biochemical and molecular basis for heavy metal tolerance (Prasad and Strzalka, 2002).

Metal transporters and interactions in membranes at molecular level

Plants and humans require adequate amounts of micronutrients like iron and zinc (Cakmak and Marschner, 1987), but accumulation of an excess or uptake of non-essential metals like cadmium or lead can be extremely harmful. Proteins of the CDF (cation diffusion facilitator) family (now termed as cation efflux family) are involved in the homeostasis of $Cd^{2+,}$ Co^{2+}, Fe^{2+} and Zn^{2+} in microbes, animals and plants (Lasat et al. 2000). Therefore, elucidation of the role of CDF proteins in *Arabidopsis thaliana* would be advantageous to the success of phytoremediaiton. Complementary DNAs are to be functionally expressed in appropriate mutants of *Saccharomyces cerevisiae* to test their function. In a reverse genetics approach several representative *Arabidopsis* CDFs will be e used in RNA interference technology (Kramer et al. 1997; Persans et al. 2001). Regulation and localization of these CDFs need to be investigated by expressing promoter: GUS fusions and epitope-tagged fusion proteins in *A. thaliana*, and by development and use of specific antibodies. Very a little of information is available about protein-protein interactions of membrane. Such interactions might be vital for CDF function because their substrate metal cations are thought to be bound to metallochaperone proteins in the cytoplasm.

Molecular genetic and transgenic strategies for phytoremediation hype

Genetic strategies and transgenic plant and microbe production and field trials will fetch phytoremediaition field applications (Misra and Gedamu, 1989; Stomp et al. 1994; Ow, 1996; Arazi et al. 1999; Cai et al. 1999; Karenlampi et al. 2000; Mengoni et al. 2000; Kramer and Chardonnens, 2001; Pence et al. 2000; Palmer et al. 2001). Mercury is a world wide problem as a result of its many diverse uses in industry. Mercury has been used in bleaching operations (chlorineproduction, paper, textiles, etc.) as a catalyst, a pigment for paints, for gold mining, as well as a fungicide and antibacterial agent in seeds and bulbs. Elemental mercury, Hg (0), can be a problem because it is oxidized to Hg^{2+} by biological systems and subsequently is leached into wetlands, waterways, and estuaries. Additionally, mercury can accumulate in animals as methyl mercury (CH_3-Hg^+), dimethylmercury ($CH_3)_2$-Hg) or other organomercury salts. Organic mercury, produced by some anaerobic bacteria, is 1-2 orders of magnitude more toxic in some eukaryotes, is more likely to biomagnify than ionic mercury, and efficiently permeates biological membranes. Monomethyl-Hg is responsible for severe neurological degeneration in birds, cats, and humans.

Certain bacteria are capable of pumping metals out of their cell, and or oxidizing, reducing, or modifying the metal ions to less toxic species. One example is the mer operon. The mer operon contains genes that sense mercury (merB), transport mercury (merT), sequester mercury to the periplasmic space (merP), and reduce mercury (merA). MerB is a subset of the mer operon and is capable of catalyzing the breakdown of various forms of organic mercury to Hg^{2+}. MerB encodes an enzyme, organomercurial lyase, that catalyses the protonolysis of the carbon-mercury bond. One of the products of this reaction is ionic mercury (Rugh et al 1996; Heaton et al. 1998; Pilon-Smits and Pilon, 2000):

Hg^{2+}. R-CH_2-Hg^+ ----merB---->R-CH_3 + Hg(II)

Hg(II) + NADPH ----merA---->Hg(0) + $NADP^+$ + H^+

Hg (0) (elemental mercury) can be volatilized by the cell

Chelator enhanced phytoremediation technology

Use of soil amendments such as synthetics (ammonium thiocyanate) and natural zeolites have yielded promising results (Huang et al. 1996; Anderson et al. 1998; Churchmann et al. 1999; Huttermann, 1999; Zorpas et al. 1999). EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate and acetate etc. have been used as chelators for rapid mobility and uptake of metals from contaminated soils by plants. Use of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots facilitating phytoextraction of the metals from low grade ores. Synthetic cross-linked polyacrylates, hydrogels have protected plant roots from heavy metals toxicity and prevented the entry of toxic metals into roots. Application of low cost the synthetics and natural zeolites on large scale are applied to the soil through irrigation at specific stages of plant growth which might be beneficial to accelerate metal acumulation (Blaylock et al. 1997).

A major factor influencing the efficiency of phytoextraction is the ability of plants to absorb large quantities of metal in a short period of time. Hyperaccumulators accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil (Baker, 1981), as long as the metal in question is present. This property is unlike moderate accumulators now being used for phytoextraction where the quantity of absorbed metal is a reflection of the concentration in the soil. Although the total soil metal content may be high, it is the fraction that is readily available in the soil solution that determines the efficiency of metal absorption by plant roots. To enhance the speed and quantity of metal removal by plants, some researchers advocate the use of various chemicals for increasing the quantity of available metal for plant uptake. Chemicals that are suggested for this purpose include various acidifying agents (Brown et al. 1994; Cunningham and Ow, 1996; Huang et al. 1998; Blaylock and Huang, 2000; Chen et al.

2000; Kamnev and Van de Lelie, 2000; Chen and Cutright, 2001), fertilizer salts (Lasat et al. 1997; Lasat et al. 1998) and chelating materials (Blaylock et al. 1997; Huang et al. 1997a). These chemicals increase the amount of bioavailable metal in the soil solution by either liberating or displacing metal from the solid phase of the soil or by making precipitated metal species more soluble. Research in this area has been moderately successful, but the wisdom of liberating large quantities of toxic metal into soil water is questionable.

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake (Marschner, 1995). Under acidic conditions, H^+ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed (*i.e*. specific adsorption; McBride, 1994). The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn (McBride, 1994; Blaylock and Huang, 2000). It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil (Brown et al. 1994; Salt et al. 1995a; Blaylock and Huang, 2000). Possible amendments for acidification include NH_4-containing fertilizers, organic and inorganic acids, and elemental S. Trelease and Trelease, 1935 indicated that plant roots acidify hydroponic solutions in response to NH_4nutrition and cause solutions to become more alkaline in response to NO_3nutrition. Metal availability in the soil can be manipulated by the proper ratio of $NO_3$to NH_4used for plant fertilization by the effect of these N sources on soil pH, but no phytoremediation research has been conducted on this topic to date. The acidification of soil with elemental S is a common agronomic practice, which can be used to mobilize metal cations in soil. Brown et al. 1994 acidified a Cd- and Zn-contaminated soil with elemental S and observed that accumulation of these metals by plants was greater than when the amendment was not used. Acidifying agents are also used to increase the availability of radioactive elements in the soil for plant uptake. Huang et al. 1998 reported that the addition of citric acid increases U accumulation in Indian mustard (*B. juncea*) tissues more than nitric or sulfuric acid although all acids decrease soil pH by the same amount (Figure 13). These authors speculated that citric acid chelates the soil U, thereby enhancing its solubility and availability in the soil solution. The addition of citric acid causes a 1000-fold increase of U in the shoots of *B. juncea* compared to accumulation in the control (no citric acid addition). Despite the promise of some acidifying agents for use in phytoextraction, little research is reported on this subject.

The addition of chelating materials to soil, such as EDTA, HEDTA, and EDDHA, is the most effective and controversial means of liberating labile metal-contaminants into the soil solution. Chelates complex the free metal ion in solution, allowing further dissolution of the sorbed or precipitated phases until an equilibrium is reached between the complexed metal, free metal, and insoluble metal fraction (Norvell, 1991). Chelates are used to enhance the phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb, and Zn (Blaylock et al. 1997; Huang et al. 1997a; Huang et al. 1997b). Huang et al. 1997a suggested that chelates are able to induce Pb accumulation in agronomic crops such as corn (*Zea mays* L.) and pea (*Pisum sativum* L.). These authors reported a 1000-fold increase of Pb in the soil solution after HEDTA application in comparison to soil solution of a control (no HEDTA addition). Under these conditions Pb concentrations in the shoots of corn and pea increases from less than 500 mg A kg - to more than 10,000 mg A kg^{-1} within one week after HEDTA application. This chelate-assisted accumulation of toxic quantities of metal in a non-accumulator species is termed "chelate-induced hyperaccumulation" (Huang et al. 1997a). These researchers explained that when chelate-induced hyperaccumulation is the goal, metals on site are initially immobilized to allow for rapid establishment and growth of an agronomic crop such as corn. When the crop accumulates sufficient biomass, chelating materials are applied to the soil to result in the liberation of large quantities of metal into the soil solution. Massive amounts of metal are absorbed by plant roots and are translocated to the shoot tissue where they accumulate to toxic levels. After death, plants are harvested and removed from the site. Chelate-induced hyperaccumulation is in contrast to the normal practice of phytoextraction where plants are given a gradual exposure to non-toxic quantities of metal in solution, and accumulation occurs gradually over time as the plants grow. The controversy surrounding the use of chelates deals with the fate of the residual chelate in the soil after metal absorption occurs (Brooks, 1998a). The massive liberation of chelate-bound metals into the soil solution makes them subject to leaching into deeper soil layers. Metals which migrate downward beyond the root zone of plants cannot be recovered through means of phytoremediation and may require the use of more expensive conventional remediation methods. The primary concern is that the liberated metals have the ability to migrate into uncontaminated areas, possibly groundwater reservoirs (Cunningham et al. 1997). The scientific literature lacks appreciable information concerning the appropriate amount of chelate to apply under different levels of contamination and for different plant species. Further research is required to determine the fate of the chelate-metal complex in soil before the use of these amendments are accepted widely for use in phytoextraction. Some positively charged metals and radionuclides may be bound to the soil CEC by weak electrostatic forces and may be displaced by other cations in the soil solution (Sparks, 1995).

As a plant- based technology, the success of phytoextraction is inherently dependent upon proper plant

selection. As previously discussed, plants used for phytoextraction must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissue (Kumar et al. 1995a; Cunningham and Ow, 1996; Blaylock et al. 1997; McGrath, 1998). Many plant species have been screened to determine their usefulness for phytoextraction. Researchers initially applied hyperaccumulators to clean metal polluted soils (Chaney, 1983). At present, there are nearly 400 known hyperaccumulators (Salt and Kramer, 2000), but most are not appropriate for phytoextraction because of their slow growth and small size. Several researchers have screened fast-growing, high- biomass accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Dushenkov et al. 1995; Kumar et al. 1995a; Salt et al. 1995b; Bañuelos et al. 1997; Blaylock et al. 1997; Ebbs et al. 1997; Ebbs and Kochian, 1998; Huang et al. 1997a; Huang et al. 1997b; Lasat et al. 1997; Ebbs and Kochian, 1998; Lasat et al. 1998). Many metal-tolerant plant species, particularly grasses, escape toxicity through an exclusion mechanism and are therefore better suited for phytostabilization than phytoextraction (Baker, 1981; Ebbs et al. 1997). However, barley (Hordeum vulgare L.) and oat (Avena sativa L.) are tolerant of metals such as Cu, Cd, and Zn, and accumulate moderate to high amounts of these metals in their tissues (Ebbs and Kochian, 1998). Many herbaceous species, including members of the Brassicaceae, also accumulate moderate amounts of various metals in their shoots. A list of promising plant species for phytoextraction of metals and radionuclides is given (Table 3). One of the most promising, and perhaps most studied, non- hyperaccumulator plant for the extraction of heavy metals from contaminated sites is Indian Mustard (*B. juncea*). Many hyperaccumulators belong to the Brassica family. Once it was suspected that known hyperaccumulators were not suited for phytoextraction, researchers looked to other high biomass- accumulating members of the Brassicaceae for plants which accumulated large quantities of toxic metals (Dushenkov et al. 1995; Kumar et al. 1995a). Kumar et al. 1995a tested many fast growing Brassicas for their ability to tolerate and accumulate metals, including Indian mustard (*B. juncea*), black mustard (*Brassica nigra* Koch), turnip (*Brassica campestris* L.), rape (*Brassica napus* L.), and kale (*Brassica oleracea* L). Although all Brassicas accumulated metal, *B. juncea* showed a strong ability to accumulate and translocate Cu, Cr VI, Cd, Ni, Pb, and Zn to the shoots. Kumar et al. 1995a also investigated possible genetic variation of different *B. juncea* accessions in hope of finding some that had more phytoextraction potential than others. The term, accession, refers to seeds that have been gathered from a particular area and are now part of a collection at a seed bank or plant- introduction laboratory/institute. Once in the collection, seeds are assigned a number that identifies the particular accession. Although all Indian mustard accessions are *B. juncea* Czern., they may exhibit different phenotypes as a result of being from different regions where environmental factors may have influenced the natural selection of this species. Kumar et al. 1995a determined that accessions 426308, 211000, 426314, and 182921 are among the best suited for phytoextraction. Several researchers have confirmed the phytoremediation potential of these and other *B. juncea* accessions (Dushenkov et al. 1995; Salt et al., 1995b; Blaylock et al. 1997; Ebbs and Kochian, 1998). The USDA-ARS Plant Introduction Station of Iowa State now maintains, and distributes, metal-accumulating accessions which are considered useful for phytoremediation. Indian mustard is an oilseed *Brassica* crop for which cultivation extends from India through western Egypt and Central Asia to Europe (Nishi, 1980)

According to Prakash, 1980, the oldest reference to *B. juncea* in Sanskrit literature is by the name `Rajika', and carbonized seeds of this species have been found in the ancient sites of the Harappan civilization (2300-1750 B.C.). Despite the efforts of historians and researchers, the precise origin of this crop remains an enigma. Perhaps the most likely place or places of origin are those regions where its parents, *B. nigra* and *B. campestris*, overlap in their distribution. Possible centers of origin include Africa (Zeven and Zhukovsky, 1975), China (Chen et al. 1995), the Middle East, Southwest Asia, and India (Sauer, 1993). Indian mustard is eaten as a leafy vegetable in China but is grown in India primarily for its oil-containing seeds (~ 40% oil; Prakash, 1980), which serve as a source of cooking oil and spice (Nishi, 1980; Krzymañski, 1997). Indian mustard is capable of producing 18 tons of biomass per hectare per crop (Kumar et al. 1995a). Plants perform very well in nutrient solution culture, progressing from the four- leaf stage to fully grown plants (up to 50 g shoot fresh mass) in as little as 21 days (personal observations). Although short day conditions (<12 hrs light) result in a more compact growth habit, shorter height, and limited leaf production (Bhaskar and Vora, 1994), biomass accumulation is greater than under long day conditions (9-10 hrs light optimal; Neelam et al. 1994). Long day conditions promote early flowering (Bhaskar and Vora, 1994) but are not required for flower development. These plants have indeterminate growth and continue to branch from the nodes and to accumulate biomass after flower and siliquae (seed pod) development. The recommended fertility rate for maximum growth of *B. juncea* under un-contaminated conditions is 75 to120 kg N ha^{-1} and 30 to 50 kg P_2O_5 per hectare (Thakral et al. 1995; Gurjar and Chauhan, 1997; Tomar et al. 1997). Zaurov et al. 1999 reported that biomass accumulation *of B. juncea* was greatest when plants in soil are supplied with 200 kg N, 100 kg P_2O_5, and 66 kg K_2 O per hectare. However, Cd concentration in the tissue was greatest when no N was supplied. Indian mustard is given considerable attention by present day researchers, geneticists, and plant breeders in particular, because of its unique polyploid genome. Several accessions of *B. juncea* have been identified as moderate accumulators of metallic elements and are maintained by the USDA- ARS Plant Introduction Station at Iowa State University, Ames, Iowa. The benefit of *using B. juncea* seed from the plant introduction station is that the genetic integrity of the accessions is preserved

through appropriate breeding techniques. Experiments that utilize these seeds have more precision than those conducted with seeds from commercially available sources. Precision is also greater, because future researchers can obtain the same accessions for their experiments. The USDA-ARS Plant Introduction Station maintains a world-wide collection of *B. juncea* accessions that are known metal-accumulators, and the seeds are distributed to public and private research institutions at no cost.

