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Review:

Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives*

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Abstract: Environmental pollution affects the quality of pedosphere, hydrosphere, atmosphere, lithosphere and biosphere. Great efforts have been made in the last two decades to reduce pollution sources and remedy the polluted soil and water resources. Phytoremediation, being more cost-effective and fewer side effects than physical and chemical approaches, has gained increasing popularity in both academic and practical circles. More than 400 plant species have been identified to have potential for soil and water remediation. Among them, *Thlaspi*, *Brassica*, *Sedum alfredii* H., and *Arabidopsis* species have been mostly studied. It is also expected that recent advances in biotechnology will play a promising role in the development of new hyperaccumulators by transferring metal hyperaccumulating genes from low biomass wild species to the higher biomass producing cultivated species in the times to come. This paper attempted to provide a brief review on recent progresses in research and practical applications of phytoremediation for soil and water resources.

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INTRODUCTION

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers (McGrath *et al*., 2001; Nriagu and Pacyna, 1988; Schalscha and Ahumada, 1998). Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001; Gisbert *et al*., 2003). Therefore, heavy metal pollution poses a great potential threat to the environment and human health.

In order to maintain good quality of soils and waters and keep them free from contamination, continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. Physicochemical approaches have been

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widely used for remedying polluted soil and water, especially at a small scale. However, they experience more difficulties for a large scale of remediation because of high costs and side effects. The use of plant species for cleaning polluted soils and waters named as phytoremediation has gained increasing attention since last decade, as an emerging cheaper technology. Many studies have been conducted in this field in the last two decades. Numerous plant species have been identified and tested for their traits in the uptake and accumulation of different heavy metals. Mechanisms of metal uptake at whole plant and cellular levels have been investigated. Progresses have been made in the mechanistic and practical application aspects of phytoremediation. They were reviewed and reported in this paper.

SOURCES OF HEAVY METALS AND SOIL-WATER POLLUTION

Land and water pollution by heavy metals is a worldwide issue. All countries have been affected, though the area and severity of pollution vary enormously. In Western Europe, 1400000 sites were affected by heavy metals (McGrath *et al*., 2001), of which, over 300000 were contaminated, and the estimated total number in Europe could be much larger, as pollution problems increasingly occurred in Central and Eastern European countries (Gade, 2000). In USA, there are 600000 brown fields which are contaminated with heavy metals and need reclamation (McKeehan, 2000). According to government statistics, coal mine has contaminated more than 19000 km of US streams and rivers from heavy metals, acid mine drainage and polluted sediments. More than 100000 ha of cropland, 55000 ha of pasture and 50000 ha of forest have been lost (Ragnarsdottir and Hawkins, 2005). The problem of land pollution is also a great challenge in China, where one-sixth of total arable land has been polluted by heavy metals, and more than 40% has been degraded to varying degree due to erosion and desertification (Liu, 2006). Soil and water pollution is also severe in India, Pakistan and Bangladesh, where small industrial units are pouring their untreated effluents in the surface drains, which spread over near agricultural fields. In these countries raw sewage is often used for producing vegetables near big cities.

Heavy metals that have been identified in the polluted environment include As, Cu, Cd, Pb, Cr, Ni, Hg and Zn. The sources of various heavy metals are listed in Table 1. The presence of any metal may vary from site to site, depending upon the source of individual pollutant. Excessive uptake of metals by plants may produce toxicity in human nutrition, and cause acute and chronic diseases. For instance, Cd and Zn can lead to acute gastrointestinal and respiratory damages and acute heart, brain and kidney damages. High concentrations of heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Garbisu and Alkorta, 2001; Schmidt, 2003; Schwartz *et al*., 2003). Soil contamination with heavy metals may also cause changes in the composition of

