PHYTOREMEDIATION OF METAL MINE WASTE

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(Received 24th April 2008 ; accepted 28th May 2010)

Abstract: Phytoremediation is a group of technologies that use plants to reduce, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim to clean-up contaminated areas. In this review paper, different types of phytoremediation processes, typical plants used and their application for clean-up of metal contaminated sites were reviewed. Plant responses to heavy metals and mechanisms of metal uptake and transport were also discussed. Phytoremediation of Pb, Zn, Cu and Fe tailings and mine spoils were carried out by grasses, herbs and shrubs, which could be categorised as As accumulator (*Paspalum, Eriochloa, Holcus, Pennisetum Juncus, Scirpus* and *Thymus),* Pb accumulator *(Brassica juncea, Vetiveria, Sesbania, Minuartia, Juncus, Scirpus* and *Thymus),* Cu accumulator *(Ammania baccifera, Scleranthus)* Zn and Cd accumulator *(Vetiveria, Sesbania, Viola, Sedum, Rumex).* The research work showed that, bioavailability and metal uptake by plants could be accomplished by ameliorating pH, addition of organic amendment, fertiliser and chelating agents. Further research is required to develop fast growing high biomass plants with improved metal uptake ability, increased translocation and tolerance of metals through genetic engineering for effective phytoremediation of metal mine wastes.

Keywords: *Phytoextraction, mine tailings, metal accumulator plants.*

Introduction

Phytoremediation, the use of plants for environmental restoration, is an emerging cleanup technology. Both metal and non-metal mining activities generate huge quantity of waste rocks, which damages the aesthetics of the area. Particularly, in case of metal mining, activites such as crushing, grinding, washing, smelting and all the other process used to extract, concentrate metals, generate a large amount of waste rocks and tailings which scars the landscape, disrupts ecosystems and destroys microbial communities. Waste materials or spoils that remain after the extraction of usable ores are dumped on the surrounding land, which is the sources of toxic metals, leave the land devoid of topsoil, nutrients and supportive microflora and vegetation, thus remains barren (Das and Maiti, 2008).

Most of the conventional remedial technologies like leaching of pollutant, vitrification, electrokinetical treatment, excavation and off-site treatment are expensive and technically limited to relatively small areas (Barceló and Poschenrieder, 2003). Moreover, they deteriorates the soil fertility, which subsequently causes negative impacts on the ecosystem. Establishment of vegetation cover can fulfill the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings (Freitas et al., 2004). The use of plants for purifying contaminated soils and water has been developed much more recently. In the 1970s, reclamation initiatives of mining sites developed technologies for covering soil with vegetation for stabilization purposes and reduction of visual impact (Williamson and Johnson, 1981). It was not until the 1990s, that the concept of phytoremediation emerged as a new technology that uses plants to reduce, remove, degrade or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. The generic term ‘phytoremediation’ consists of the Greek prefix “phyto” (plant), attached to the Latin root “remedium” (to correct or remove evil) (Cunningham and Ow, 1996). Phytoremediation is a cost effective, environmental friendly, aesthetically pleasing approach with long term applicability. Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable (Williamson and Johnson, 1981).

However, adverse factors such as acidity, nutrient deficiencies, toxic heavy metal ions, poor physical structure, and their interaction in most mine tailings inhibit plant establishment and growth on the tailings (Pichtel and Salt, 2003). Mine spoil or tailing dumps usually have barren surfaces, with rare plants that show signs of suffering such as stunted growth, chlorosis, necrosis and anomalous development of roots with respect to shoots (Dinelli and Lombini, 1996). Low specific diversity in the mine spoil area is the result of severe environmental conditions, but some plants can tolerate high concentration without sign of stress. Collecting plant species from contaminated soil and the evaluation of plant metal concentrations can be used to get information about specific plant behavior in this environment and to complete data about metal dispersion, with reference to their mobility to the biomass. Some plants phytostabilise heavy metals in the rhizophere through root exudates immobilization (Blaylock and Huang, 2000), whilst other species incorporate them into root tissues (Khan, 2001). Some plants also transfer metals to their above ground tissues, potentially allowing the soil to be decontaminated by harvesting the aboveground parts. Therefore, plant community established on mine spoil / tailings could be useful to minimize the impacts of mining. Moreover knowing the diversity of plant responses in contaminated sites having different metals and toxicity levels, is important to study the composition of plant community that was established on degraded soils or mine spoil, which would serve as a basic approach for mine remediation.

Phytoremediation processes

Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes and the build-up of organic carbon in the soil. For metal contaminates, plants show the potential for phytoextraction (uptake and recovery of contaminates into above-ground biomass), filtering metals from water into root systems (*rhizofiltration*), or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water (*phytostabilization*) and so on (Cunningham and Ow, 1996). There are a number of different forms of phytoremediation, discussed below. All phytoremediation processes are not exclusive and may be used simultaneously. The different forms of phytoremediation may apply to specific types of contaminants or contaminated media and may require different types of plants as shown in *Table 1*.