CONCLUDING REMARKS

There are certain limitations to implement phytoremediation with the use of biodiversity (Cunningham et al 1995; Cunningham and Ow, 1996; Chaney et al. 1997; Clemens et al. 2002). To a considerable extent theseinclude: potential contamination of the vegetation and food chain, and often extremely difficult to establish and maintain vegetation on contaminated sites for. *i.e.* mine tailings with high level of residual metals. For metal contaminants, plants show the potential for phytoextraction (uptake and recovery of contaminants into above-ground biomass) (Anderson et al. 1998; Bañuelos et al. 1999; Huang and Cunningham, 1996), filtering metals from water onto root systems (rhizofiltration), or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water (phytostabilization) (Terry and Bañuelos, 2000; Heijden and Sanders, 2002). After the plants have been allowed to grow for some time, they are harvested and either incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfil. Finally, phytoremediation in some countries has limited acceptance by the local government and takes long duration of time to mitigate the contaminant. Metal hyperaccumulators are generally slow-growing with a small biomass and shallow root systems. Plant biomass must be harvested and removed, followed by proper disposal. Plants experience stress due to prevailing high concentrations of metals.

One of the main advantages of phytoextraction is, that the plant biomass containing the extracted contaminant can be a resource. For example, i) biomass that contains selenium (Se), an essential nutrient, has been transported to areas that are deficient in Se and used for animal feed (Bañuelos and Meek, 1989; Bañuelos et al. 1993a), ii) metal hyperaccumulators are of special significance in biogeochemical prospecting of minerals.

Rhizofiltration has the following limitations i) terrestrial or aquatic plants are used for this purpose. Although terrestrial plants require support, such as a floating plat-form, they generally remove more contaminants than aquatic plants. This system can be either *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system). An ex situ system can be placed any where because the treatment does not have to be at the original location of contamination (Dushenkov et al. 1995; Dushenkov et al. 1997b).

Rhizofiltration has the following disadvantages: i) the pH of the influent solution may have to be continually adjusted to obtain optimum metals uptake. ii) the chemical speciation and interaction of all species in the influent have to be understood for proper application, iii) A well-engineered system is required to control influent concentration and flow rate, iv) plants (especially terrestrial plants) may have to be grown in a greenhouseor nursery and then placed in the rhizofiltration system, v) periodic harvesting and plant disposal are required, vi) metal immobilization and uptake results from laboratory and greenhouse studies might not be achievable in the field.

In phytovolatilization has the following advantages: i) contaminants could be transformed to less toxic forms, such as elemental mercury and dimethyl selenite gas; ii) contaminants ormetabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation (Azaizeh et al. 1997).

Phytovolatilization limitations: i) the contaminant (such as Se) might be released into the atmosphere (Azaizeh et al. 1997; Bañuelos et al. 1993a; Bañuelos et al. 1999).Therefore, adequate planning in needed for phytoremediation-based systems integrated with the environment *i.e.*, green belts (invaluable ecological niches, particularly in urban industrial areas); constructed wetlands in which *Eichhornia crassipes* (water hyacinth), *Hydrocotyle umbellata* (pennywort), *Lemma minor* (duckweed) and *Azolla pinnata* (water velvet), are maintained and managed which can take up Pb, Cu, Cd, Fe and Hg from aqueous solutions (Carbonell, 1998).

Nicotianamine (NA), a plant nonproteinogenic amino acid is an efficient complexing agent for Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and other divalent transition metals. Genetic manipulation of genes involved in the biosynthesis of metal sequestering compounds and introduction to desirable plant species might attract phytoremediation strategies (Prasad and Strazalka, 2002). It is very advantageous to use commonly cultivated crops such as *Brassica juncea, Armoracia rusticana* and *Helianthus annuus*, and which are reported to accumulate many toxic metals. Plants that are amenable to genetic manipulation and *in vitro* culture play significant role for the success of phytoremediation (Figure 14) (Gleba et al. 1999).

REFERENCES

ALCANTARA, E.; BARRA, R.; BENLLOCH, M.; GINHAS, A.; JORRIN, J.; LOPEZ. J.A.; LORA, A.; OJEDA, M.A.; PUJADAS, A.; REQUEJO, R.; ROMERA, J.; SANCHO, E.D.; SHILEV, S. and TENA, M. Phytoremediation of a metal contaminated area in southern

Spain. In: *Intercost workshop*. (15th - 18th November, 2000, Sorrento, Italy). 2000. p. 121-123.

ANDERSON, C.W.N.; BROOKS, R.R.; STEWART, R.B. and SIMCOCK, R. Harvesting a crop of gold in plants, *Nature,* 1998, vol. 395, no. 6702, p. 553-554.

ARAZI, T.; SUNKAR, R.; KAPLAN, B. and FROMM, H. A tobacco plasma membrane calmodulin-binding transporter confers Ni^{2+} tolerance and Pb^{2+} hypersensitivity in transgenic plants. *The Plant Journal*, 1999, vol. 20, no. 2, p. 171-82.

ARIANOUTSOU, M.; RUNDEL, P.W. and BERRY, W.L. Serpentine endemics as biological indicators of soil elemental concentrations. In: Markert ed. *Plants as Biomonitors. Indicators for Heavy Metals in the Terrestrial Environment*. VCH-Publisher, Weinheim, 1993, p. 179-189.

ARISI, A.C.M.; MOCQUOT, B.; LAGRIFFOUL, A.; MENCH, M.; FOYER, C.H. and JOUANIN, L. Responses to cadmium in leaves of transformed poplars overexpressing g-glutamylcysteine synthetase. *Physiologia Plantarum*, 2000, vol. 109, no. 2, p. 143-149.

ARISI, A.C.M.; NOCTOR, G.; FOYER, C.H. and JOUANIN, L. Modification of thiol contents in poplars (*Populus tremula* x *P. alba*) overexpressing enzymes involved in glutathione synthesis. *Planta*, 1997, vol. 203, no. 3, p 362-372.

ATKINSON, R.; ASCHMANN, S.M.; HASEGAWA, D.; EAGLE-THOMPSON, E.T. and FRANKENBERGER, JR. W.T. Kinetics of the atmospherically important reactions of dimethylselenide. *Environmental Science and Technology*, 1990, vol 24, p.1326-1332.

AZAIZEH, H.A.; GOWTHAMAN, S. and TERRY, N. Microbial selenium volatilization in rhizosphere and bulk soils from a constructed wetland. *Journal of Environmental Quality*, 1997, vol. 26, no. 3, p. 666-672.

BADRI, M. and SPRINGUEL, I. Biogeochemical prospecting in the south-easter desert of Egypt. *Journal of Arid Environment*, 1994, vol. 28, p. 257-264.

BAKER, A.J.M.; REEVES R.D. and MCGRATH S.P. *In situ* decontamination of heavy metal polluted soils using crops of metal-accumulating plants: a feasibility study. In: R.L. Hinchee and R.F. Olfenbuttel eds. *In situ bioreclamation*. Boston, Butterworth - Heinemann, 1991, p. 600-605.

BAKER, A.J.M. and BROOKS, R.R. Terrestrial higher plants which hyperaccumulate metal elements: A review of their distribution, ecology, and phytochemistry. *Biorecovery*, 1989, vol. 1, p. 81-126.

BAKER, A.J.M. Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, 1981, vol. 3, p. 643-654.

BAÑUELOS, G.S.; SHANNON, M.C.; AJWA, H.; DRAPER, J.H.; JORDAHL, J. and LICHT, L. Phytoextraction and accumulation of boron and selenium by poplar (*Populus*) hybrid coles. *International Journal of Phytoremediation*, 1999, vol. 1, no. 1, p. 81-96.

BAÑUELOS, G.S.; AJAW, H.A.; MACKEY, B.; WU, L.; COOK, C.; AKOHOUE, S. and ZAMBRUZUSKI, S. Evaluation of different plant species used for phytoremediation of high soil selenium. *Journal of Environmental Quality,* 1997, vol 26, no. 3, p. 639-646.

BAÑUELOS, G.S.; CARDON, G.; MACKEY, B.; BEN-ASHER, J.; WU, L.P.; BEUSELINCK, P., AKOHOUE, S. and ZAMBRZUSKI, S. Boron and selenium removal in B-laden soils by four sprinkler irrigated plant species. *Journal of Environmental Quality*, 1993a, vol. 22, no. 4, p. 786-797.

BAÑUELOS, G.S.; MEAD, R.R. and HOFFMAN, G.J. Accumulation of selenium in wild mustard irrigated with agricultural effluent. *Agriculture, Ecosystems & Environment,* 1993b, vol. 43, no. 2, p. 119-126.

BAÑUELOS, G.S. and MEEK, D.W. Selenium accumulation in selected vegetables. *Journal of Plant Nutrition,* 1989, vol. 12, no. 10, p. 1255-1272.

BARGAGLI, R. Trace elements in terrestrial plants - an ecophysiological approach to biomonitoring and biorecovery. Heidelberg, Springer-Verlag, 1998. 324 p.p. ISBN3-540-64551-9.

BEGONIA, G.B.; DAVIS, C.D.; BEGONIA, M.F.T. and GRAY, C.N. Growth responses of Indian Mustard [*Brassica juncea* (L.) Czern.] and its phytoextraction of lead from a contaminated soil. *Bulletin of Environmental Contamination and Toxicology*, 1998, vol. 61, p. 38-43.

BERTI, W.R. and CUNNINGHAM, S.D. Phytostabilization of metals. In: I. Raskin and B.D. Ensley eds. *Phytoremediation of toxic metals: using plants to clean-up the environment.* New York, John Wiley & Sons, Inc., 2000, p. 71-88.

BHASKAR, V.V. and VORA, A.B. Growth and yield analysis of *Brassica juncea* L. under three photoperiods. *Journal of Environmental Biology*, 1994, vol. 15, p. 55-61.

BIZILY, S.P.; RUGH, C.L.; SUMMERS, A.O. and MEAGHER, R.B. Phytoremediation of methylmercury pollution: *Mer B* expression in *Arabidopsis thaliana* confers resistance to organomerculials. *Proceedings of the National Academy of Sciences of the United States of America,* 1999, vol. 96, p. 6808-6813.

BLAYLOCK, M.J. and HUANG, J.W. Phytoextraction of metals. In: I. Raskin and B.D. Ensley eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, John Wiley & Sons, Inc., 2000, p. 53-70.

BLAYLOCK, M.J.; SALT, D.E.; DUSHENKOV, S.; ZAKHAROVA, O.; GUSSMAN, C.; KAPULNIK, Y.; ENSLEY, B.D. and RASKIN. I. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environmental Science and Technology*, 1997, vol. 31, no. 3, p.860- 865.

BOYD, R.S.; SHAW, J.J. and MARTENS, S.N. Nickel hyperaccumulation in *S. Polygaloids* (Brassicaceae) as a defense against pathogens. *American Journal of Botany*, 1994, vol. 81, no. 3, p. 294-300.

BROOKS, R.R. General Introduction. In: R.R. Brooks ed. *Plants that hyperaccumulate heavy metals:their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining.* New York, CAB International, 1998a, p. 1-14.

BROOKS, R.R. (ed). *Plants that hyperaccumulate heavy metals*. Wallingford, CAB International. 1998b, p. 384.

BROOKS, R.R. *Serpentine and its vegetation: A multidisciplinary approach*. Oregon, Portland, Discorides Press, 1987, p. 454.

BROOKS, R.R. *Biological Methods of Prospecting for Minerals.* New York, Wiley-Interscience, 1983. pp. 313.

BROWN, S.L.; CHANEY, R.L.; ANGLE, J.S. and BAKER, A.J.M. Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium-contaminated soil. *Journal of Environmental Quality*, 1994, vol. 23, no. 6, p. 1151-1157.

CAI, X.H.; BOWN, C.; ADHIYA, J.; TRAINA, S.J. and SAYRE, R.T. Growth and heavy metal binding properties of transgenic *Chlamydomonas* expressing a foreign metallothionein gene. *International Journal of Phytoremediation*, 1999, vol. 1, no. 1, p. 53-65.

CAKMAK, I. and MARSCHNER, H. Mechanism of phosphorus-induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants. *Physiologia Plantarum,* 1987, vol. 70, p. 13-20.

CARBONELL, A.A.; AARABI, M.A.; DELAUNE, R.D.; GAMBRELL, R.P. and PATRICK, JR., W.H. Bioavailability and uptake of arsenic by wetland vegetation: Effects on plant growth and nutrition. *Journal of Environmental Science and Health,* 1998, vol. A33, no. 1, p. 45- 66.

CARMAN, E.P. CROSSMAN, T.L. and GATLIFF, E.G. Phytoremediation of no. 2 fuel oil- contaminated soil. *Journal of Soil Contamination*, 1998, vol. 7, p. 455-466.

CHANEY, R.L.; MALIK, M.; LI, Y.M.; BROWN, S.L.; BREWER, E.P.; ANGLE J.S. and BAKER, A. J.M. Phytoremediation of soil metals. *Current Opinions in Biotechnology*, 1997, vol. 8, no. 3, p 279.

CHANEY, R.L. Plant uptake of inorganic waste constitutes. In: PARR, J.F.; MARSH, P.B. and KLA, J.M. eds. *Land treatment of hazardous wastes.* Park Ridge, NJ, Noyes Data Corp., 1983, p. 50-76.

CHEN, H. and CUTRIGHT, T. EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus*. *Chemosphere*, 2001, vol. 45, no. 1, p. 21-28.

CHEN, H.M.; ZHENG, C.R.; TU, C. and SHEN, Z.G. Chemical methods andphytoremediation of soil contaminated with heavy metals. *Chemosphere*, 2000, vol. 41, no. 1-2, p. 229-234.

CHEN, C.L.; ZHOU, G.F.; FAN, Y.H.; ZHOU, Y. and CHEN, X.Q. Discussion on the origin of mustard (*Brassica juncea*) in China. *Acta horticulturae*, 1995, vol. 402, p. 317- 320.