Table 1 Different sources of heavy metals

Heavy metals	Sources
As	Semiconductors, petroleum refining, wood preservatives, animal feed additives, coal power plants, herbicides, volcanoes, mining and smelting (Nriagu, 1994; Walsh *et al*., 1979)
Cu	Electroplating industry, smelting and refining, mining, biosolids (Liu *et al*., 2005)
Cd	Geogenic sources (Baize, 1997), anthropogenic activities (Nriagu and Pacyna, 1988), metal smelting and refining, fossil fuel burning, application of phosphate fertilizers, sewage sludge (Alloway, 1995; Kabata-Pendias, 2001)
Cr	Electroplating industry, sludge, solid waste, tanneries (Knox *et al*., 1999)
Pb	Mining and smelting of metalliferous ores, burning of leaded gasoline, municipal sewage, industrial wastes enriched in Pb, paints (Gisbert *et al*., 2003; Seaward and Richardson, 1990)
Hg	Volcano eruptions, forest fire, emissions from industries producing caustic soda, coal, peat and wood burning (Lindqvist, 1991)
Se	Coal mining, oil refining, combustion of fossil fuels, glass manufacturing industry, chemical synthesis (e.g., varnish, pigment formulation)
Ni	Volcanic eruptions, land fill, forest fire, bubble bursting and gas exchange in ocean, weathering of soils and geological materials (Knox *et al*., 1999)
Zn	Electroplating industry, smelting and refining, mining, biosolids (Liu *et al*., 2005)

soil microbial community, adversely affecting soil characteristics (Giller *et al*., 1998; Kozdrój and van Elsas, 2001; Kurek and Bollag, 2004).

TECHNOLOGIES FOR THE RECLAMATION OF POLLUTED SOILS

The cleaning of contaminated soils from heavy metals is the most difficult task, particularly on a large scale. The soil is composed of organic and inorganic solid constituents, water and mixture of different gases present in various proportions. The mineral components vary according to parent materials on which the soil had been developed under a particular set of climatic conditions. Therefore, soils vary enormously in physical, chemical and biological properties. Soil water movement is controlled by physical properties, such as soil structure and texture. The soil moisture has great bearing in controlling solute movement, salt solubility, chemical reactions and microbiological activities and ultimately the bioavailability of the metal ions. A successful phytoremediation program, therefore, must take into consideration variations in soil properties of the specific site.

Different approaches have been used or developed to mitigate/reclaim the heavy metal polluted soils and waters including the landfill/damping sites. These may be broadly classified into physicochemical and biological approaches.

The physicochemical approach includes excavation and burial of the soil at a hazardous waste site, fixation/inactivation (chemical processing of the soil to immobilize the metals), leaching by using acid solutions or proprietary leachants to desorb and leach the metals from soil followed by the return of clean soil residue to the site (Salt *et al*., 1995), precipitation or flocculation followed by sedimentation, ion exchange, reverse osmosis and microfiltration (Raskin *et al*., 1996). The physicochemical approaches are generally costly and have side effects (Raskin *et al*., 1997; McGrath *et al*., 2001).

Biological approaches of remediation include: (1) use of microorganisms to detoxify the metals by valence transformation, extracellular chemical precipitation, or volatilization [some microorganism can enzymatically reduce a variety of metals in metabolic processes that are not related to metal assimilation], and (2) use of special type of plants to decontaminate soil or water by inactivating metals in the rhizosphere or translocating them in the aerial parts. This approach is called phytoremediation, which is considered as a new and highly promising technology for the reclamation of polluted sites and cheaper than physicochemical approaches (Garbisu and Alkorta, 2001; McGrath *et al*., 2001; Raskin *et al*., 1997).