Table 1. *Typical Plants Used in Various Phytoremediation Processes*

Process	Mechanism	Media	Contaminants	Typical Plants
1. Phyto-extraction	Hyper-accumulation	Soil, Brownfields, Sediments	Metals (Pb, Cd, Zn, Ni, Cu) with EDTA addition for Pb, Selenium.	Sunflowers, Indian mustard, Rape seed plants, Barley, Hops, Crucifers, Serpentine plants
2. Rhizo-filtration	Rhizosphere accumulation	Groundwater, Water and Wastewater in Lagoons or Created Wetlands	Metals (Pb, Cd, Zn, Ni, Cu) Radionuclides (^{137}Cs, ^{90}Sr, ^{238}U) Hydrophobic organics	Aquatic Plants: - Emergents (bullrush, cattail, pondweed, arrowroot, duckweed); - Submergents (algae, stonewort, parrot feather, *Hydrilla*)
3. Phyto - stabilization	Complexation	Soil, Sediments	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) Hydrophobic Organics (PAHs, PCBs, dioxins, furans, pentachlorophenol, DDT, dieldrin)	Phreatophyte trees to transpire large amounts of water for hydraulic control; Grasses with fibrous roots to stabilize soil erosion; Dense root systems are needed to sorb / bind contaminants
4. Phyto-volatization	Volatization by leaves	Soil, Groundwater, Sediments	Mercury, Selenium, Tritium	Poplar, Indian mustard, Canola, Tobacco plants.
5. Phyto-degradation	Degradation in plant	Soil, Groundwater, Landfill leachate, Land application of wastewater	Herbicides (atrazine, alachlor) Aromatics (BTEX) Chlorianated aliphatics (TCE) Nutrients (NO_3^-, NH_4^+, PO_4^{3-}) Ammunition wastes (TNT, RDX)	Phreatophyte trees (poplar, willow, cottonwood); Grasses (rye, Bermuda, sorghum, fescue); Legumes (clover, alfalfa, cowpeas)
6. Rhizo-degradation	Degradation by plant rhizosphere microorganisms	Soil, Sediments, Land application of wastewater	Organic contaminants (pesticides, aromatics and polynuclear aromatic hydrocarbons [PAHs])	Phenolics releasers (mulberry, apple, orange); Grasses with fibrous roots (rye, fescue, Bermuda) for contaminants 0-3 ft deep; Phreatophyte trees for 0-10 ft; Aquatic plants for sediments

Phytoextraction

This process reduces soil metal concentrations by cultivating plants with a high capacity for metal accumulation in shoots (Barceló and Poschenrieder, 2003). The plants must extract large concentrations of heavy metals into their roots, translocate the heavy metals to above ground shoots or leaves and produce large quantity of plant biomass that can be easily harvested; when plants are harvested contaminants are removed from the soil. Recovery of high price metals from the harvested plant material may be cost effective (eg. phytomining of Ni, Tl or Au). If not, the dry matter can be burnt and the ash disposed of under controlled conditions. Phytoextraction is also known as phytoaccumulation, phytoabsorption and phytosequestration. Phytoextraction can be divided into two categories: continuous and induced (Salt et al., 1998). Continuous phytoextraction requires the use of plants that accumulate particularly high levels of the toxic contaminants throughout their lifetime (hyperaccumulators), while induced phytoextraction approaches enhance toxin accumulation at a single time point by addition of accelerants or chelators to the soil.

Rhizofiltration

This technique is used for cleaning contaminated surface waters or waste waters such as industrial discharge, agricultural runoff, or acid mine drainage by absorption or precipitation of metals onto roots or absorption by roots or other submerged organs of metal tolerant aquatic plants. For this purpose plants must not only be metal resistant but also have a high absorption surface and must tolerate hypoxia (Dushenkov et al., 1995). Contaminant should be those that sorb strongly to roots, such as hydrophobic organics, lead, chromium(III), uranium and arsenic(V). Plants like sunflower, Indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove lead from effluent, with sunflower having the greatest ability (Raskin and Ensley, 2000).

Phytostabilization

It refers to the holding of contaminated soils and sediments in place by vegetation, and to immobilizing toxic contaminants in soils. Phytostabilization is also known as in-place inactivation or phytoimmobilization. Phytostabilization can occur through the sorption, precipitation, complexation or metal valence reduction (Ghosh and Singh, 2005). Metals do not ultimately degrade, so capturing them in situ is sometimes the best alternative at sites with low contamination levels or at vast contaminated areas where a large scale removal action or other in situ remediation is not possible. Plants with high transpiration rates, such as grasses, sedges, forage plants and reeds are useful for phytostabilization by decreasing the amount of ground water migrating away from the site carrying contaminants. Combining these plants with hardy, perennial, dense rooted or deep rooting trees (popular, cottonwoods) can be an effective combination (Berti and Cunningham, 2000).