CHURCHMANN, G.J.; SLADE, P.G.; RENGASAMY, P.; PETER, P.; WRIGHT, M. and NAIDU, R. Use of fine grained minerals to minimize the bioavaliability of metal contaminants. Environmental impacts of metals. Int Workshop, Tamil Nadu Agricultural University, Coimbatore, India, 1999, p. 49-52.

CLEMENS, S.; PALMGREN, M.G. and KRÄMER, U. A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science*, 2002, vol. 7, p. 309-315.

CLEMENS, S. Moleclar mechanisms of plant metal tolerance and homeostasis. *Planta*, 2001, vol. 212, no. 4, p. 475-486.

CLEMENS, S.; KIM, E.J.; NEUMANN, D. and SCHROEDER, J.I. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. *EMBO J.,* 1999, vol. 18, no. 12, p. 3325-3333.

COLE, S. The emergence of treatment wetlands. *Environmental Science and Technology,* 1998, vol. 32, no. 9, p. 218A-223A.

COMIS, D. Green remediation: Using plants to clean the soil. *Journal of soil and water conservation,* 1996, vol. 51, no. 3, p. 184-187.

COMIS, D. Metal-scavenging plants to cleanse the soil. *Agricultural Research*, 1995, vol. 43, p. 4-9.

CRITES, R.W.; DOMBECK,G.D. and WILLIAMS, C.R. Removal of metals and ammonia in constructed wetlands. *Water Environment Research,* 1997, vol. 69, no. 2, p. 132-136.

CUNNINGHAM, S.D.; SHANN, J.R.; CROWLEY, D.E. and ANDERSON, T.A. Phytoremediation of contaminated water and soil. In: KRUGER, E.L.; ANDERSON, T.A. and COATS, J.R. eds. Phytoremediation of soil and water contaminants. ACS symposium series 664. Washington, DC, American Chemical Society, 1997, p. 2-19.

CUNNINGHAM, S.D. and OW, D.W. Promises and prospects of phytoremediation. *Plant Physiology*, 1996, vol. 110, no. 3, p. 715-719.

CUNNINGHAM, S.D.; BERTI, W.R. and HUANG, J.W. Phytoremediation of contaminated soils. *Trends in Biotechnology,* 1995, vol. 13, no. 9, p. 393-397.

DESOUZA, M.P.; PILON-SMITS, E.A.H. and TERRY, N. The physiology and biochemistry of selenium volatilization by plants. In: RASKIN, I. and ENSLEY, B.D. eds. Phytoremediation of toxic metals: using plants to clean-up the environment. New York, John Wiley & Sons, Inc., 2000, p. 171-190.

DESOUZA, M.P.; HUANG, C.P.; CHEE, N. and TERRY, N. Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. *Planta*, 1999, vol. 209, no. 2, p. 259-263.

DELORME, T.A.; GAGLIARDI, J.V.; ANGLE, J.S. and CHANEY, R.L. Influence of the zinc hyperaccumulator *Thlaspi caerulescens* J. & C. Presl. and the nonmetal accumulator *Trifolium pratense* L. on soil microbial populations. *Canadian Journal of Microbiology*, 2001, vol. 47, no. 8, p. 773-776.

DIERBERG, F.E.; DEBUSK, T.A. and GOULET JR., N.A. Removal of copper and lead using a thin-film technique.In: REDDY, K.R. and SMITH W.H. eds. *Aquatic plants for water treatment and resource recovery*. Magnolia Publishing, 1987, p. 497-504.

DUSHENKOV, S. and KAPULNIK, Y. Phytofilitration of metals. In: RASKIN, I.and Ensley, B.D. eds. *Phytoremediation of toxic metals - using plants to clean-up the environment*. New York, John Wiley & Sons, Inc., 2000, p. 89-106.

DUSHENKOV, S.; VASUDEV, D.; KAPULNIK, Y.; GLEBA, D.; FLEISHER, D.; TING K.C. and ENSLEY. B. Removal of uranium from water using terrestrial plants. *Environmental Science and Technology*, 1997a, vol. 31, no. 12, p. 3468-3474.

DUSHENKOV, S.; VASUDEV, D.; KAPULNIK, Y.; GLEBA, D.; FLEISHER, D.; TING, K.C. and ENSLEY, B. Phytoremediation: A novel approach to an old problem. In: WISE, D.L. ed. *Global environmental biotechnology*. Amsterdam, Elsevier Science B.V., 1997b, p. 563-572.

DUSHENKOV, V.; KUMAR P.B.A.N.; MOTTO, H. and RASKIN, I. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environmental Science and Technology*, 1995, vol. 29, p. 1239-1245.

EBBS, S.D. and KOCHIAN. L.V. Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). *Environmental Science and Technology*, 1998, vol. 32, no. 6, p. 802-806.

EBBS, S.D.; LASAT, M.M.; BRANDY, D.J.; CORNISH, J.; GORDON, R. and KOCHIAN, L.V. Heavy metals in the environment: Phytoextraction of cadmium and zinc from a contaminated soil. *Journal of Environmental Quality,* 1997, vol. 26, p. 1424-1430.

ENSLEY, B.D. Rational for use of phytoremediation. In: RASKIN, I. and ENSLEY, B.D. eds. *Phytoremediation of toxic metals: using plants to clean- up the environment*. New York, John Wiley & Sons, Inc., 2000, p. 3-12.

ENTRY, J.A.; WATRUD, L.S. and REEVES, M. Accumulation of ^{137}Cs and ^{90}Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi. *Environmental Pollution*, 1999, vol. 104, p. 449-457.

EPA (Environmental Protection Agency). Electrokinetic laboratory and field processes applicable to radioactive and hazardous mixed waste in soil and groundwater. EPA 402/R- 97/006. Washington, DC. 1997.

ERNST, W.H.O., Revolution of metal hyperaccumulation and phytoremediation hype. *New Phytologists*, 2000, vol 146, p. 357-358.

FLATHMAN, P.E. and LANZA, G.R. Phytoremediation: current views on an emerging green technology. *Journal of Soil Contamination*, 1998, vol. 7, no. 4, p. 415-432.

FREITAS, H., PRASAD, M.N.V. and PRATAS, J. Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications. *Environment International*, 2004a, vol. 30, p. 65-72.

FREITAS, H; PRASAD, M.N.V. and PRATAS, J. Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation – relevance to the management of mine environment. *Chemosphere*, 2004b, vol. 54, p. 1625-1642.

FREITAS, H.; NABAIS, V. and PAIVA, J. Heavy metals pollution in the urban areas and roads of Portugal using *Nerium oleander* L. In: *Proceedings of the International Conference Heavy metals in the Environment*. (8º, 12th - 16th September, 1991) CEP consultants Ltd. Edinburgh. FARMER, J.G. ed., 1991. vol. 1, p. 240-242.

GLASS, D.J. Economic potential of phytoremediation. In: RASKIN, I. and ENSLEY, B.D. eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, John Wiley & Sons, 2000a, p. 15-32.

GLASS, D.J. *The 2000 Phytoremediation Industry*. Needham, Mass., D. Glass Associates, 2000b, p. 100. chapter or book?.

GLASS, D.J. *U.S. and international markets for phytoremediation , 1999-2000.* Needham, Mass., D. Glass Associates, 1999, p. 266.

GLEBA, D.; BORISJUK, M.V.; BORISJUK, L.G.; KNEER, R.; POULEV, A.; SKARZHINSKAYA, M.; DUSHENKOV, S.; LOGENDRA, S.; GLEBA, Y.Y. and RASKIN, I. Use of plant roots for phytoremediation and molecular farming. *Proceedings of the National Academy of Sciences of the United States of America*, 1999, vol. 96, no. 11, p. 5973-5977.

GORDON, M.; CHOE, N.; DUFFY, J.; EKUAN, G.; HEILMAN, P.; MUIZNIEKS, I.; NEWMAN, L.; RUSZAJ, M.; SHURTLEFF, B.B.; STRAND, S. and WILMOTH J. Phytoremediation of trichloroethylene with hybrid poplars. In: KRUGER, E.L.; ANDERSON, T.A. and COATS, J.R. eds. *Phytoremediation of soil and water contaminants*. Washington, DC, American Chemical Society, 1997, p. 177-185.

GURJAR, B.S. and CHAUHAN, D.V.S. Yield attributes and seed yield of Indian mustard (*Brassica juncea*) as influenced by varieties, fertility levels and spacing in Harsi Command area. *Indian Journal of Agronomy*, 1997, vol. 42, no. 1, p. 142-144.

HAMLIN, R.L. Phytoremediation literature review. Environmental awareness in the United States. 2002. Available from: http://www.umass.edu/umext/soilsandplant/.

HANSEN, D.; DUDA, P.J.; ZAYED, A. and TERRY, N. Selenium removal by constructed wetlands: role of biological volatilization. *Environmental Science and Technology*, 1998, vol. 32, no. 5, p. 591-597.

HARRISON, S. Local and regional diversity in a patchy lanscape: Native, alien, and endemic herbs on serpentine. *Ecology*, 1999, vol. 80, p. 70-80.

HEATON, A.C.P.; RUGH, C.L.; WANG, N. and MEAGHER. R.B. Phytoremediation of mercury - and methylmercury - polluted soils using genetically engineered plants. *Journal of Soil Contamination*, 1998, vol. 7, no. 4, p. 497-510.

HEIJDEN, M.G.A. and SANDERS, I.R. (eds). *Mycorrhizal Ecology*. Heidelberg, Springer-Verlag, 2002, 469 p. ISBN 3-54-042407-5.

HUANG, J.W.; BLAYLOCK, M.J.; KAPULNIK, Y. and ENSLEY, B.D. Phytoremediation of Uranium-Contaminated Soils: role of organic acids in triggering uranium hyperaccumulation in plants. *Environmental Science and Technology*, 1999, vol. 32, no. 13, p. 2004-2008.

HUANG, J.W.; CHEN, J.; BERTI, W.R. and CUNNINGHAM, S.D. Phytoremediation of lead contaminated soil: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology,* 1997a, vol. 31, no. 3, p. 800-805.

HUANG, J.W.; CHEN, J. and CUNNINGHAM, S.D. Phytoextraction of lead from contaminated soils. In: KRUGER, E.L.; ANDERSON, T.A. and COATS, J.R., eds. *Phytoremediation of soil and water contaminants*. Washington, DC, American Chemical Society, 1997b. p. 283-298

HUANG, J.W. and CUNNINGHAM, S.D. Lead phytoextraction: Species variation in lead uptake and translocation. *New Phytologists*, 1996, vol. 134, p. 75-84.

HUTTERMANN, A.; ARDUINI, I. and GODBOLD, D.L. Metal pollution and forest decline. In: Prasad, M.N.V. and Hagemeyer, J. *Heavy metal stress in plants - From molecules to ecosystems*. Berlin, Heidelberg, New York, Springer-Verlag, 1999, p. 253-272.

KADLEC, R.H.; KNIGHT, R.L.; VYMAZAL, J.; BRIX, H.; COOPER, P. and HABERT, R. *Constructed wetlands for pollution control: processes, performance, design and operation.* London, IWA publishing, 2000, 156 p. ISBN 1900222051.

KALTSIKES, P.J. *Phytoremediation - State of the art in Europe, an international comparison.* In: *COST Action 837, Workshop*. (6^{th} - 8^{th} April, 2000, Crete, Grece). Agricultural University of Athens, 2000.

KAMNEV, A.A. and VAN DER LELIE, D. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Bioscience Reports*, 2000, vol. 20, no. 4, p 239-258.

KARENLAMPI, S.; SCHAT, H.; VANGRONSVELD, J.; VERKLEIJ, J.A.C.; VAN DER LELIE, C.; MERGEAY, M. and TERVAHAUTA, A.I. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environmental Pollution*, 2000, vol. 107, no. 2, p. 225-231.

KAY, S.H.; HALLER, W.T. and GARRARD, L.A. Effect of heavy metals on water hyacinths [*Eichhornia crassipes* (Mart.) Solms]. *Aquatic Toxicology*, 1984, vol 5, p. 117-128.

KRÄMER, U. and CHARDONNENS, A.N. The use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Applied Microbiology and Biotechnology,* 2001, vol. 55, no. 6, p. 661-672.

KRÄMER, U.; SMITH, R.D.; WENZEL,W.; RASKIN, I. and SALT, D.E. The role of metal transport and metal tolerance in nickel hyperaccumulation by *Thlaspi goesingense* Hálácsy. *Plant Physiology,* 1997, vol. 115, no. 4, p.1641-1650.

KRZYMAÑSKI, J. Agronomy of oilseed Brassicas. In: THOMAS, G. and MONTEIRO, A.A. eds. *Proceedings of the international symposium on Brassicas*. Acta Horticultureae, no. 459, ISHS, Rennes, France. 1997, p. 55-60.

KRUCKEBERG, A.R. California serpentines: Flora, vegetation, geology, soils and management problems. Berkeley, University of California Press, Putah and Cache, 1984, 180 p. ISBN 0-52-009701-7.

KUMAR, P.B.A.N; DUSHENKOV, V.; MOTTO, H. and RASKIN, I. Phytoextraction: The use of plants to remove heavy metals from soils. *Environmental Science and Technology*, 1995a, vol. 29, no. 5, p. 1232-1238.

KUMAR, P.B.A.N.; MOTTO, H. and RASKIN, I. Rhizofiltration: The Use of Plantsto Remove Heavy Metals from Aqueous Streams. *Environmental Science and Technology*, 1995b, vol. 29, no. 5, p.1239-1245.

LAMBERT, M.; PIERZYNSKI, G.; ERICKSON, L. and SCHNOOR, J. Remediation of lead, zinc and cadmium contaminated soils. In: Hester, R.E. and Harrison, R.M., eds. *Issues in environmental science and technology: contaminated land and its reclamation*. Letchworth, U.K., The Royal Society of Chemistry, 1997, p. 91-102.

LASAT, M.M.; PENCE, N.S.; GARVIN, D.F.; EBBS, S.D. and KOCHIAN, L.V. Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*, 2000, vol. 51, no. 342, p. 71-79.

LASAT, M.M.; FUHRMANN, M.; EBBS, S.D.; CORNISH, J.E. and KOCHIAN, L.V. Phytoremediation of a radiocesium contaminated soil: evaluation of cesium- 137 bioaccumulation in the shoots of three plant species. *Journal of Environmental Quality,* 1998, vol. 27, no. 1, p. 165-168.

LASAT, M.M.; NORVELL, W.A. and KOCHIAN, L.V. Potential for phytoextraction of ^{137}Cs from a contaminated soil. *Plant Soil*, 1997, vol. 195, no. 1, p. 99-106.

LEWIS, B.G.; JOHNSON, C.M. and DELWICHE, C.C. Release of volatile selenium compounds by plants: collection procedures and preliminary observations. *Journal of Agricultural and Food Chemistry*, 1966, vol. 14, p. 638- 640.

LIANG ZHU, Y.; PILON-SMITS, E.A.; JOUANIN, L. and TERRY, N. Overexpression of glutathione synthetase in indian mustard enhances cadmium accumulation and tolerance. *Plant Physiology*, 1999, vol. 119, no. 1, p. 73-80.