Phytoremediation, also referred as botanical bioremediation (Chaney *et al*., 1997), involves the use of green plants to decontaminate soils, water and air. It is an emerging technology that can be applied to both organic and inorganic pollutants present in the soil, water or air (Salt *et al*., 1998). However, the ability to accumulate heavy metals varies significantly between species and among cultivars within species, as different mechanisms of ion uptake are operative in each species, based on their genetic, morphological, physiological and anatomical characteristics. There are different categories of phytoremediation, including phytoextraction, phytofiltration, phytostabilization, phytovolatization and phytodegradation, depending on the mechanisms of remediation. Phytoextraction involves the use of plants to remove contaminants from soil. The metal ion accumulated in the aerial parts that can be removed to dispose or burnt to recover metals. Phytofiltration involves the plant roots or seedling for removal of metals from aqueous wastes. In phytostabilization, the plant roots absorb the pollutants from the soil and keep them in the rhizosphere, rendering them harmless by preventing them from leaching. Phytovolatization involves the use of plants to volatilize pollutants from their foliage such as Se and Hg. Phytodegradation means the use of plants and associated microorganisms to degrade organic pollutants (Garbisu and Alkorta, 2001). Some plants may have one function whereas others can involve two or more functions of phytoremediation.

PLANT SPECIES FOR PHYTOREMEDIATION

To identify plant populations with the ability to accumulate heavy metals, 300 accessions of 30 plant species were tested by Ebbs *et al*.(1997) in hydroponics for 4 weeks, having moderate levels of Cd, Cu and Zn. The results indicate that many *Brasssica* spp. such as *B. juncea* L., *B. juncea* L. Czern, *B. napus* L. and *B. rapa* L. exhibited moderately enhanced Zn and Cd accumulation. They were also found to be most effective in removing Zn from the contaminated soils. To date, more than 400 plant species have been identified as metal hyperaccumulators, representing less than 0.2% of all angiosperms (Brooks, 1998; Baker *et al*., 2000). The plant species that have been identified for remediation of soil include either high biomass plants such as willow (Landberg and Greger, 1996) or those that have low biomass but high hyperaccumulating characteristics such as *Thlaspi* and *Arabidopsis* species. On worldwide basis, the number of species identified to have ability to accumulate one or more metals >1000 mg/kg dry weight is listed in Table 2 (Reeves, 2003).

Table 2 The number of plant species that are reported to have hyperaccumulation traits (metal concentration >1000 mg/kg dry weight) (Reeves, 2003)

Metals	Number of species	Metals	Number of species
As	04	Pb	14
Cd	01	Ni	>320
Co	34	Se	20
Cu	34		

The hyperaccumulators that have been most extensively studied by scientific community include *Thlaspi* sp., *Arabidopsis* sp., *Sedum alfredii* sp. (both genera belong to the family of Brassicaceae and Alyssum). *Thlaspi* sp. are known to hyperaccumulate more than one metal, i.e., *T. caerulescens* for Cd, Ni, Pb and Zn, *T. goesingense* for Ni and Zn, *T. ochroleucum* for Ni and Zn, and *T. rotundifolium* for Ni, Pb and Zn (Prasad and Freitas, 2003). Among the genus *Thlaspi*, the hyperaccumulator plant *Thlaspi caerulescens* received much attention and has been extensively studied as potential candidates for Cd and Zn contaminated soils. Robinson *et al*.(1998) found *T. caerulescens* as hyperaccumulator for Cd and Zn could remove as high as 60 kg Zn/ha and 8.4 kg Cd/ha. It can accumulate as high as 2600×10^{-6} Zn without showing any injury (Brown *et al*., 1995) and extract up to 22% of soil exchangeable Cd from the contaminated site. It also showed remarkable Cd tolerance (Sneller *et al*., 2000; Escarre *et al*., 2000; Lombi *et al*., 2000). *T. caerulescens* has higher uptake of Cd due to specific rooting strategy and a high uptake rate resulting from the existence in this population of Cd-specific transport channels or carriers in the root membrane (Schwartz *et al*., 2003).