Phytovolatization

It involves the use of plants to take up contaminants from the soil transforming them into volatile form and transpiring them into the atmosphere. Selenium (Se) is a special case of a metal that is taken up by plants and volatilized. Neumann et al. (2003) found that an axenically cultured isolate of single celled freshwater microalgae (*Chlorella* sp.) metabolized toxic selenate to volatile dimethylselenide at exceptionally high rates when transferred from mineral solution to water for 24h, than those similarly measured for wetland macroalgae and higher plants. Hyper-volatilization of selenate by microalgae cells may provide a novel detoxification response. Uptake and evaporation of Hg is achieved by some bacteria. The bacterial genes responsible have already been transferred to *Nicotiana* or *Brassica* species, and these transgenic plants may become useful in cleaning Hg-contaminated soils (Meager et al., 2000).

Phytodegradation

It involves uptake, metabolisation and degradation of contaminants within the plant, or the degradation of contaminants in the soil sediments, sludges, groundwater or surface water by enzymes produced and released by the plant. Phytodegradation is not dependent on microorganisms associated with the rhizosphere. Phytodegradation is also known as phytotransformation, and is a contaminant destruction process. For instance, the major water and soil contaminant trichloroethylene (TCE) was found to be taken up by hybrid poplar trees (*Populas deltoids nigra*), which breaks down the contaminant into its metabolic components (Newman et al., 1997).

Rhizodegradation

Rhizodegradation is the breakdown or organics in the soil through microbial activity of the root zone (rhizosphere). Enhanced rhizosphere degradation uses plants to stimulate the rhizosphere microbial community to degrade organic contaminants (Kirk et al., 2005). Grasses with high root density, legumes and alfalfa that fix nitrogen and have high evapotranspiration rates are associated with different microbial populations. Significantly higher populations of total heterotrophs, denitrifers, were found in rhizosphere soil around hybrid poplar trees in a field pot than in non-rhizosphere soil (Jordahl et al., 1997).

Phytorestoration

It involves the complete remediation of contaminated soils to fully functioning soils (Bradshaw, 1997). In particular, this subdivision of phytoremediation uses plants that are native to the particular area, in an attempt to return the land to its natural state.

Hydraulic control

It is the use of vegetation to influence the movement of ground water and soil water, through the uptake and consumption of large volumes of water. Hydraulic control reduces or prevents infiltration and leaching and induces upward flow of water from the water table through the vadose zone. Vegetation water uptake and transpiration rates are important for hydraulic control. The application of different phytoremediation technologies for cleaning up of metal contaminated sites are given in *Table 2.*

Table 2. *Phytoremediation application*

Location	Application	Plants	Contaminants	Performance
Trenton, NJ	Phytoextraction demonstration 200 ft x 300 ft plot Brownfield location	Indian mustard *(Brassica juncea)*	Pb	Pb cleaned-up to below action level in one season.
Dearing, KS	Phytostabilization demonstration one acre test plot abandoned smelter, barren land	Poplars *(Populus* sp.)	Pb, Zn Cd Concs. > 20,000 ppm for Pb and Zn	50% survival after 3 years. Site was successfully revegetated.
Whitewood Cr., South Dakota	Phytostabilization demonstration one acre test plot mine wastes	Poplars *(Populus* sp.)	As, Cd	95% of trees died. Inclement weather, toxicity caused die-off.
Pennsylvania	Phytoextraction mine wastes	*Thlaspi caerulescens*	Zn, Cd	Uptake is rapid but difficult to decontaminate soil.
San Francisco, CA	Phytovolatization refinery wastes and agricultural soils	*Brassica* sp.	Se	Selenium is partly taken-up and volatilized, but difficult to decontaminate soil.

Plant response to heavy metals

When categorizing plants that can grow in the presence of toxic elements, the terms "metal excluder", "metal indicator", and "metal accumulator" are used. Metal excluders prevent metal from entering their aerial parts or maintain low and constant metal concentration over a broad range of metal concentration in soil; they mainly restrict metal in their roots. The plant may alter its membrane permeability, change metal binding capacity of cell walls, or exclude more chelating substances (Lasat, 2000).

A metal indicator species is one that actively accumulates metal in their aerial tissues and generally reflects metal level in soil. They tolerate the existing concentration level of metals by producing intracellular metal binding compounds (chelators), or alter metal compartmentalization pattern by storing metals in non-sensitive parts (Ghosh and Singh, 2005). Indicator species have been used for mine prospecting to find new ore bodies.