MA, J.F.; RYAN, P. and DELHAIZE, E. Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Science,* 2001a, vol. 6, no. 6, p. 273-278.

MA, L.Q.; KOMAR, K.M.; TU, C.; ZHANG, W.; CAI, Y. and KENNELLEY, E.D. A fern that hyperaccumulates arsenic. *Nature*, 2001b, vol. 409, no. 6820, p. 579.

MADEP (Massachusetts Department of Environmental Protection Publication). *310 CMR 40.0000: Massachusetts Contingency Plan (MCP)*. Boston, 1993.

MARKERT, B. *Plants as biomonitors: indicators for heavy metals in the terrestrial environment*. New York, VCH Publishers, 1993, 644 p. ISBN 3-52-73000-15.

MARSCHNER, H. *Mineral nutrition of higher plants*. 2nd ed. New York, Academic Press, 1995, 889 p. ISBN 0-12-473542-8.

MCBRIDE, M.B., *Environmental chemistry of soils*. New York, Oxford University Press, 1994, 406 p., ISBN 0-19-507011-9.

MCGRATH, S.P. Phytoextraction for soil remediation. In: BROOKS, R.R., ed. *Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining*. New York, CAB International, 1998, p. 261-288.

MCINNES, B.I.A.; DUNN, C.; CAMERON, E.M. and KAMEKO, L. Biogeochemical exploration for gold in tropical rain forest regions of Papua New Guinea. *Journal of Geochemical Exploration, 1996, vol. 57, no. 1-3, 227-243*.

MEJARE, M. and BŰLOW, L. Metal binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends in Biotechnology,* 2001, vol. 19, no. 2, p. 67-75.

MENGONI, A.; GONNELLI, C.; GALARDI, F.; GABBRIELLI, R. and BAZZICALUPO, M. Genetic diversity and heavy metal tolerance in populations of *Silene paradoxa* L. (Caryophyllaceae): a random amplified polymorphic DNA analysis. *Molecular Ecology*, 2000, vol. 9, no. 9, p. 1319-1324.

MISRA, S. and GEDAMU, L. Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L.

plants. *Theoretical and Applied Genetics*, 1989, vol. 78, p. 161-168.

MO, S.C.; CHOI, D.S. and ROBINSON, J.W. Uptake of mercury from aqueous solution by duckweed: the effect of pH, copper, and humic acid. *Journal of Environmental Health*, 1989, vol. 24, p. 135-146.

MOFFAT, A.S. Engineering plants to cope with metals. *Science*, 1999, vol. 285, p.369-370.

MYERS, N. The biodiversity challenge: expanded hot-spots analysis. *The Environmentalist*, 1990, vol. 10, no. 4, p. 243-256.NEDELKOSKA, T.V. and DORAN, P.M. Hyperaccumulation of cadmium by hairy roots of *Thlaspi caerulescens*. *Biotechnology and Bioengineering*, 2000, vol. 67, no. 5, p. 607-615.

NEELAM, K.; BHARGAVA, S.C.; BHARDWAJ, S.N. and KUMARI, N. Physiological basis of yield and its components in rapeseed-mustard with reference to photoperiod. *Indian Journal of Plant Physiology*, 1994, vol. 37, no. 3, p. 142-146.

NEGRI, C.M. and HINCHMAN, R.R. The use of plants for the treatment of radionuclides. In: RASKIN, I. and ENSLEY, B.D., eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York, Wiley-Interscience, Chapter 8, 2000.

NISHI, S. Differentiation of Brassica crops in Asia and the breeding of `Hakuran': a newly synthesized leafy vegetable. In: Tsunoda, S. et al. ed. *Brassica crops and wild allies: biology and breeding*. Tokyo, *Japanese Science Society Press*, 1980, p. 133-150.

NORVELL, W.A., Reactions of metal chelates in soil and nutrient solution. In: MORTVEDT, J.J. et al., eds. *Micronutrients in agriculture*. 2nd ed. Madison, WI, Soil Science Society of America, 1991, p. 187-228.

NRC. Metals and radionuclides: technologies for characterization, remediation, and containment. In: Groundwater and soil cleanup: improving management of persistent contaminants. Washington, DC, National Academy Press, 1999, p. 72-128.

NRC. Challenges of groundwater and soil cleanup. In: *Innovations in Groundwater and Soil Cleanup*. Washington, DC, National Academy Press, 1997, p. 18-41.

NRIAGU, J.O. Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. *Nature,* 1979, vol. 279, p. 409- 411.

ODUM, H. T.; WOUCIK, W. and PRITCHARD, L. *Heavy metals in the environment: using wetlands for their removal*. Boca Raton, Lewis Publishers, 2000, 326 p. ISBN 1-56-670401-4.

OW, D.W. Heavy metal tolerance genes: prospective tools for bioremediations. *Resources Conservation and Recycling*, 1996, vol. 18, no. 1-4, p. 135-149.

PALMER, E.F.; WARWICK, F and KELLER, W. Brassicaceae (Cruciferae) family, plant biotechnology and phytoremediation. *International Journal of Phytoremediation*, 2001, vol 3, no. 3, p. 245-287.

PENCE, N.S.; LARSEN, P.B.; EBBS, S.D.; LETHAM, D.L.; LASAT, M.M.; GARVIN, D.F.; EIDE, D. and KOCHIAN, L.V. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences of the United States of America,* 2000, vol. 97, no. 9, p. 4956-4960.

PERSANS, M.W.; SALT, D.E.; KIM, S.A. and GUERINOT, M.L. Phylogenetic relationships within cation transporter families of Arabidopsis. *Plant Physiology*, 2001, vol. 126, no. 4, p. 1646-1667.

PILON-SMITS, E.A.H. and PILON, M. Breeding mercury-breathing plants for environmental cleanup. *Trends in Plant Science*, 2000, vol. 5, no. 6, p. 235-236.

PILON-SMITS, E.A.H.; DESOUZA, M.P.; HONG, G.; AMINI, A.; BRAVO, R.C.; PAYABYAB, S.T. and TERRY, N. Selenium volatilization and accumulation by twenty aquatic plant species. *Journal of Environmental Quality*, 1999a, vol. 28, no. 3, p. 1011-1017.

PILON-SMITS, E.A.H.; HWANG, S.; LYTLE, C.M.; ZHU, Y.L.; TAY, J.C.; BRAVO, R.C.; CHEN, Y.; LEUSTEK, T. and TERRY, N. Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. *Plant Physiology*, 1999b, vol. 119, no. 1, p. 123-132.

PRAKASH, S. Cruciferous oilseeds in India. In: TSUNODA, S.; HINATA, K. and GÓMEZ-CAMPO,C., eds. *Brassica crops and wild allies: biology and breeding*. Tokyo, Japan Scientific Societies Press, 1980, p. 151-166.

PRASAD, M.N.V. Phytoremediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants. In: *Heavy metal stress inlants: from biomlecules to ecosystems*. Heidelberg, Springer-Verlag, 2nd ed., 2004, pp. 345-392.

PRASAD, M.N.V. and STRZALKA, K. *Physiology and biochemistry of metal toxicity and tolerance in plants*. Dordrecht, Kluwer Academic Publishers, 2002, 432 p. ISBN 1-40-200468-0.

PRASAD, M.N.V. *Metals in the environment: analysis by biodiversity*. New York, Marcel Dekker, 2001, 487 p. ISBN 0-58-540412-7. 2001a. pp. 504.

PRASAD, M.N.V. Bioremediation Potential of Amaranthaceae. In: *Proceedings of the 6th International In Situ and On-Site Bioremediation Symposium*. (4th - 7th June, 2001, San Diego, California, USA).Phytoremediation, Wetlands, and Sediments, Battelle Press, Columbus, OH. LEESON, A.; FOOTE, E.A.; BANKS, M.K. and MAGAR, V.S., eds. 2001b, vol. 6, no. 5, p. 165-172.

PRASAD, M.N.V.; GREGER, M. and LANDBERG, T. *Acacia nilotica* L. bark removes toxic metals from solution: Corroboration from toxicity bioassay using *Salix viminalis* L. in hydroponic system. *International Journal of Phytoremediation*, 2001, vol. 3, no. 3, p. 289-300.

PRASAD, M.N.V. and FREITAS, H. Removal of toxic metals from the aqueous solution by the leaf, stem and root phytomass of *Quercus ilex* L. (Holly Oak). *Environmental Pollution*, 2000, vol. 110, no. 2, p. 277-283.

PRASAD, M.N.V. and FREITAS, H. Feasible biotechnological and bioremediation strategies for serpentine soils and mine spoils. *Electronic Journal of Biotechnology* [online], 15 April 1999, vol. 2, no. 1, p. 35-50. Available from Internet: http://www.ejbiotechnology.info/content/vol2/issue1/full/5/index.html.

RASKIN, I. and ENSLEY, B.D. eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. New York, John Wiley and Sons, 2000, 352 p. ISBN0-47-119254-6.

RASKIN, I. Plant genetic engineering may help with environmnetal cleanup. *Proceedings of the National Academy of Sciences of the United States of America*, 1996, vol. 93, no. 8, p. 3164-3166.

RASKIN, I.; KUMAR, P.B.A.N.; DUSHENKOV, S. and SALT, D.E. Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology,* 1994, vol. 5, no. 3, p. 285-290.

REEVES, R.D. and BAKER, A.J.M. Metal- accumulating plants. In: RASKIN, I. and ENSLEY, B.D., eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, John Wiley and Sons, 2000, p. 193-230.

REEVES, R.D.; BAKER, A.J.M.; BORHIDI, A. and BERAZAIN, R. Nickel hyperaccumulation in the serpentine flora of Cuba. *Annals of Botany*, 1999, vol. 83, no. 1, p. 29-38.

REEVES, R.D.; BAKER, A.J.M.; BORHIDI, A. and BERAZAIN, R. Nickel-accumulating plants from the ancient serpentine soils of Cuba. *New Phytologist,* 1996, vol. 133, no. 2, p. 217-224.

REEVES, R.D. and BROOKS, R.R. Hyperaccumulation of lead and zinc by two metallophytes from a mining area of Central Europe. *Environmental Pollution Series A*, 1983, vol. 31, p. 277-287.

RUGH, C.L.; BIZILY, S.P. and MEAGHER, R.B. Phytoreduction of environmental mercury pollution. In: RASKIN, I. and ENSLEY, B.D., eds. *Phytoremediation of toxic metals: using plants to clean- up the environment*. New York, John Wiley and Sons, 2000, p. 151-170.

RUGH, C.L.; GRAGSON, G.M.; MEAGHER, R.B. and MERKLE, S.A. Toxic mercury reduction and remediation using transgenic plants with a modified bacterial gene. *Hortscience,* 1998, vol. 33, no. 4, p. 618-621.

RUGH, C.L.; WILDE, H.D.; STACKS, N.M.; THOMPSON, D.M.; SUMMERS, A.O. and MEAGHER. R.B. Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. *Proceedings of the National Academy of Sciences of the United States of America*, 1996, vol. 93, no. 8, p. 3182-3187.

SALT, D.E. and KRAMER, U. Mechanisms of metal hyperaccumulation in plants. In: RASKIN, I. and ENSLEY, B.D., eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. John Wiley & Sons, Inc., New York. 2000, p. 231-246.

SALT, D.E.; SMITH, R.D. and RASKIN, I. Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, 1998, vol. 49, p. 643-668.

SALT, D.E.; BLAYLOCK, M.; KUMAR, N.P.B.A.; DUSHENKOV, V.; ENSLEY, D.; CHET, I. and RASKIN, I. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology,* 1995a, vol. 13, p. 468-474.

SALT, D.E.; PRINCE, R.C.; PICKERING, I.J. and RASKIN, I. Mechanisms of cadmium mobility and accumulation in Indian Mustard. *Plant Physiology,* 1995b, vol. 109 p. 1427-1433.

SAUER, J.D. *Historical geography of crop plants - a select roster*. CRC Press, Boca Raton, FL. 1993. p. 320. ISBN: 0-8493-8901-1.

SAXENA, P.K.; KRISHNARAJ, S.; DAN, T.; PERRAS, M.R. and VETTAKKORUMA-KANKAV, N.N. Phytoremediation of metal contaminated and polluted soils. In: PRASAD, M.N.V. and Hagemeyer, J., eds. *Heavy metal stress in plants - From molecules to ecosystems*. Springer-Verlag, Heidelberg, 1999, p. 305-329.

SCHÄFER, H.J.; HAAG-KERWER, A. and RAUSCH, T. cDNA cloning and expression analysis of genes encoding GSH synthesis in roots of the heavy-metal accumulator *Brassica juncea* L.: evidence for Cd-induction of a putative mitochondrial g-glutamyl cysteine synthetase isoform. *Plant Molecular Biology*, 1998, vol. 37, p. 87-97.

SCHNOOR, J.L. Phytostabilization of metals using hybrid poplar trees. In: RASKIN, I. and ENSLEY, B.D., eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, John Wiley & Sons, Inc., 2000, p. 133- 150.

SCHNOOR, J.L.; LIGHT, L.A.; MCCUTCHEON, S.C.; WOLFE, N.L. and CARREIRA, L.H. Phytoremediation of organic and nutrient contaminants. *Environmental Science and Technology*, 1995, vol. 29, no. 7, p. 318A-323A.

SCHWARTZ, C. and MOREL, J.L. How can agricultural practices improve phytoremediation of soils contaminated with heavy metals?. In: *Proceedings of the International Conference on Contaminated Soil*. (6º, DATE, 1998, PLACE). London, Thomas Telford Ltd., 1998, vol. 1. p. 607-617.

SIEGEL, F.R. *Environmental Geochemistry of potentially toxic metals*. Heidelberg, Springer-Verlag, 2002. 218 p. ISBN 3540420304.

SMITH, R.A.H. and BRADSHAW, A.D. Stabilization of toxic mine wastes by the use of tolerant plant populations. Transactions of the Institution of Mining and Metallurgy, 1992, vol. 81, A230-A237.

SPARKS, D.L. *Environmental chemistry of soils*. New York, Academic Press, 1995. ISBN: 0195070119.

STOMP, A.M.; HAN, K.H.; WILBERT, S.; GORDON, M.P. and CUNNINGHAM, S.D. Genetic strategies for enhancing phytoremediation. *Annals of New York Academy of Sciences,* 1994. vol. 721, p. 481-491.

SUSZCYNSKY, E.M. and SHANN, J.R. Phytotoxicity and accumulation of mercury subjected to different exposure routes. *Environmental Toxicology and Chemistry*, 1995, vol. 14, p. 61-67.

TERRY, N. and BAÑUELOS, G. *Phytoremediation of Contaminated Soil and Water*. Lewis Publishers, Inc., 2000, 408 p. ISBN: 1566704502.

TERRY, N.; CARLSON, C.; RAAB, T.K. and ZAYED, A. Rates of selenium volatilization among crop species. *Journal of Environmental Quality*, 1992, vol. 21, p. 341-344.