METAL HYPERACCUMULATION IN VARIOUS PLANT SPECIES

The hyperaccumulation of metals in various plant species has been extensively investigated and to date substantial progress has been made. It becomes clear that different mechanisms of metal accumulation, exclusion and compartmentation exist in various plant species. In *T. caerulescens*, Zn is sequestered preferentially in vacuoles of epidermal cells in a soluble form (Frey *et al*., 2000). In *A. halleri* leaves, Zn was found to be accumulated in the mesophyll cells (Kupper *et al*., 2000; Zhao *et al*., 2000; Sarret *et al*., 2002). Cosio *et al*.(2004) investigated the mechanisms of Zn and Cd accumulation in three different plant species through ion compartmentation by measuring the short term ^{109}Cd and ^{65}Zn uptake in mesophyll protoplast of *T. caerulescens* “Ganges” and *A. halleri*. Their study suggests the existence of regulation mechanism on the plasma membrane of leaf mesophyll protoplast.

Puschenreiter *et al*.(2003) investigated chemical changes in the rhizosphere of hyperaccumulators *T. goesingense* and *T. caerulescens* and the metal excluder *T. arvense* with a rhizosphere bag experiment on the contaminated and non-contaminated soils. Hyperaccumulation and depletion of labile Zn in the rhizosphere were observed for *T. goesingense* grown on the contaminated soil. In the non-contaminated soil, Zn was accumulated but labile Zn in the rhizosphere was not changed. Nickel present in background concentration in both soils was accumulated by *T. goesingenseonly* when grown on non-contaminated soil. In contrast, labile Ni in the rhizosphere increased in both soils, suggesting a general tendency of Ni mobilization by *T. goesingense*. Uneo *et al*.(2004a) studied the interaction between Zn and Cd in *T. caerulescens* in solution culture and in pot soil. Results from long term (4 weeks) and short term (1 week) solution culture experiments indicate that Cd accumulation in the shoot was not affected by the supply of a 4~10-fold excess of Zn, whereas the Cd concentration of the roots decreased with increasing Zn concentrations in the solution. The results suggest that the Ganges ecotype of *T. caerulescens* displayed different uptake systems for Cd and Zn and that Cd competed with Zn uptake while Zn did not compete with Cd uptake. Uneo *et al*.(2004b) investigated the uptake of Cd and Zn by *T. caerulescens* (the Ganges ecotype) from enriched soil with different insoluble and soluble sources of Cd and Zn. The data show that there was no significant differences in the shoot Cd concentration between the treatments with soluble or insoluble Cd compounds, even though Cd concentration in the soil solution was in the order of $CdSO_4$>>$CdCO_3$>CdS. *Thlaspi caerulescens* grown on the ZnS-enriched soil accumulated up to 6900 mg Zn/kg in the shoots, although Zn accumulation was 1.5 times higher with the addition of more soluble compounds $Zn_3(PO_4)_2$ or $ZnSO_4$. These results indicate that the Ganges ecotype of *T. caerulescens* is able to utilize insoluble Cd and Zn compounds in soils.

Whiting *et al*.(2000) found that the plants from *T. carerulescens* population that accumulated Cd also showed increased root biomass and root length after allocation into Cd-enriched soil, whereas plants from the population that did not accumulate Cd showed no such increase.

T. caerulescens was grown with *H. vulgare* and *L. heterophyllum* in the field to examine the effect of rhizosphere interaction on metal uptake. The data show that the Cd concentration in *H. vulgare* was increased by a factor of 2.4 when it was grown along the sides of *T. caerulescens* without a barrier. In contrast, the uptake of Zn by *H. vulgare* was significantly decreased, probably through metal depletion within the zone of the Zn-hyperaccumulator rhizosphere. These results suggest that *T. caerulescens* may alter conditions in the shared rhizospheres and thereby affect the availability of selected metals to neighboring plants (Gove *et al*., 2002). On the other hand, when *S. alfredii* was intercropped with a grain crop, *Z. mays*, heavy metals (Zn and Cu) accumulated in the grains were significantly reduced, as compared to monoculture cropping, and the intercropping improved the growth of both plant species (Liu *et al*., 2005).