Metal accumulators can concentrate metal in their aerial parts, to levels far exceeding than soil. By definition, hyperaccumulators are herbaceous or woody plants that accumulate and tolerate without visible symptoms a hundred times or greater metal concentrations in shoots than those usually found in non-accumulators. Baker and Brooks (1989) established 0.1% as the minimum threshold tissue concentrations for plants considered Co, Cu, Cr, Pb or Ni hyperaccumulators, while for Zn or Mn the threshold is 1%. For cadmium and other rare metals, it is 0.01% by dry weight. Hyperaccumulators are found in 45 different families, with the highest occurrence among the Brassicaceae (Reeves and Baker, 2000). These plants are quite varied, from perennial shrubs and trees to small annual herbs. Some of the hyperaccumulators and their metal accumulation capabilities are listed in *Table 3.*

Table 3. *Metal concentrations (on a dry weight basis) in known hyperaccumulators*

Metal	**Plant Species**	**Concentrations in "Harvestable" Material from Plants Grown in Contaminated Soil (dry wt basis)**
Cd	*Thlaspi caerulescens*	1,800 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Cu	*Ipomoea alpine*	12,300 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Co	*Haumaniastrum robertii*	10,200 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Pb	*T. rotundifolium*	8,200 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Mn	*Macadamia neurophylla*	51,800 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Ni	*Psychotria douarrei,* *Sebertia acuminate*	47,500 mg kg $^{-1}$ in shoots (Baker and Walker, 1990) 25% by wt of dried sap (Jaffre et al., 1976)
Zn	*T. caerulescens*	51,600 mg kg $^{-1}$ in shoots (Brown et al., 1994)

How do plants take up and transport metal?

The process of metal accumulation involves several steps; one or more of which are responsible for the hyperaccumulation in plants:

Solubilization of the metal from the soil matrix

Many metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal. Plants have evolved these processes to liberate essential metals from the soil, but soils with high concentrations of toxic metals will release both essential and toxic metals to solution (Lasat, 2000).

Uptake into the root

Soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells. While it is possible for solutes to travel up through the plant by apoplastic flow, the more efficient method of moving up the plant is through the vasculature of the plant, called the xylem. To enter the xylem, solutes must cross the Casparian strip, a waxy coating, which is impermeable to solutes, unless they pass through the cells of the endodermis. Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channel. Most toxic metals are thought to cross these membranes through pumps and channels intended to transport essential elements. Excluder plants survive by enhancing specificity for the essential element or pumping the toxic metal back out of the plant (Hall, 2002).

Transport to the leaves

Once loaded into the xylem, the flow of the xylem sap will transport the metal to the leaves, where it must be loaded into the cells of the leaf, again crossing a membrane. The cell types where the metals are deposited vary between hyperaccumulator species.

Detoxification and/or chelation

At any point along the pathway, the metal could be converted to a less toxic form through chemical conversion or by complexation. Various oxidation states of toxic elements have very different uptake, transport, and sequestration or toxicity characteristics in plants. Chelation of toxins by endogenous plant compounds can have similar effects on all of these properties as well. As many chelators use thiol groups as ligands, the sulfur (S) biosynthetic pathways have been shown to be critical for hyperaccumulator function (Van Huysen et al., 2004) and for possible phytoremediation strategies.

Sequestration and volatilization

The final step for the accumulation of most metals is the sequestration of the metal away from any cellular processes it might disrupt. Sequestration usually occurs in the plant vacuole, where the metal/metal-ligand must be transported across the vacuolar membrane. Metals may also remain in the cell wall instead of crossing the plasma membrane into the cell, as the negative charge sites on the cell walls may interact with polyvalent cations (Wang and Evangelou, 1994). Selenium may also be volatized through the stomata.

Phytoremediation of metal mine waste

The selection of trace element tolerant species is a key factor to the success of remediation of degraded mine soils. For long-term remediation, metal tolerant species are commonly used for revegetation of mine tailings (Lan et al., 1997), and herbaceous legumes can be used as pioneer species to solve the problem of nitrogen deficiencies in mining wastelands because of their N_2 fixing ability (Lan et al., 1997).

Singh et al. (2004) describes the impact of young high-density plantations of two native leguminous (*Albizia procera* and *A. lebbeck*) and one non-leguminous timber tree (*Tectona grandis*) species on the soil redevelopment process during the early phase of mine restoration in a dry tropical environment. There was a general improvement in soil properties due to establishment of plantations. Highest soil organic C values were found in *A. lebbeck* plantations and lowest in *T. grandis* plantations. Both *A. lebbeck* and *A. procera* substantially increased levels of nitrogen in soil. However, *A. procera*, with slow decomposing litter, was not as effective in raising N levels in the soil as *A. lebbeck*, indicating that all N fixers may not be equally efficient in raising soil N levels.