THAKRAL, S.K.; SINGH, B.P.; FARODA, A.S.; GUPTA, S.K. and KUMAR, S. Fatty acids composition in seeds of Brassica species as affected by moisture and fertility levels. *Crop Research*, 1995, vol. 10, no. 2, p. 137-140.

TIMOFEEV-RESOVSKY, E.A.; AGAFONOV, B.M. and TIMOFEEV-RESOVSKY, N.V. Fate of radioisotopes in aquatic environments (In Russian*). Proceedings of Bilogical Institute USSR Academy of Sciences*, 1962, vol. 22, p. 49-67.

TOMAR, T.S.; SINGH, S.; KUMAR, S. and TOMAR, S. Response of Indian mustard (*Brassica juncea*) to nitrogen, phosphorus and sulfur fertilization. *Indian Journal of Agronomy,* 1997, vol. 42, no. 1, p. 148-151.

TRAMPCZYNSKA, A.; GAWRONSKI, S.W. and KUTRYS, S. *Canna x generalis* as a plant for phytoextraction of heavy metals in urbanized area. *Zeszyty Naukowe Politechniki Slaskiej*, 2001, vol. 45, p. 71-74.

TRELEASE, S.F, and TRELEASE, H.M. Changes in hydrogen- ion concentration of culture solutions containing nitrate and ammonium nitrogen. *American Journal of Botany*, 1935, vol. 22, p. 520-542.

TSAO, D. *Phytoremediation.* Heidelber, Springer-Verlag, 2003. 206 p. ISBN 3540433856.

VALDES, J.J. *Bioremediation*. Kluwer Academic Publishers, 2002. p. 169. ISBN 0792364597.

VANGRONSVELD, J. and CUNNINGHAM, S.D. *Metal-contaminated soils: in-situ in activation and phytorestoration*. Springer-Verlag, Berlin, Heidelberg, 1998. 265 p. ISBN 3540642080.

WATANABE, M.E. Phytoremediation on the brink of commercialization. *Environmental Science and Technology*, 1997, vol. 31, p. 182-186.

WENZEL, W.W.; LOMBI, E. and ADRIANO, D.C. Biogeochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. In: PRASAD, M.N.V. and HAGEMEYER, J. eds. *Heavy metal stress in plants - From molecules to ecosystems*. Berlin, Heidelberg, New York, Springer-Verlag, 1999, p. 271-303.

WILBER, C.G. Toxicology of selenium: a review. *Clinical Toxicology*, 1980, vol. 17, p. 171-230.

WISE, D.L.; TRANTOLO, D.J.; CICHON, E.J.; INYANG, H.I. and STOTTMEISTER, U. *Bioremediation of contaminated soils*. New York, Marcel Dekker Inc., 2002. p. 903. ISBN 0824703332.

ZAUROV, D.E.; PERDOMO, P. and RASKIN, I. Optimizing soil fertility and pH to maximize cadmium removed by Indian mustard from contaminated soils. *Journal of Plant Nutrition*, 1999, vol. 22, no. 6, p. 977-986.

ZEVEN, A.C. and ZHUKOVSKY, P.M. Dictionary of cultivated plants and their centres of diversity - excluding ornamentals, forest trees and lower plants. Pudoc, Wageningen, 1975. ISBN 90 220 0549 6.

ZHU, Y.L.; PILON-SMITS, E.A.; TARUN, A.S.; WEBER, S.U.; JOUANIN, L. and TERRY, N. Cadmium tolerance and accumulation in Indian mustard is enhanced by over expressing gamma-glutamylcysteine synthetase. *Plant*

Physiology, 1999a, vol. 121, p. 1169-1178.

ZHU, Y.L.; ZAYED, A.M.; QUIAN, J.H.; DE SOUZA, M. and TERRY, N. Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. *Journal of Environmental Quality,* 1999b, vol. 28, p. 339-344.

ZORPAS, A.A.; CONSTANTINIDES, T.; VLYSSIDES, A.G.; ARALAMBOUS, I. and LOIZIDOU, M. Heavy metal uptake by natural zeolite and metals partitioning in sewage sludge compost. *Bioresource Technology*, 1999, vol. 71, no. 2, p. 113-119.

APPENDIX

Tables

Table 1. Biodiversity exhibiting resistance to metals and with potential to cleanup toxic metals in all the three compartments of the environment viz., atmosphere, hydrosphere and lithosphere. Several of the listed organisms are used for laboratory and filed experiments. The results obtained are found to be useful to advance the knowledge of bioremediation and metal monitoring in the environment (Markert, 1993; Bargagli, 1998; Prasad, 2001a) (The list is not exhaustive).

Bacteria

Acinetobacter
Agrobacterium
Alcaligenes eutrophus
A. faecalis
Arthrobacter sp.
Bacillus spp
Citribacter freundii
Comamonas sp.
Desulfobulbus spp
Desulfomicrobium
Desulfovibrio spp
Enterobacter colacae
Leptospirillum sp.
Pseudomonas aeruginosa
P. putida
P. syringae var. tomato
Photobacterium phosphoreum
Ralstonia eutropha
R. metallidurans
Salmonella typhimurium
S. aureus
Thiobacillus ferrooxidans

Mycorrhizae

Albatrellus ovinus
Amanita muscaria
Chantharellus tubaeformis
C. ciliarius
Cortinarius sp.
Dermocybe sp.
Glomus mosseae
Gomphidius sp.
Hebeloma sp.
Hydnum sp.
Hymenoscyphus ericae
Laccaria laccata
Leccinum spp
Oidiodendron maius
Paxillus involutus
Pisolithus tinctorius
Russula sp.
Scleroderma sp.
Suillus bovinus
S. luteus
Thelephora terrestris

Freshwater algae

Achanthes microcephala
A.minutissima
Anabaena cylindrica
A. doliolum
A. inaequalis
A. lutea
Anacystis nidulans
Ankistridesmus falcatus
Aphanocapsa sp. Asterococcus sp
Chlamydomonas acidophila
C. ampla
C. bacilus
C. pyrenoidisa
C. reinhardtii
C. subglobosus
C. vulgaris
C. vulgaris
Chamaesiphon minutus
Chara corallina
Chlamydocapsa bacillus
Chlamydocapsa cf. petrify
Chlorella fusca var vacuolata
Cladophora glomerata
Cosmarium sp.
Cyanidium caldarium
Dictyococcus sp.
Dunaliella bioculata
Eisenia bicylis
Euglena gracilis
E. mutabilis
Eunotia exigua
Gleocapsa turfosa
Gleochrysis acoricola
Gleococcus
Gleocystis gigas
Gomphenema
Hormidium rivulare
Hypnomonas
Klebsormidium klebsii
K. rivulare
Microspora pachyderma
M. stagnorum
M. stagnosum
M. strictissimum
M. tumidula
M. willeana
M. floccosa
Microthamnion kutzingianum
Mougeotia
Navicula
Nitchia palea
Nostoc calcicola
Oedogonium sp.
O. nephrocytioides
Oocystis elliptica
O. lacustris
Oscillatoria
Phormidium foveolarum
P. luridum
Pinnularia acoricola
Plectonema
Plerococcus
Pseudoanabaena catenata
Pseudococcomyxa adhaerens
Scenedesmusobliqus
S.quadricauda
S. subspicatus
Scenedesmus acutiformis
Schizothrix sp.
Selenastrum capricornutum
Snechococcus sp.
Spartina maritima
Spirogyra sp.
Spirulina platensis
Stegeoclonium sp.
Stegioclonium sp.
Stichococcus sp.
Stichococcus bacillaris
Stigeoclonium tenue
Surirella angustata
Synechocystis aquatilis
Synedra filiformis

Bryophytes

Barbula recurvirostrata
B. acuminatum

B. philonotula
Bryum argenteum
B. rubens
Campylopus bequartii
Cephalozia bicuspidata
Cephaloziella hampeana
C. masalongi
C. nicholsonii
C. rubella
C. stellulifera
C. integerrima
Ditrichum cornubicum
D. plumbicola
Funaria hygrometrica
Grimmia atrata
Gymnocolea acutiloba
Mielichhoferia macrocarpa
M. elongata
M. nitida
M. mieilichhoferi
Pholia nutans
P. andalusica
P. nutans
Pottia sp.
Scopelophila cataractae
S. ligulata
S. cataractae
S. cataractae
Scapania undulata

Pteridophytes

A*splenium adiantum-nigrum*
A. cuneifolium
A. hybrida
A. presolanense
A. ruta-muraria
A. septiontrionale
A. trichomanes
A. viride
Ceratopteris cornuta
Cehaloziella calyculata
Cheilanthes hirta
C. inaequalis var. lanopetiolata
C. inaequalis var inaequalis
C. hirtaMohria lepigera
Nardia scalaris
Nothalaena marantae
Oligotrichum hercynicum
Ophiglossum lancifolium
Pellea calomelanos
Pteris vittata

Lichens

Bryoria fuscescens
Diploschistes muscorum
Flavoparmelia baltimorensis
Hypogyminia physodes
Lobaria pulmonaria
Parmelia caperata
Peltigera canina
Ramalina duriaei
R. farinaceae
R. fastigata

Gymnosperms

Abies Chamaecyparis
Chamaecyparis
Cryptomeria
Gingko
Juniperus
LarixPicea
Pinus ponderosa
Pseudotsuga
Taxodium

Angiosperms

Acer saccharinum
Aeollanthus biformifolius
Agrostis capillaris
A. giganteaA. tenuis
Alyssum heldreichii
A. lesbiacum
A. perenneA. akamasicum
A. alpestre
A. americanum
A. anatolicum
A. argenteum
A. bertlonii
A. bertolonii subsp.scutarinum
A. callichroum
A. carcium
A. cassiumA. chondrogynum
A. cilicium
A. condensatum
A. constellatum
A. corsicum
A. crenulatum
A. cypricum
A. davisianum
A. discolor
A. dubertretii
A. eriophyllum
A. euboeum
A. fallacinum
A. floribundum
A. giosnanum
A. heldreichii
A. huber-morathii
A. janchenii
A. lesbiacum
A. malacitanum
A. markgrafii
A. masmenaeum
A. murale
A. obovatum
A. oxycarpum
A. penjwinensis
A. pinifolium
A. pintodasilave
A. pterocarpum
A. robertianum
A. samariferum
A. serpyllifolium
A. singarense
A. smolikanum
A. stolonifera
A. syriacum
A. tenium
A. trapeziforme
A. troodii
A. virgatum
A. wulfenianum
A. montanum
A. serpyllifolium sub sp. malacinatum
Amaranthus retroflexus
Anthoxanthum odoratum
Arabidopsis halleri
A. thaliana
Arabis stricta
Armeria maritima sub. sp. elongata
Arrhenatherum pratensis
Astragalus racemosus
Avenella flexuosa
Berkheya coddi
Betula papyrifera
Bornmuellera glabrescens
B. tymphea
B. baldaccii subsp. baldacci
B. baldaccii subsp. markgrafii
B. baldaccii baldaccii subsp. Rechingeri
Brassica nigra
B. pendula
B. pubescens
B. rapa
B. campestris
B. hordeaceus
B. japonica
B. juncea
B. napus
B. narinosa
B. pekinensis
B. ramosus
Brachypodium chinensis
Brachypodium sylvaticum
Calystegia sepium
Cardamine resedifolia
Cardminopsis halleri
Carex echinata

Chrysanthemum morifolium
Cochlearia aucheri
C. pyrenaica
C. sempervium
C. pyrenaica
Colocasia esculenta
Cynodon dactylon
Danthonia decumbens
D. linkii
Datura innoxia
Deschampsia caespitosa
Echinochloa colona
Epilobium hirsutum
Eriophorum angustiflolium
Eschscholtzia californica
Fagopyrum esculentum
Fagus sylvatica
Festuca rubra
Fraxinus angustifolia
Gossypium hirsutum
Haumaniastrum katangense
Helianthus annuus
Holcus lanatus
Hordelymus europaeus
Hybanthus floribundus
Hydrangea
Hydrocotyle umbellata
Limnobium stoloniferum
Lolium multiflorum
L. perenne
Macadamia neurophylla
Medicago sativa
Melilotus officinalis
Mimulus guttatus
Minuartia hirsute
Nardus stricta
Noccaea aptera
N. boeotica
N. eburneosa
N. firmiensis
N. tymphaea
Pelargonium
Peltaria dumulosa
P. emarginata
Pinus pinaster
Podophyllum peltatum
Polygonum cuspidatum
Populus tremula
Pseudosempervium aucheri
Quercus rubra
Q. ilex
Ranunculus baudotti
Raparia
Rauvolfia serpentina
Ricinus communis
Rumex hydrolapathum
Salix viminalis
Sebertia acuminata
Senecio cornatus
Silene cucubalus
S. compacta
S. italica
Solanum nigrum
Sorghum sudanense sub. sp. Halleri
S. sudanens sub. sp. maritima
Stanleya sp.
Streptanthus polygaloides
Thlaspi alpestre subsp. virens
T. arvense
T. brachypetalum
T. bulbosum
T. bulbosum
T. caerulescens
T. calaminare
T. Cepaefolium
T. cepaeifolium subsp. cepaefolium
T. cypricum
T. elegans
T. epirotum
T. goesingense
T. graecum
T. idahoense
T. japonicum
T. jaubertii
T. kovatsii
T. liliaceum
T. limosellifolium
T. magallanicum
T. montanum
T. montanum var.montanum
T. ochroleucum
T. oxyceras
T. parvifolium
T. praecox
T. repens
T. rotundifolium
T. rotundifolium subsp. cepaefolium
T. rotundifolium var. corymbosum
T. stenocarpum
T. sylvium
T. tatraense
T. tymphaeum
T. violascens
Thinopyrum bessarabicum
Trifolium pratense
Viola calaminaria
Viola arvensis

Aquatic macrophytes

Arenicola christata
A. marina
Carex sp.
Ceratophyllum demersum
Glyceria fluitans
Hydrilla verticillata
Ipomea aquatica
Lemna minorL. trisulca
Myriophyllum spicatum
Najas sp
Phragmites australis
Potamogeton pectinatus
P. perfoliatum
Ruppia sp.

Tree crops

Acer pseudoplatanus
Betula alleghanensis
B. papyrifera
B. pendula
B. tauschii
Cryptomeria japonica
Eucalyptus camaldulensis
Fagus japonica
F. sylvatica
Liriodendron tulipifera
P. nigra x *P. maximowiczi*
P. deltoides x *P. nigra*
P. maximowiczii
P. nigra
P. taeda
P. trichocarpa x *P. deltoides*
Picea abies
Pinus strobus
Populus alba
Prunus virginiana
Salix arenaria
S. burjatica cv. aquatica
S. x *caprea*
S. viminalis
S. triandra
S. dasyclados

Table 2. Vascular plants growing on mine refuse in Portugal. (Freitas et al 2004a,b)

1. Apiaceae

Daucus crinitus L.
D. carota L. subsp. *maritimus* (Lam.) Batt.
Eryngium campestre L.
E. tenue Lam.
Foeniculum vulgare Miller subsp. *piperitum* (Ucria) Coutinho
Oenanthe crocata L.
Pimpinella villosa Schousb.
Seseli peixotianum Samp.