Studies on the role of rhizosphere process in metal hyperaccumulation of Ni in *T. geosingense* Halacsy by Wenzel *et al*.(2002) indicate that root exudates of organic ligands may contribute to Ni hyperaccumulation in *T. geosingense* Halacsy. This was attributed to the ligand-induced dissolution of Ni bearing minerals in the rhizosphere of *T. geosingense* and appeared to be less effective in the rhizosphere of excluder *Silene vulgaris* and *Rumex acetosella* growing on the same site.

Sedum alfredii Hance was identified in China as hyperaccumulator for Cd and Zn and has been intensively investigated by various researchers in their studies conducted in hydroponics and/or the uncontaminated and contaminated soils (Li H. *et al*., 2005; Li T.Q. *et al*., 2005a; Liu *et al*., 2005; Xiong *et al*., 2004; Yang *et al*., 2004; 2006). The data show that the concentrations of Cd and Zn in leaves and stems increased with increasing Cd and Zn supply levels. The distributions of the metals in different plant parts decreased in the order: stem>leaf>root for Zn and leaf>stem>root for Cd. These results indicate that *S. alfredii* has an extraordinary ability to tolerate Cd/Zn toxicities, and to absorb and hyperaccumulate Cd and Zn under a range of Cd/Zn combining levels. The uptake and accumulation of Cd by the mined and the non-mined ecotypes of *S. alfredii* indicated that the plants of the mined ecotype (ME) have higher tolerance to Cd than those of the non-mined ecotypes (NME) in terms of dry matter yield (Xiong *et al*., 2004).

Zinc compartmentation studies involving hyperaccumulating and non-hyperaccumulating *S. alfredii* plants using radioactive tracer flux technique indicate that *S. alfredii* H. can accumulate Zn in shoots over 2% of dry weight. Leaf and stem Zn concentrations of the hyperaccumulating ecotype (HE) were 24- and 28-fold higher, respectively, than those of the non-hyperaccumulating ecotype (NHE), whereas 1.4-fold more Zn was accumulated in the roots of the NHE. Approximately 2.7-fold more Zn was stored in the root vacuoles of the NHE, and thus became unavailable for loading into the xylem and subsequent translocation to shoots. These results also indicate that the altered Zn transport across tonoplast in the root and the stimulated Zn uptake in the leaf cells are the major mechanisms involved in the strong Zn hyperaccumulation observed in *S. alfredii* H. (Yang *et al*., 2006).

The root morphology and Zn^{2+} uptake kinetics of

HE and NHE of *S. alfredii* H. were investigated using hydroponic methods and the radiotracer flux technique. The results indicate that the root length, root surface area and root volume of NHE decreased significantly with increasing Zn^{2+} concentration in growth media, whereas the root growth of HE was not adversely affected, and even promoted, by 500 μmol/L Zn^{2+}. The concentrations of Zn^{2+} in both ecotypes of *S. alfredii* H. were positively correlated with root length, root surface area and root volumes, but no such correlation was found with root diameter. The uptake kinetics for $^{65}Zn^{2+}$ in the roots of both ecotypes of *S. alfredii* were characterized by a rapid linear phase during the first 6 h and a slower linear phase during the subsequent period of investigation. The concentration-dependent uptake kinetics of the two ecotypes of *S. alfredii* could be characterized by the Michaelis-Menten equation, with the V_{max} (maximum uptake speed) for $^{65}Zn^{2+}$ influx being 3-fold greater in the HE than that in the NHE, indicating that enhanced absorption into the root was one of the mechanisms involved in Zn hyperaccumulation. A significantly larger V_{max} value suggested that there was a higher density of Zn transporters per unit membrane area in HE roots (Li H. *et al*., 2005).

Li T.Q. *et al*.(2005b) investigated the root morphological and physiological response of the HE of *S. alfridii* H. from the mined area and the NHE of *S. alfridii* from the agricultural area to the supplied Zn and Pb in hydroponics. The results show that Zn concentrations in the leaves and the stems of HE were 34 and 41 times higher, whereas Pb concentrations were 1.9 and 2.4 times higher, respectively than those of the NHE when grown at 1224 μmol/L Zn and/or 200 μmol/L Pb. The study also shows that the tolerance and hyperaccumulation of the HE of *S. alfridii* H. to Zn and Pb appear to be closely related to its high adaptation of root growth, morphology and physiology to Pb and Zn toxicity. Through its root excretion of some special substances, the plant can activate Pb and Zn in the mined soil, thus increasing their mobilization and bioavailability.