Yang et al. (2003) conducted a field trial at Lechang Pb/Zn mine tailings of Guangdong Province, Southern China to compare growth performance, metal accumulation of Vetiver (*Vetiveria zizanioides*) and two legume species (*Sesbania rostrata* and *Sesbania sesban*) grown on the tailings amended with domestic refuse and/or fertilizer. It was revealed that domestic refuse alone and the combination of domestic refuse and artificial fertilizer significantly improved the survival rates and growth of *V. zizanioides* and two *Sesbania* species, especially the combination. However, artificial fertilizer alone did not improve both the survival rate and growth performance of the plants grown on tailings. Roots of these species accumulated similar levels of heavy metals, but the shoots of two *Sesbania* species accumulated higher (3-4 folds) concentrations of Pb, Zn, Cu and Cd than shoots of *V. zizanioides*. Most of the heavy metals in *V. zizanioides* were accumulated in roots, and the translocation of metals from roots to shoots was restricted.

Metal uptake capacity by Caryophyllaceae species (genera Dianthus, Minuartia, Scleranthus and Silene) were studied from metalliferous soils in northern Greece, having different concentrations of Cu, Pb, Zn, Cd, Ni, Cr, Fe, Mn, Ca, Mg (Konstantinou and Babalonas, 1996). They concluded that *Scleranthus perennis subsp. perennis* showed the highest Cu concentration (205 mg kg^{-1}), whereas *Minuartia cf. bulgarica* hyperaccumulated Pb (1175 mg kg^{-1}). Ca concentrations in plants were in most cases much higher than those in soil, whereas the contrary was true for Mg. As a result the Ca/Mg ratio, which was in almost all cases lower than 1 in the soil, was much increased in the plants.

Mine spoil dump material and plants *Silene armeriu* (Caryophyllaceae), *Salix spp*. (Salicaceae) and *Populus nigra* (Salicaceae) were sampled at 4 different growing stages from the pyrite-chalcopyrite mining area of Vigonzano (Northern Apennines, Italy). Mine spoils have high concentrations of Fe, Mg, Cu, Cr, Co and Ni, and are characterized by moderately to strongly acid environmental conditions. Water leaching tests indicate the following order of extraction: $Zn \geq Cu > Ni > Fe \geq Cr$ (Dinelli and Lombini, 1996). The results indicate that metal concentrations increase with plant ageing, the highest concentrations being observed in leaves. The variations of BAC (Biological Accumulation Coefficient) for the plants growing on the Vigonzano mine spoil area indicates that Zn is the element most easily absorbed by plants. An absorption sequence Zn >Co >Cu >Ni > Fe >Cr can be generalized for plants growing on the mine spoil area indicating the importance of soil solution composition in plant absorption.

Selection of plant materials is an important factor for successful field phytoremediation. Zhuang et al. (2007) conducted a field experiment to evaluate the phytoextraction abilities of six high biomass plants – *Vertiveria zizanioides*, *Dianthus chinensis*, *Rumex K-1* (*Rumex upatientia* × *R. timschmicus*), *Rumex crispus*, and two populations of *Rumex acetosa* – in comparison to metal hyperaccumulators (*Viola baoshanensis*, *Sedum alfredii*). The paddy fields used in the experiment were contaminated with Pb, Zn, and Cd. Results indicated that *Viola baoshanensis* accumulated 28 mg Cd kg^{-1} and S. alfredii accumulated 6279 mg Zn kg^{-1} (dry weight) in shoots, with bioconcentration factors up to 4.8 and 6.3, respectively. The resulting total extractions of *V. baoshanensis* and *S. alfredii* were 0.17 kg ha^{-1} for Cd and 32.7 kg ha^{-1} for Zn, respectively, with one harvest without any treatment. The phytoextraction rates of *V. baoshanensis* and *S. alfredii* for Cd and Zn were 0.88 and 1.15 %, respectively. Among the high biomass plants, *R. crispus* extracted Zn and Cd of 26.8 and 0.16 kg ha^{-1}, respectively, with one harvest without any treatment, so it could be a candidate species for phytoextraction of Cd and Zn from soil. No plants were proved to have the ability to phytoextract Pb with such high efficiency.

Bech et al. (2002) reported the results of the screening of plant species from three different mining areas in South America: a copper mine in Peru ("Mina Turmalina"), a silver mine in Ecuador ("Mina San Bartolomé") and a copper mine in Chile ("Mina El Teniente"). The accumulation of heavy metals viz. As in shoots as a function of extractable metal concentrations in the soils was analyzed in field samples. The different plant species collected from the severely polluted soils exhibited large differences in accumulation of heavy metals and As. Among the grass species (Poaceae), the highest concentration of As was observed in the shoots of *Paspalum sp.* (> 1000 mg kg^{-1}) and *Eriochloa ramosa* (460 mg kg^{-1}) from the Cu mine in Peru, and in *Holcus lanatus* and *Pennisetum clandestinum* (> 200 mg kg^{-1}) from the silver mine in Ecuador. *Paspalum racemosum* also accumulated considerable concentrations of Cu and Zn. The species from the genus Bidens (Asteraceae) were not only able to accumulate high concentration of As in shoots (> 1000 mg kg^{-1} in *B. cynapiifolia* from Peru), but also considerable amounts of Pb (*B. humilis* from Chile). The highest concentration of Cu was found in the shoots of *Mullinum spinosum* (870 mg kg^{-1}) and in *B. cynapiifolia* (620 mg kg^{-1}). The accumulation of Zn was highest in the shoots of *Baccharis amdatensis* (> 1900 mg kg^{-1}) and in *Rumex crispus* (1300 mg kg^{-1}) from the silver mine in Ecuador.