2. Aristolochiaceae

Aristolochia longa L

3. Aspleniaceae

Asplenium adiantum-nigrum L. var. *corunnense* Christ

4. Asteraceae

Carlina corymbosa L. subsp. corymbosa
Crepis capillaris (L.) Wallr.
Dittrichia viscosa (L.) W.Greuter
Filago lutescens Jordan subsp. *lutescens*
Helichrysum stoechas (L.) Moench.
Hieracium peleteranum Mérat subsp. *ligericum* Zalm
Hispidella hispanica Lam.
Hypochaeris radicata L
L.viminea (L.) J.& C. Presl. subsp. *viminea*
L.virosa L.
Lactuca viminea (L.) J. & C.Presl
Lapsana communis L. subsp. *communis*
Leontodon taraxacoides (Vill.) Mératsubsp. *longirostris* Finch. & P. D. Sell
Logfia gallica (L.) Cosson & Germ.
L. minima (Sm.) Dumort.
Santolina semidentata Hoffm. & Link
Senecio gallicus Vill.

5. Boraginaceae

Anchusa arvensis (L.) Bieb. subsp. *arvensis*
Echium lusitanicum L. subsp. *Lusitanicu*m
E.plantagineum L.

6. Brassicaceae

Alyssum serpyllifolium Desf. subsp. *lusitanicum* Dudley & Pinto da Silva
Lepidium heterophyllum Bentham
Erysimum linifolium (Pers.) Gay subsp. *linifolium*

7. Campanulaceae

Campanula rapunculus L.
Jasione crispa (Pourret) Samp. subsp. *serpentinitic*a P. Silva

8. Caprifoliaceae

Lonicera periclymenum L. subsp. *periclymenum*
Sambucus nigra L.

9. Caryophyllaceae

Agrostemma githago L.
Arenaria montana L. subsp. montana
Aquerioides Pourret ex Willk. subsp.*fontequeri* (P. Silva) R. Afonso
Dianthus laricifolius Boiss. & Reuter subsp. *marizii* (Samp.) Franco
Ortegia hispanica Loefl.
Petrorhagia nanteuellii (Burn.) P. W. Ball& Heywood
Saponaria officinalis L.
Silene scabriflora Brot. *subsp. scabriflora*
S.coutinhoi Rothm. & Pinto da Silva
S. scabriflora Brot. *subsp. scabriflora*
Spergula pentandra L
S.purpurea (Pers.) G.Don. fil.

10. Chenopodiaceae

Chenopodium album L. *subsp. album*

11. Cistaceae

Cistus ladanifer L.
C. salvifolius L.
Tuberaria guttata (L.) Fourr.

12. Clusiaceae

Hypericum perforatum L

13. Convolvulaceae

Convolvulus arvensis L. subsp. *arvensis*

14. Crassulaceae

Sedum arenarium Brot.
S.forsteranum Sm
S.tenuifolium Strob.

15. Dioscoreaceae

Tamus communis L.

16. Elatinaceae

Elatine macropoda Guss.

17. Euphorbiaceae

Euphorbia falcata L.

18. Fagaceae

Castanea sativa Miller

Quercus faginea Lam. subsp. Faginea
Q. ilex L. subsp. *ballota* (Desf.) Samp.
Q. pyrenaica Willd.

19. Gentianaceae

Centaurium erythraea Rafin subsp. *majus* (Hoffmans.& Link) Meldéris

20. Geraniaceae

Geranium purpureum Vill.

21. Haloragaceae

Myriophyllum alterniflorum DC.

22. Hypolepidaceae

Pteridium aquilinum (L.) Kuhn subsp. *aquilinum*

23. Lamiaceae

Clinopodium vulgare L.
Dorycnium pentaphyllum Scop. subsp. *transmontanum* Franco
Lavandula stoechas L. subsp. *pedunculata* (Miller) Samp. & Rozeira
L. stoechas L. subsp. *sampaiana* Rozeira
L.stoechas L. subsp. stoechas
Mentha pulegium L.
M. spicata L.
M. suaveolens Ehrh.
Origanum virens Hoffmanns & Link
Phlomis lychnitis L.
Prunella vulgaris L. subsp. vulgaris
Salvia verbenaca L.
Teucrium scorodonia L. subsp. scorodonia
Thymus mastichina L.

24. Fabaceae

A. stoechas L.
Acacia dealbata Link
Adenocarpus complicatus (L.) J.Gay
Anthyllis lotoides L.
C. multiflorus (L'Hér.) Sweet
C. striatus (Hill.) Rothm.
Cytisus grandiflorus (Brot.) DC.
G. polyanthos Willk. subsp. hystrix(Lange) Franco
Genista triacanthos Brot.
Lotus. tenuis Willd.
L. uliginosus Schkuhr.
Lotus corniculatus L. var. *corniculatus*
O.spinosa L. *subsp. antiquorum* (L.)Arcangeli
Ononis cintrana Brot.
Ornithopus compressus L.
Phagnalon saxatile (L.) Cass.
Pisum sativum L. *subsp. elatius* (Bieb.)Ascherson & Graebner
Pterospartum tridentatum L
Trifolium arvense L. var. *arvense*
T. glomeratum L.
T. repens L. *subsp. repens*
T.campestre Schreber
Tolpis barbata (L.) Gaertner
Vicia sativa L. *subsp. nigra* (L.) Ehrh.
V. laxiflora Brot

25. Liliaceae

Allium vineale L.
A. sphaerocephalos L. *subsp. sphaerocephalos*

26. Lythraceae

Lythrum hyssopifolia L.

27. Malvaceae

Malva sylvestris L.

28. Oleaceae

Fraxinus angustifolia Vahl

29. Ongagraceae

Epilobium tetragonum L. subsp. *tetragonum*

30. Orchidaceae

Serapias lingua L.

31. Papaveraceae

Papaver rhoeas L.

32. Pinaceae

Pinus pinaster Aiton

33. Plantaginaceae

Plantgo lanceolata L.
P.radicata Hoffm. & Link *subsp. radicata*

34. Plumbaginaceae

Armeria langei Boiss. *subsp. langei*

35. Poaceae

Aegilops triuncialis L.
Agrostis curtisii Kerguélen
Arrhenatherum elatius (L.) J. & C. Presl.
Avena sterilis L.
Briza maxima L.
Bromus hordeaceus L. *subsp. Hordeaceus*
Festuca pseudotricophylla Patzke
Holcus lanatus L.
Melica ciliata L. *subsp. ciliata*
Phleum pratense L. *subsp. bertolonii* (DC.) Bornm.
Sanguisorba minor Scop. subsp. *magnolii* (Spach) Coutinho
Setariopsis verticillata Samp
Trisetaria ovata (Cav.) Paunero

36. Polygonaceae

Polygonum arenastrum Boreau
P. minus Hudson
Rumex pulcher L.
R. crispus L.
R. acetosella L. *subsp. angiocarpus* (Murb.) Murb.
R. induratus Boiss. & Reuter

37. Portulacaceae

Portulaca oleraceae L. *subsp. Oleraceae*

38. Primulaceae

Anagalis monelli L. var. *linifolia* (L.) Lange

39. Resedaceae

Reseda virgata Boiss. & Reuter

40. Rosaceae
Agrimonia procera Wallr.
Crataegus monogyna Jacq.
C.monogyna Jacq. subsp. *brevispina* (G.Kunze) Franco
Filipendula vulgaris Moench
Potentilla erecta (L.) Rauschel
Rosa canina L.
Rubus caesius L.
R. ulmifolius Schott
Sanguisorba verrucosa (Link) Ces.

41. Rubiaceae

Asperula aristata L. fil. *subsp.* scabra (J. & C. Presl) Nyman
Galium palustre L.

42. Salicaceae

Salix salvifolia Brot.
S. triandra L.

43. Scrophulariaceae

Anarrhinum bellidifolium (L.) Willd.
Digitalis purpurea L. *subsp. purpurea*
Linaria aeruginea (Gouan) Cav.
L. spartea (L.) Willd. *subsp. virgatula* (Brot.) Franco
Odontites tenuifolia (Pers.) G. Don fil.
Scrophularia auriculata
Verbascum virgatum Stokes
Digitalis thapsi L.

44. Thymelaeaceae

Daphne gnidium L.

45. Valerianaceae

Centranthus calcitrapae (L.) Dufresne subsp. *calcitrap*

Table 3. Plants with potential for the phytoextraction of various metals and radionuclides.

Metal or radionuclide	Plant species	Reference
Cd	*Brassica juncea* (L.) Czern	Kumar et al. 1995a Huang et al. 1997a Ebbs et al. 1997 Salt et al. 1995b
Cr (VI)	*B. juncea*	Kumar et al. 1995a Huang et al. 1997a
^{137}Cs	*Amaranthus retroflexus* L.; *B. juncea, B. oleracea* L.; *Phalaris arundinacea* L.; *Phaseolus acutifolius* A.Gray.	Lasat et al. 1997 Lasat et al. 1998 Negri and Hinchman, 2000
Cu	*B. juncea*	Ebbs and Kochian, 1997
Ni	*B. juncea*	Ebbs and Kochian, 1997
Pb	*B. campetris* L.; *B. carinata* A. Br.; *B. juncea*; *B. napus* L.; *B. nigra* (L.) Koch.; *Helianthus annuus* L.; *Pisum sativum* L.; *Zea mays* L.	Begonia et al. 1998 Baylock et al. 1997 Ebbs and Kochian, 1998
Se	*B. napus* L.; *Festuca arundianacea* Schreb; *Hibiscus cannabinus* L.	Bañuelos et al. 1997
U	*B. chinensis* L; *B. juncea*; *B. narinosa* L., *Amaranthus* spp.	Huang et al. 1998
Zn	*Avena sativa*; *B. juncea*; *B. napus* L. *Hordeum vulgare*, *B. rapa*	Ebbs et al. 1997 Ebbs and Kochian, 1998

Figures

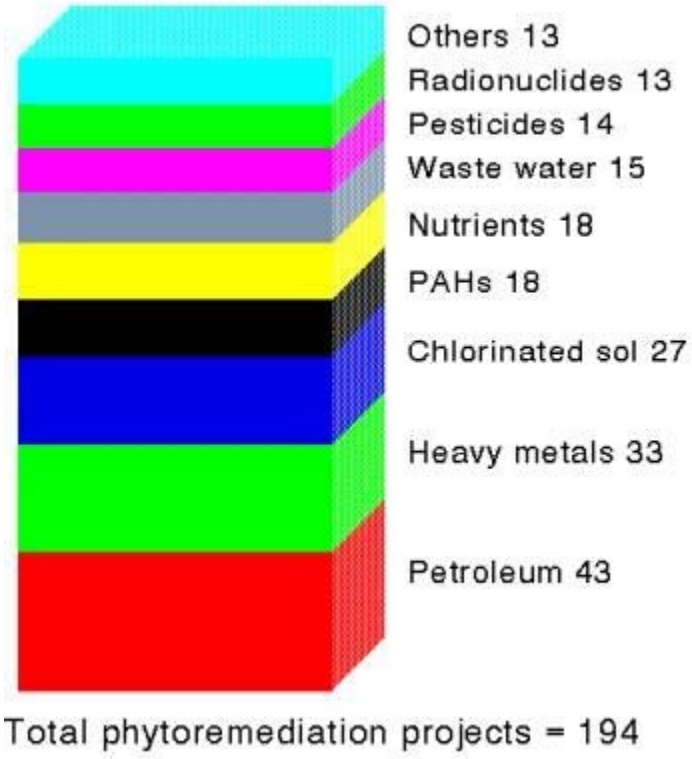

Figure 1. In a report of the year 2000, the USA environmental protection agency (EPA) listed about 194 ongoing bioremediation field research projects. Heavy metals and radionuclides represent about 30% of this activity supporting that bioremediation is a feasible technology to decontaminate the environment. Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment. The conventional remediation technologies that are used to clean heavy metal polluted environments are soil *in situ* vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification and stabilization electrokinetic systems. Each of the conventional remediation technology has specific benefits and limitations.

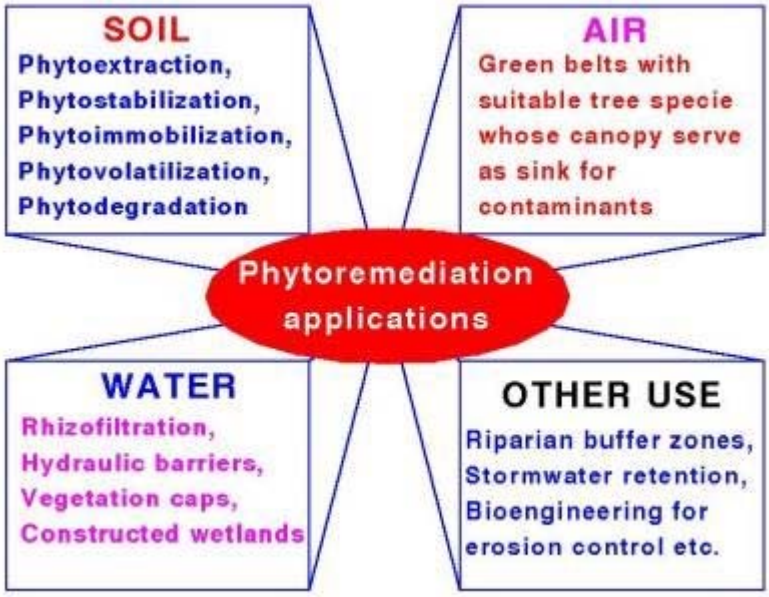

Figure 2. The important phytoremediation technologies applied are rhizofiltration, phytostabilization, phytovolatilization, and phytoextraction. The term phytoremediation ("phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore) actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments. One of the primary objectives behind the development of phytoremediation technologies is its potential for application at a low-cost. Althouh the term, phytoremediation, is of a relatively recent origin, the practice is not.