The *Alyssum* species has been extensively studied for the hyperaccumulation of Ni. Kupper *et al*.(2001) studied the Ni uptake and cellular compartmentation in three Ni hyperaccumualtors: *A. bertolonii* (Desv), *A. lesbiacum* (Candargy), and *T. goesingense* (Halacsy). These three species showed similar hyperaccumulation of Ni, but *T. goesingense* was less tolerant to Ni than the other two species. Addition of 500 mg Ni/kg to a nutrient-rich growth medium significantly increased shoot biomass of all species. X-ray microanalysis of frozen-hydrated tissues of leaves and stems of all species showed that Ni in all species was distributed preferentially in the epidermal cells, most likely in the vacuoles of the leaves and stem. Kidd and Monterroso (2005) investigated the efficiency of *Alyssum serpyllifolium* ssp. *lusitanicum* (Brassicaceae) for use in phytoextraction of polymetal-contaminated soils. The plant was grown on two mine spoil soils, one contaminated with Cr (283 mg/kg) and the other moderately contaminated with Cr (263 mg/kg), Cu (264 mg/kg), Pb (1433 mg/kg) and Zn (377 mg/kg). The results suggest that *A. serpyllifolium* could be suitable for phytoextraction uses in polymetal-contaminated soils, provided that Cu concentrations were not phytotoxic.

Among different fern species, three accessions of *P. vitta*, two cultivars of *P. cretica*, *P. longifolia* and *P. umbrosa* were grown with 0~500 mg As/kg added to the substrate. The results show that in addition to *P. vitta*, *P. cretica*, *P. longifolia* and *P. umbrosa* also hyperaccumulate As to a similar extent. This study identified three new species of As hyperaccumulators in the *Pteris* genus (Zhao *et al*., 2002). In another study, the speciation and distribution of As of Brake fern was investigated by Zhang *et al*.(2002), which was grown for 20 weeks in As contaminated soil. The results show that As recoveries of 85% to 100% were obtained from most parts of the plant (rhizomes, fiddle heads, young fronds and old fronds), and for roots, the corresponding value was approximately 60%. The result also demonstrates the ability of Blake fern as As hyperaccumulator, which can transfer As rapidly from soil to above ground biomass with minimal As concentration in the roots. As is found to be predominantly as inorganic species. Caille *et al*.(2005) conducted a pot experiment with 0~500 mg/kg As added as arsenate and another short term (8 h) uptake experiment with 5×10^{-6} arsenate under phosphorus sufficient conditions, and grew hyperaccumulator *Pteris vitta* and the nonhyperaccumulator *Pteris tremula*. The results show that in both experiments *P. vitta* accumulated much more As than *P. tremula* without any visual toxicity symptoms.

PHYTOREMEDIATION OF POLLUTED WATER

Rhizofiltration is the removal of pollutants from the contaminated waters by accumulation into plant biomass. Several aquatic species have been identified and tested for the phytoremediation of heavy metals from the polluted water. These include sharp dock (*Polygonum amphibium* L.), duck weed (*Lemna minor* L.), water hyacinth (*Eichhornia crassipes*), water lettuce (*P. stratiotes*), water dropwort [*Oenathe javanica* (BL) DC], calamus (*Lepironia articulate*), pennywort (*Hydrocotyle umbellate* L.) (Prasad and Freitas, 2003). The roots of Indian mustard are found to be effective in the removal of Cd, Cr, Cu, Ni, Pb and Zn, and sunflower can remove Pb, U, Cs-137 and Sr-90 from hydroponic solutions (Zaranyika and Ndapwadza, 1995; Wang *et al*., 2002; Prasad and Freitas, 2003).