Maiti et al. (2005) conducted a study with the aim to identify pioneering species that naturally colonize Fe tailings and accumulate heavy metals. Total, bioavailable, acid extractable and water-soluble fractions were studied. After the second year onwards, along with nine herbaceous pioneering species, four tree species (*Tectona grandis*, *Alstonia scholaris*, *Azadirachta indica* and *Peltaphorum*) were found growing naturally. The study shows that some species could accumulate relatively high metal concentrations indicating internal detoxification of metals. The study revealed that *T. grandis* accumulated a higher concentration of metals than *A. scholaris* in the Fe tailings, but all concentrations were within the normal range. Native naturally colonizing plant species may be used for the bioremediation of iron tailings as initial cover species to stabilize and reduce erosion.

A pilot scale study conducted on the Fe tailings of Noamundi, Tata- Steel by Maiti and Nandhini (2005) reported that nine plant species was able to grow naturally on the Fe tailings, out of which 4 species namely *Borhevia repens*, *Oxalis corniculata*, *Blumea lacera* and *Avera aspera* were analysed for total metal contents in the whole plant. The total metal contents in the natural vegetation varied widely between 1530-8412 mg Fe kg^{-1}, 17-102 mg Mn kg^{-1}, 28-110 mg Zn kg^{-1}, 10.8-18.8 mg Cu kg^{-1}, 5.2-35.8 mg Pb kg^{-1}, 12-32 mg Ni kg^{-1} and 5.5-31.8 mg Co kg^{-1}. Maximum accumulation of Fe was found in *Oxalis* (7442 mg kg^{-1}) whereas Mn and Zn were observed maximum in *Blumea lacera* (88 mg kg^{-1}) and *Avera aspera* (109 mg kg^{-1}) respectively. The variation of BAC (Biological Accumulation Coefficient = total metals in plants/ DTPA metals in soil) for plants growing in the Fe tailings indicated that Fe was the element most easily absorbed by the plants. An absorption sequence was in the order of Fe> Ni> Pb> Zn> Cu> Mn> Co.

Das and Maiti (2007a) conducted a field studies in an abandoned copper mine tailings (Rakha mine, Jharkhand, India), to find out accumulation of metals (Cu, Ni, Mn, Zn, Pb, Cd and Co) in the naturally colonising vegetation. They found that, out of 11 species, *Ammania baccifera* growing on copper tailings, levels of Cu accumulation in the root parts was found even more than 1000 mg kg^{-1} dry weight (DW). Metals accumulated by *A. baccifera* were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. Thus, establishment of such plant on copper tailings can be a safe method to stabilize the metals.

Das and Maiti (2007b) analyzed metal accumulation in above and underground tissues of plants belonging to 5 genera and 4 families from the abandoned Cu-tailing ponds of Rakha mines, Jharkhand, India. Tailings have high concentration of Cu, Ni and characterized by moderately acid environment and low nutrient contents. Plant communities respond differently, depending on their ability to uptake or exclude a variety of metals. Accumulated metals were mostly retained in root tissue indicating that an exclusion mechanism for metal tolerance widely exists in them. Retention of some metals more than toxic level in the above ground tissues of some plants suggests the presence of internal metal detoxification and tolerance mechanisms in them.

Freitas et al. (2004) studied the metal accumulation in the natural vegetation in the degraded copper mine of São Domingos, SE Portugal. Plants belonging to 24 species, 16 genera and 13 families were collected and samples were analyzed for total Ag, As, Cu, Ni, Pb, and Zn. The highest concentrations of metals in Cu mine soil (DW) were 11217.5 mg Pb kg^{-1}, 1829 mg Cu kg^{-1}, 1291 mg As kg^{-1}, 713.7 mg Zn kg^{-1}, 84.6 mg Cr kg^{-1}, 54.3 mg Co kg^{-1}, 52.9 mg Ni kg^{-1} and 16.6 mg Ag kg^{-1}. With respect to plants, the higher concentrations of Pb and As were recorded in the semi-aquatic species *Juncus conglomeratus* with 84.8 and 23.5 mg kg^{-1} DW respectively, *Juncus efusus* with 22.4 and 8.5 mg kg^{-1} DW, and *Scirpus holoschoenus* with 51.7 and 8.0 mg kg^{-1} DW, respectively. *Thymus mastichina* also showed high content of As in the aboveground parts, 13.6 mg kg^{-1} DW. Overall, the results indicates accumulation of various metals by different plant species, with some of these metals being partitioned to the shoots.