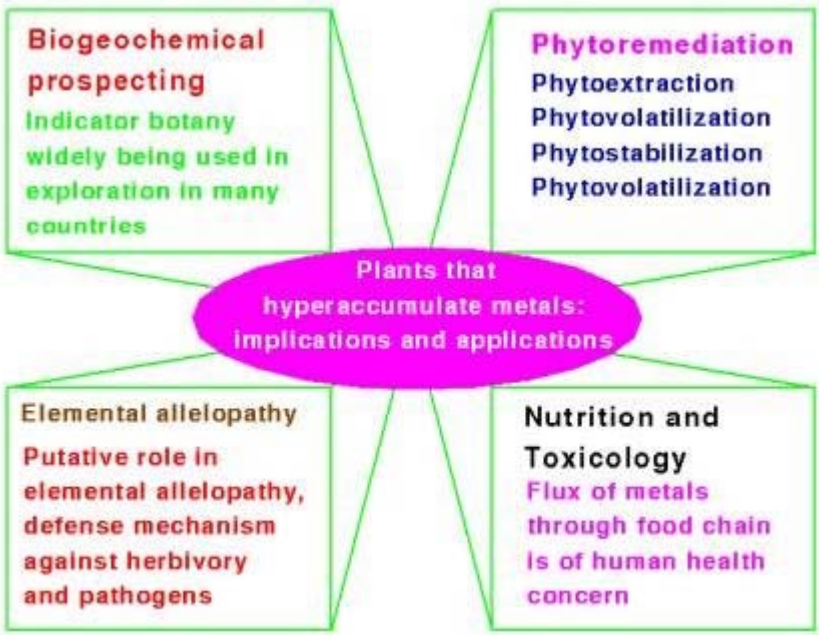

Figure 3. Metal hyperaccumulators were believed to have limited potential in the area of phytoremediation owing to their slow growth, low biomass production which limit the speed of metal removal. By definition, a hyperaccumulator must accumulate at least 100 mg g^{-1} (0.01% dry wt.), Cd, As and some other trace metals, 1000 mg g^{-1} (0.1 dry wt.) Co, Cu, Cr, Ni and Pb and 10,000 mg g^{-1} (1% dry wt.) Mn and Ni. Plants that hyperaccumulate metals have other applications and implications. The most important applications are phytoremediation and biogeochemical prospecting. The other implications are elemental allelopathy and nutrition and toxicology which is of human health related subject.

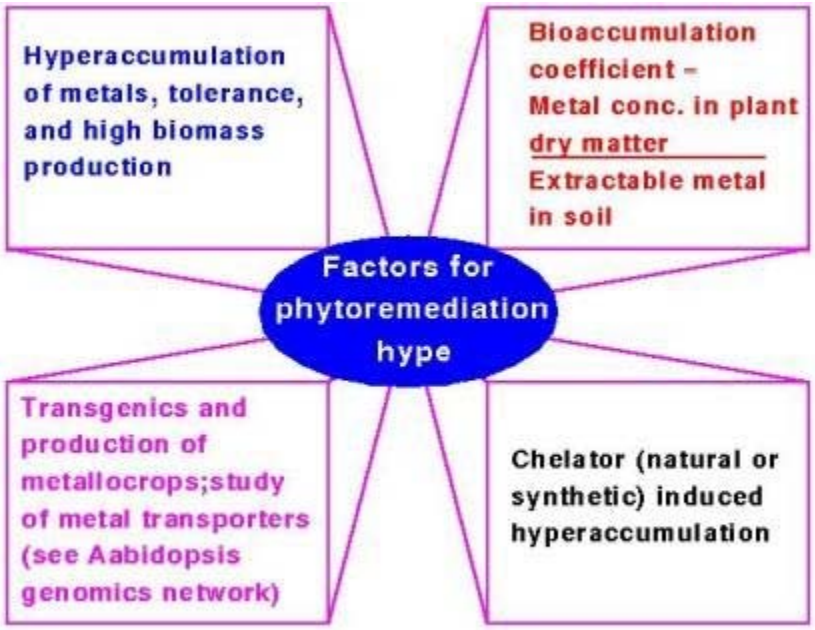

Figure 4. Several factors would accelerate phytoremediation technology. The prime being: genetic engineering and production of transgenics having tolerance and metal accumulation ability for use in phytoremediation, facilitating the factors that would influence the metal bioaccumulation coefficient which inturn will depends upon heavy metal availability in the soil, absorption, transport and sequestration etc, and development of low cost technologies for chelate-induced hyperaccumulation.

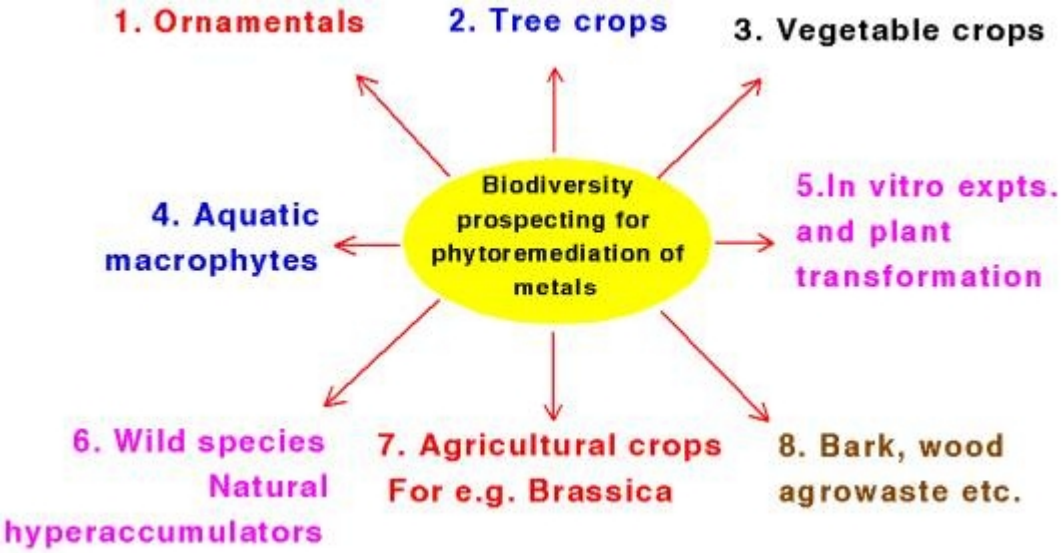

Figure 5. Biodiversity prospecting for phytoremediaiton of metals in the environment. Please see the cited references for additional information.

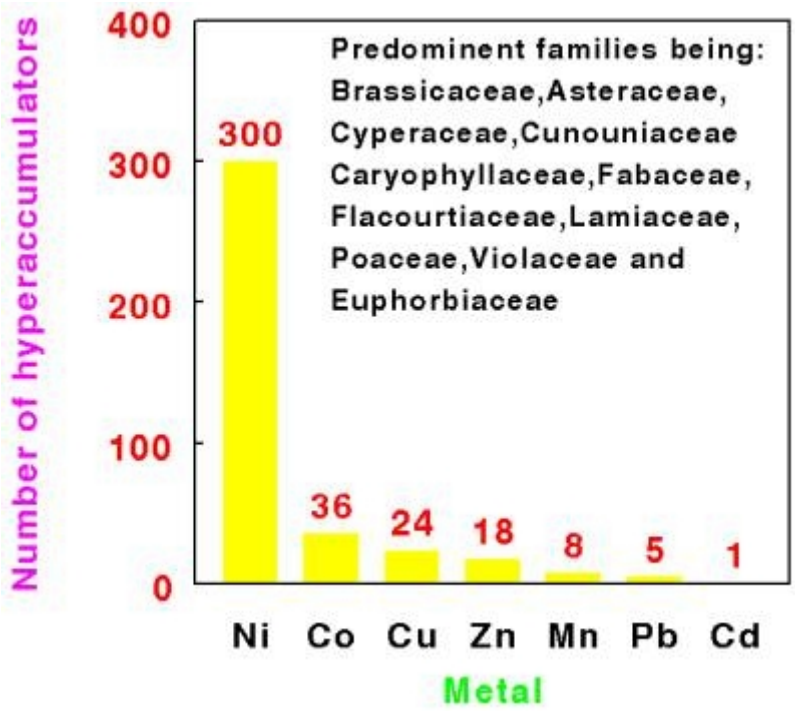

Figure 6. Taxa of various angiospermous families that hyperaccumulate metals. The families dominating the metal accumulators and hyperaccumulators being Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae.

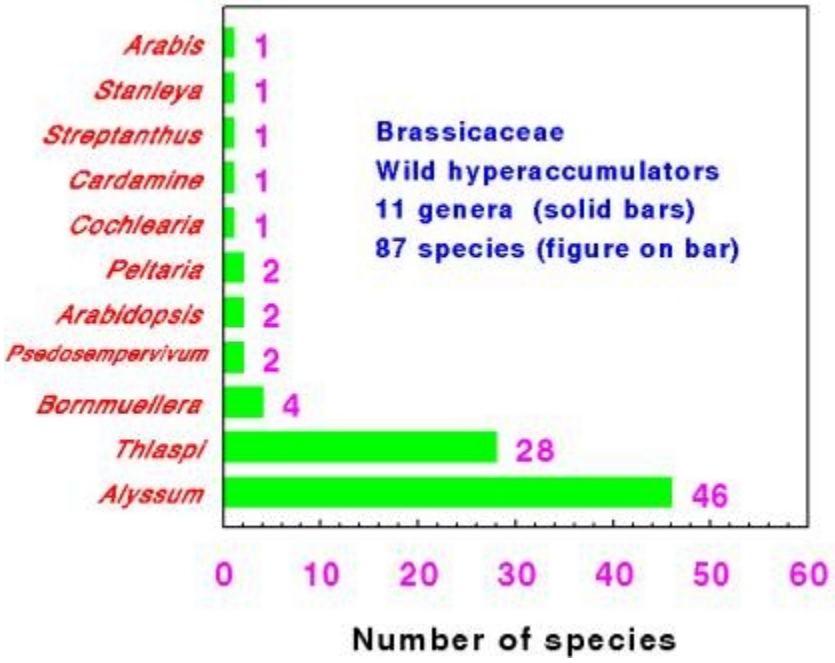

Figure 7. Among wild Brassicaceae 11 genera and 87 species are known to hyperaccumulate metals.

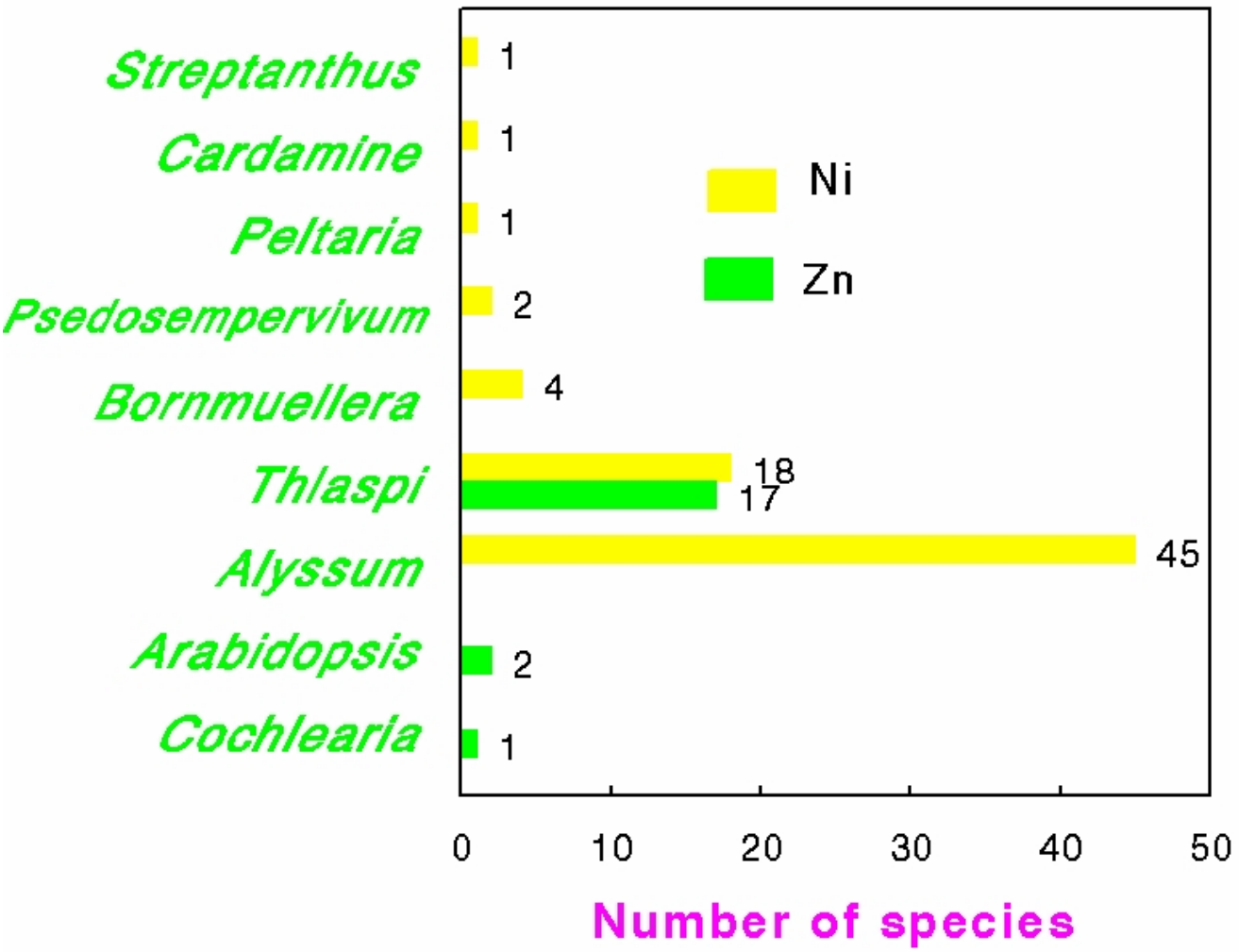

Figure 8. Brassicaceae has the largest number of nickel (7 genera and 72 species) and zinc hyperacccumulators (3 genera and 20 species).