The potential of duck weed was investigated by Zayed *et al*.(1998) for the removal of Cd, Cr, Cu, Ni, Pb and Se from nutrient-added solution and the results indicate that duck weed is a good accumulator for Cd, Se and Cu, a moderate accumulator for Cr, but a poor accumulator of Ni and Pb. Dos Santos and Lenzi (2000) tested aquatic macrophyte (*Eiochhornia crassipes*) in the elimination of Pb from industrial effluents in a green house study and found it useful for Pb removal. Water hyacinth possesses a well-developed fibrous root system and large biomass and has been successfully used in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients. This plant can also reduce the concentrations of heavy metals in acid mine water while exhibiting few signs of toxicity. Water hyacinth accumulates trace elements such as Ag, Pb, Cd, etc. and is efficient for phytoremediation of wastewater polluted with Cd, Cr, Cu and Se (Zhu *et al*., 1999).

Wang *et al*.(2002) conducted a pot experiment to test five wetland plant species, i.e., sharp dock, duckweed, water hyacinth, water dropwort and calamus for their possible use in remedying the polluted waters. The results show that sharp dock was a good accumulator of N and P. Water hyacinth and duckweed strongly accumulated Cd with a concentration of 462 and 14200 mg/kg, respectively. Water dropwort achieved the highest concentration of Hg, whereas the calamus accumulated Pb (512 m/kg) substantially in its roots. Ingole and Bhole (2003) conducted hydroponic studies to investigate the uptake of As, Cr, Hg, Ni, Pb and Zn by water hyacinth from the aqueous solution at the concentrations ranging from 5 to 50 mg/L, and observed that in aqueous solutions containing 5 mg/L of As, Cr and Hg, the maximum uptake was 26, 108 and 327 mg/kg dry weight of water hyacinth, respectively.

Among the ferns, *Pteris vitta* commonly known as Brake fern has been identified as As hyperacccumulator for As contaminated soils and waters. It can accumulate up to 7500 mg As/kg on a contaminated site (Ma *et al*., 2001) without showing toxicity symptoms. One fern cultivar is available commercially for As phytoremediation and has been successfully used in field trials (Salido *et al*., 2003).

Li H. *et al*.(2005) conducted a laboratory study in hydroponics to test different levels of Cd on the growth and Cd uptake by three hydrophytes: *Gladiolous*, *Isoetes taiwaneneses* Dwvol and *Echinodorus amazonicus*. The data show that the biomass of all the plants decreased with an increase in Cd concentration from 5 to 20 mg/L. However, Cd toxic effect was greater on *Isoetes taiwaneneses* Dwvol and *Echinodorus amazonicus* than that on *Gladiolous*. In addition, the accumulation of Cd was higher in *Gladiolous* than the other two plants. Zhang *et al*. (2005) investigated the efficiency of Cu removal from the contaminated water by *Elsholtzia argyi* and *Elsholtzi splendens* in hydroponics. The results show that *Elsholtzia argyi* showed better Cu phytofiltration than *Elsholtzi splendens*, which was associated with better ability to higher Cu concentrations and translocation to shoots.

ENHANCEMENT OF PHYTOREMEDIATION BY CHEMICAL AND BIOLOGICAL APPROACHES

In order to cope with heavy metal contaminated soils, various phytoremediation approaches (phytostabilization, phytoimmobilization and phytoextraction) can be applied. However, the choice will depend on many factors, such as plant tolerance to pollutants, soil physicochemical properties, agronomic characteristics of the plant species, climatic conditions (rainfall, temperature), and additional technologies available for the recovery of metals from the harvested plant biomass. It appears that both chemical and biological approaches are passing through their infancy and need more efforts for their effective use in the future.