In a study conducted by (Blaylock et al., 1999) at a lead-contaminated site in Trenton, New Jersey, the soil was treated for phytoremediation using successive crops of *B. juncea* combined with soil amendments. Through phytoremediation, the average surface soil Pb concentration was reduced by 13%. In addition, the target soil concentration of 400 mg/kg was achieved in approximately 72% of the treated area in one cropping season. It is found that the integration of specially selected metal-accumulating crop plants (*Brassica juncea* (L) Czern.) with innovative soil amendments allows plants to achieve high biomass and metal accumulation rates.

In a field study, mine wastes containing Cu, Pb and Zn were stabilized by grasses – *Agrostis tenuis* for acid lead and zinc mine wastes, *Agrostis tenuis* for copper mine wastes, and *Festuca rubra* for calcareous lead and zinc mine wastes (Smith and Bradshaw, 1979).

Shu et al. (2004) conducted a field experiment to compare the growth and metal accumulation in 4 grasses (*Vertiveria zizanioides*, *Paspalum notatum*, *Cynodon dactylon* and *Imparata cylindraca var major*) on the fields amended with 10 cm domestic refuse + complex fertilizer (NPK, Treatment A), 10 cm domestic refuse (Treatment B) and complex fertilizer (NPK, Treatment C), respectively, and without any amendment used as control (Treatment D). The results indicated that *V. zizanioides* was a typical heavy metal excluder, because the concentrations in shoots of the plants were the lowest among the four plant species tested. The most of metal accumulated in *V. zizanioides* distributed in its roots, and transportation of metal in this plant from root to shoot was restricted. Therefore, *V. zizanioides* was more suitable for phytostabilization of toxic mined lands than *P. notatum* and *C. dactylon*, which accumulated a relatively high level of metals in their shoots and roots. It was found that *I. cylindraca var. major* accumulated lower amounts of Pb, Zn Cu than *C. dactylon* and *P. notatum* and could also be considered for phytostabilization of tailings. Although the metal (Pb, Zn, and Cu) concentrations in shoots and roots of *V. zizanioides* were the lowest, the total amounts of heavy metals accumulated in shoots of V. zizanioides were the highest among the four tested plant species due to the highest dry weight yield of it. The results indicated that *V. zizanioides* was the best choice among the four species used for phytoremediation (for both phytostabilization and phytoextraction) of metal contaminated soils.

Chelant-enhanced phytoextraction of heavy metals is an emerging technological approach for a non-destructive remediation of contaminated soils. Komárek et al. (2006) studied the effect of the use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated soils. The main objectives of this study were (i) to assess the extraction efficiency of two different synthetic chelating agents (ethylenediamine-tetraacetic acid (EDTA) and ethylenediaminedisuccinic acid (EDDS) for desorbing Pb from two contaminated agricultural soils originating from a mining and smelting district and (ii) to assess the phytoextraction efficiency of maize (*Zea mays*) and poplar (*Populus sp.*) after EDTA application. EDTA was more efficient than EDDS in desorbing and complexing Pb from both soils, removing as much as 60% of Pb. Maize exhibited better results than poplar when extracting Pb from the more acidic (pH 4) and more contaminated (upto 1360 mg Pb kg^{-1}) agricultural soil originating from the smelting area. On the other hand, poplars proved to be more efficient when grown on the near-neutral (pH$\sim$6) and less contaminated (upto 200 mg Pb kg^{-1}) agricultural soil originating from the mining area. Furthermore, the addition of EDTA led to a significant increase of Pb content especially in poplar leaves, proving a strong translocation rate within the poplar plants.

Zhuang et al. (2005) conducted a field trial to evaluate the phytoextraction efficiencies of three plants and the effects of EDTA or ammonium addition [(NH4)2SO4 and NH4NO3] for assisting heavy metal (Pb, Zn, and Cd) removal from contaminated soil. The tested plants include *Viola baoshanensis*, *Vertiveria zizanioides*, and *Rumex K-1* (*Rumex patientia* × *R. timschmicus*). The application of EDTA soil was the most efficient to enhance the phytoavailability of Pb and Zn, but did not have significant effect on Cd. Lead phytoextraction rates of *V. baoshanensis*, *V. zizanioides* and *Rumex K-1* were improved by 19-, 2-, and 13-folds compared with the control treatment, respectively. The application of ammonium did not have obvious effects on phytoextraction of the three metals, except that the accumulations of Zn and Cd in shoot of *V. baoshanensis*. Among the three tested plants, *V. baoshanensis* always accumulated the highest concentrations of Pb, Zn, and Cd. The concentrations of Pb, Zn, and Cd in the shoots of *V. baoshanensis* treated with EDTA were 624, 795, and 25 mg kg^{-1}, respectively, and the phytoextraction efficiencies of this species for Pb, Zn, and Cd were also the highest among the three species. Results presented here indicated that *V. baoshanensis* had great potential in phytoremediation of soils contaminated by multiple heavy metals, although the dry weight yield was the lowest among the three plants.