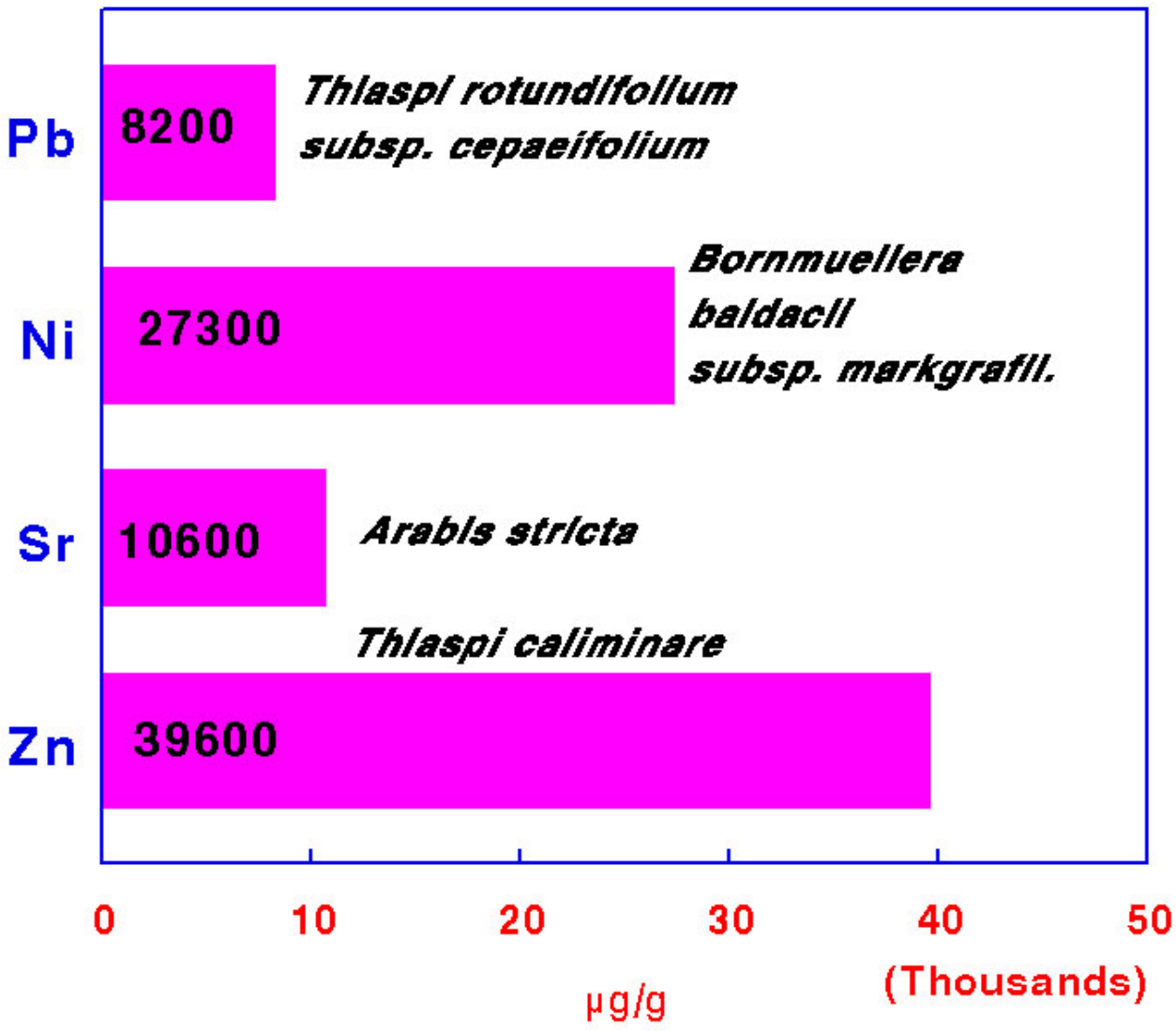

Figure 9. Selected examples of Brassicaeae that hyperaccumulate lead, nickel, strontium and zinc.

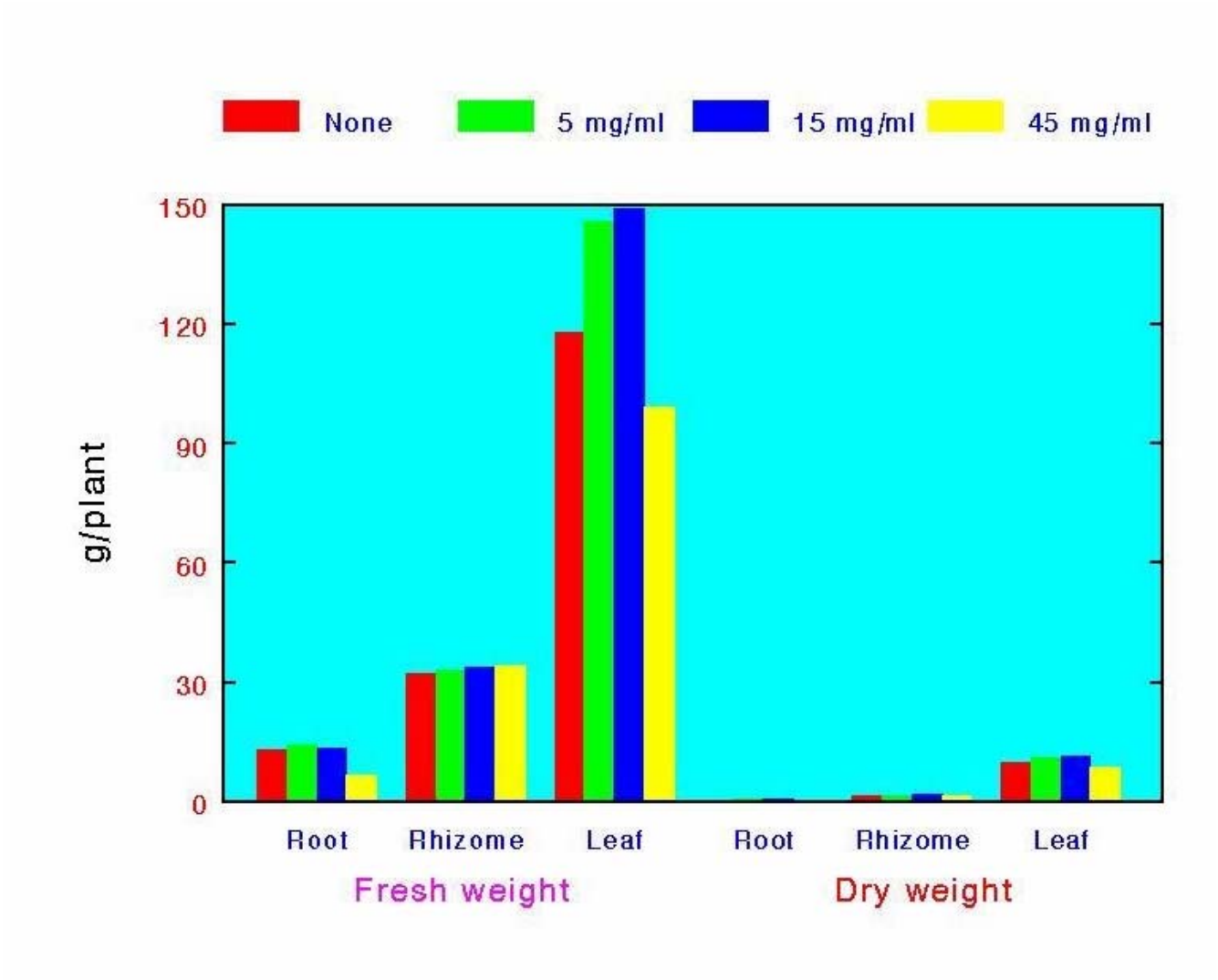

Figure 10. Fresh weight and dry weight of *Canna* x *generalis* respectively treated with lead 5, 15 and 45 mg/ml (Trampczynska et al. 2001).

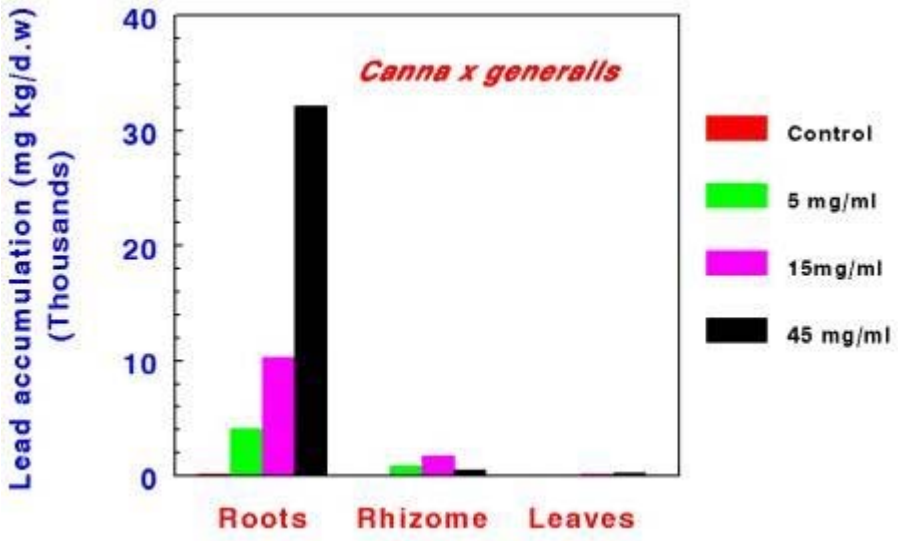

Figure 11. Lead accumulation in *Canna* x *generalis* (Trampczynska et al. 2001).

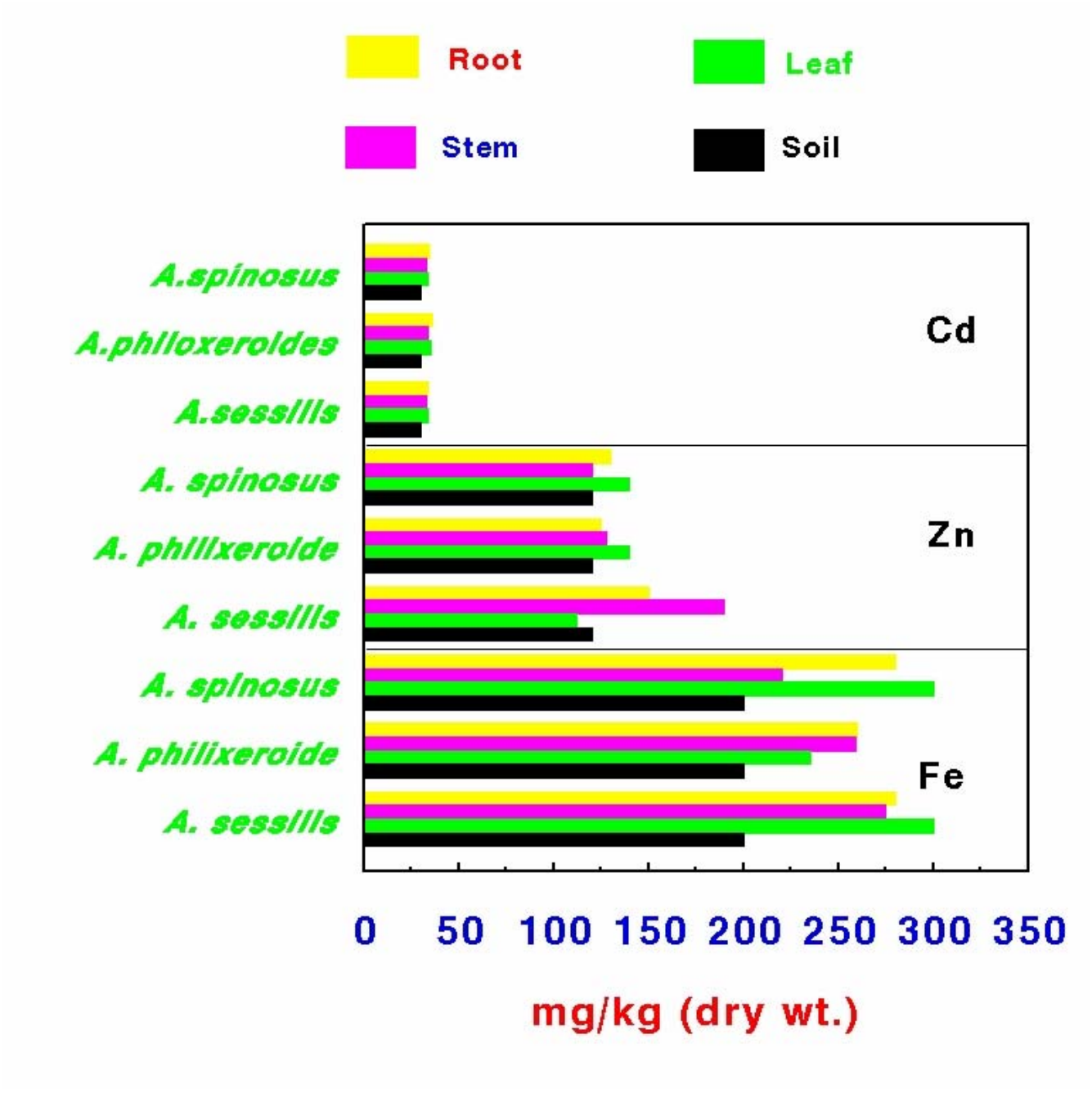

Figure 12. Bioaccumulation (mg/kg of dry matter) of Cd, Zn and Fe in plant parts of Amaranthaceae species viz., *Amaranthus spinosus, Alternanthera* philoxeroides and *Alternanthera sessilis* growing on sewage sludge in polluted Musi river, Hyderabad (Prasad, 2001).

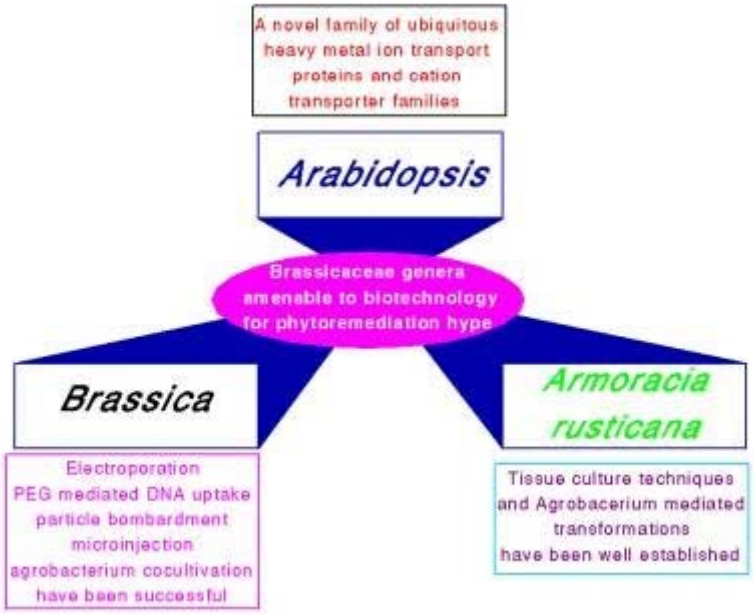

Figure 13. Citric acid is a naturally occurring chelating agent. The chelation process is water activated. EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate and acetate etc. have been used for "chelate-induced hyperaccumulation". Synthetic soil amendments such as ammonium thiocyanate and natural zeolites have yielded promising results in inducing hyperaccumulation of metals.

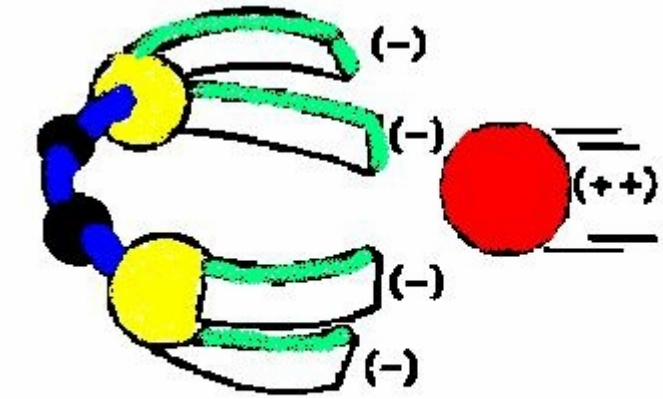

Figure 14. Biotechnology prospecting for phytoremediaiton of metals in the environment. In Brassicaceae *Arabidopsis thaliana*, *Brassica* species and *Armoracia rusticana* have been extensively studied for metal sensitivity and resistance. In *A. thaliana* a number of heavy metal accumulating and sequestering mutants have been identified. Brassicace are amenable to well-characterized biotechnological and molecular biological tools through which transgenic production can be achieved for field trials.