The solubility of heavy metals in the polluted soils can be increased by using organic and inorganic agents, thus enhancing the phytoextraction capabilities of many plant species. Ebbs *et al*.(1997) amended the contaminated soil with Grower-Power, a commercial soil amendment that improves soil structure and fertility, and the removal of Zn by plant shoots was doubled to more than 30000 mg Zn/pot (4.5 kg). Other applied enhancement materials include ethylene diamine tetraacetic acid (EDTA), citric acid, elemental sulfur or ammonium sulfate. Increases greater than 100 folds in Pb concentration in the biomass of crops were reported when EDTA was applied to the contaminated soils (Cunningham and Berti, 2000). Uranium, cadmium and zinc concentrations in plant biomass were increased by the application of citric acid, elemental sulfur or ammonium sulfate, respectively (Schmidt, 2003). In addition to the chelating material, the plant roots excrete metal-mobilizing substances called phytosiderophores. Other exudates include mugenic and deoxymugeneic acids from barley and corn, and avenic acid from oats (Welch and Norvell, 1993). Plant roots can increase metal bioavailability by exuding protons that acidify the soil and mobilize the metals. The lowering of soil pH decreases the adsorption of heavy metals and increases their concentrations in the soil solution. Soil microbes associated with plant roots are also helpful in the phytoextraction of the heavy metals in soils through the degradation of organic pollutants. These include several strains of bacillus and psedumonos, which increase the Cd accumulation in *Brassica juncea* seedlings (Salt *et al*., 1995).

Scott Angle *et al*.(2003) determined the effect of high soil moisture content on the growth and hyperaccumulation of Ni in three different species, including *Alyssum murale* and *Berkheya coddii* and Zn hyperaccumulator *T. caerulescens* cultivar AB300 and AB336. The results show that hyperaccumulators grew well under high soil moisture content and the biomass of all the tested species was generally greater at higher soil moistures and inhibited at lower soil moistures. These results suggest that for successful phytoremediation of metal polluted soils, a strategy should be developed to combine a rapid screening of plant species possessing the ability to accumulate heavy metals with agronomic practices that enhance shoot biomass production and/or increase metal bioavailability in the rhizosphere.

CONCLUSIONS AND PERSPECTIVES

The contamination of heavy metals to the environment, i.e., soil, water, plant and air is of great concern due to its potential impact on human and animal health. Cheaper and effective technologies are needed to protect the precious natural resources and biological lives. Substantial efforts have been made in identifying plant species and their mechanisms of uptake and hyperaccumulation of heavy metals in the last decade. There are genetic variations among plant species and even among the cultivar of the same species. The mechanisms of metal uptake, accumulation, exclusion, translocation, osmoregulation and copartmentation vary with each plant species and determine its specific role in phytoremediation. Variations exist for hyperaccumulation of different metals among various plant species and within populations. These variations do not correlate with either the metal concentration in the soil or the degree of metal tolerance in the plant (Pollard *et al*., 2002). In order to develop new crop species/plants having capabilities of metal extraction from the polluted environment, traditional breeding techniques, hybrid generation through protoplast fusions, and production of mutagens through radiation and chemicals are all in progress. With the development of biotechnology, the capabilities of hyperaccumulators may be greatly enhanced through specific metal gene identification and its transfer in certain promising species. This can play a significant role in the extraction of heavy metals from the polluted soils. The use of cleaning technologies is site-specific due to spatial and climatic variations and is not economically feasible everywhere. Therefore, cheaper technologies are being sought for practical use. Nevertheless, the recent advances in plant biotechnology have created a new hope for the development of hyperaccumulating species. However, much research work is needed in this respect such as metal uptake studies at cellular level including efflux and influx of different metal ions by different cell organelles and membranes. Rhizosphere studies under the control and field conditions are also needed to examine the antagonistic and synergistic effects of different metal ions in soil solution and the polluted waters. In depth soil microbial studies are required to identify the micro-organisms highly associated with metal solubility or precipitations. To date the available methods for the recovery of heavy metals from plant biomass of hyperaccumulators are still limited. Traditional disposal approaches such as burning and ashing are not applicable to volatile metals; therefore, investigations are needed to develop new methods for effective recovery of metals from the hyperaccumulatior plant biomass.

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