Enhancement of phytoremediation by plant genetic modification

The development of commercial phytoextraction technologies requires plants that produce high biomass and that accumulate high metal concentrations in organs that can be easily harvested, i.e. in shoots. It has been suggested that phytoremediation would rapidly become commercially available if metal-removal properties of hyperaccumulator plants, such as *Thlaspi caerulescens*, could be transferred to high-biomass producing species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) (Brown et al., 1995). In an effort to correct for small size of hyperaccumulator plants, Brewer et al. (1997) generated somatic hybrids between *T. caerulescens* (a Zn hyperaccumulator) and *Brassica napus* (canola), followed by hybrid selection for Zn tolerance. High biomass hybrids with superior Zn tolerance were recovered.

The use of genetic engineering to modify plants for metal uptake, transport and sequestration may open up new avenues for enhancing efficiency of phytoremediation. Metal chelator, metal transporter, metallothionein (MT), and phytochelatin (PC) genes have been transferred to plants for improved metal uptake and sequestration. For example, in tobacco (*Nicotiana tabacum*) increased metal tolerance has been obtained by expressing the mammalian metallothionein, metal-binding proteins, genes (Maiti et al., 1991).

Transgenic plants, which detoxify/accumulate Cd, Pb, Hg, As and Se have been developed. The most spectacular application of biotechnology for environmental restoration has been the bioengineering of plants capable of volatilizing Hg from soil contaminated with methylmercury. Methyl-mercury, a strong neurotoxic agent, is biosynthesized in Hg-contaminated soils. To detoxify this toxin, transgenic plants (*Arabidopsis* and tobacco) were engineered to express bacterial genes merB and merA. In these modified plants, merB catalyzes the protonolysis of the carbonmercury bond with the generation of Hg^{2+}, a less mobile mercury species. Subsequently, MerA converts Hg(II) to Hg (0) a less toxic, volatile element which is released into the atmosphere (Rugh et al., 1996). Hg reductase has also been successfully transferred to *Brassica*, tobacco and yellow poplar trees (Meager et al., 2000).

Phytoremediation efficiency of plants can be substantially improved using genetic engineering technologies. Recent research results, including overexpression of genes whose protein products are involved in metal uptake, transport, and sequestration, or act as enzymes involved in the degradation of hazardous organics, have opened up new possibilities in phytoremediation. A better understanding of the mechanisms of rhizosphere interaction, uptake, transport and sequestration of metals in hyperaccumulator plants will lead to designing novel transgenic plants with improved remediation traits. As more genes related to metal metabolism are discovered, facilitated by the genome sequencing projects, new vistas will be opened up for development of efficient transgenic plants for phytoremediation. 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.

Technology development

Phytoremediation is an emerging technology, potentially effective and applicable to a number of different contaminants and site conditions. Major limitations of the present research lacks data related to the mass balance of the metals. In addition, the problem is compounded by metal leaching away from the original source. The cost associated with phytoremediation is difficult to estimate because of lack of economic data. It is likely, however, that the cost will be very much site specific. Recently, a group of scientist ranked a variety of metals with respect to phytoextraction research status, readiness for commercialization, and regulatory acceptance of the technology (Lasat, 2000). Results of this evaluation are shown in *Table 4*.

Table 4. *Current research status, readiness for commercialization, and regulatory acceptance of phytoremediation for several metal and metalloid contaminants*

Metal	**Contaminant**							
	Ni	**Co**	**Se**	**Pb**	**Hg**	**Cd**	**Zn**	**As**
Commercial readiness*	4	4	4	4	3	2	3	1
Regulatory acceptance**	Y	Y	N	Y	N	Y	Y	N

** rating: 1- basic research underway; 2- laboratory stage; 3- field deployment; 4- under commercialization.*

*** Regulatory acceptance: Y- yes, N- no.*

Conclusions

Metal mine waste generally contain anomalous concentration of metals, which inhibit the plant colonisation. Metal being non-biodegradable, phytoremediation techniques are the only viable solution to decontaminate the metal contaminated land. Even though, there are several processes of phytoremediation and different plant species have been used, role of grasses, legumes and some tree species has been well established. Out of the several grass and legume species reported, *Vetiveria* sp., *Sesbania* sp were found to be most promising for bioremediation of tailings pond. Adding organic amendment facilitates the effective establishment and colonisation of pioneer species. The research work showed that, bioavailability and metal uptake by plants could be accompalished by ameliorating pH, adding chelating agents, using appropriate fertilisers and altering soil ion composition. Further research is required to develop fast growing high biomass plants with improved metal uptake, translocation and tolerance through genetic engineering for effective phytoremediation of metal mine wastes.

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