Global Journal of Environmental Research 4 (3): 135-150, 2010
ISSN 1990-925X

Phytoremediation of Metal Enriched Mine Waste: A Review

Sangeeta Mukhopadhyay and Subodh Kumar Maiti

Department of Environmental Sc. & Engg/ Centre of Mining Environment,
Indian School of Mines, Dhanbad-826004 (Jharkhand), India

Abstract: Reclamation of mine waste is carried out by phytoremediation process, which employes plants as a tool for stabilization and pollution control of dumps. In this review paper, different types of phytoremediation processes like, phytoextraction, rhizofiltration, phytostabilisation, phytovolatilization, phytodegradation and phytorestoration are reviewed The key factor for the success of remediation process depends on characteristics of mine waste, geo-climatic conditions, types of amendment used and selection of plant species. Adverse factors such as elevated acidity and toxic metal ions, impoverished nutrient status and poor physical structure inhibit plant establishment and growth on the mine spoil or tailings. Evaluation of different fractions of bioavailable metals, their mobility in plant parts and growth of plant species on contaminated sites could be helpful for phytoremediation of metallic wastes. In this review paper phytoavailability of metals in mine wastes and their accumulation in different plant parts are discussed. 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. The importance of genetic modifications of plants and commercialisation of phytoremediation is also highlighted.

Key words: Phytoremediation · Heavy metals · Phytoavailability of metals · Metal accumulation in plants

INTRODUCTION

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 cleanup-contaminated areas. Mining activities such as crushing, grinding, washing, smelting and all the other processes used to extract and concentrate metals generate a large amount of waste rocks and tailings are often very unstable and make elements environmentally labile through normal biogeochemical pathways, to sink such as sediments, soils or biomass [1, 2]. The direct effect will be loss of cultivated land, forest or grazing land and the overall loss of production [3]. Establishment of vegetation cover can fulfill the objectives of stabilisation, pollution control, visual improvement and removal of threats to human beings [4]. However, adverse factors such as acidity, nutrient deficiencies, toxic heavy metal ions and poor physical structure and their interaction with most mine tailings inhibit plant establishment and growth on the tailings [5]. Evaluation of metal concentrations in plants growing in contaminated sites can be used to get information about specific plant behavior in that environment, metal dispersion and mobility with reference to their biomass. Metal concentration in plants is a function not only of the total soil concentrations but depend also on the chemical speciation of metals in soil solutions [6, 7] and on the involvement of the metal in biological functions [8]. Plant species found in metal polluted/contaminated soils are expected to take up metals and eventually accumulate them [9]. Some plants phytostabilise heavy metals in the rhizosphere through root exudates immobilisation [10] whilst other species incorporate them into root tissues [11]. Some plants also transfer metals to their above ground tissues, potentially allowing the soil to be decontaminated by harvesting the above ground parts. Therefore plant community established on mine waste could be useful to minimize the impacts of mining, thus considering the diversity of plant responses in contaminated sites with different metals and toxicity levels, it is important to study the composition of plant community established on mine waste, which would serve as a basic tool for mine remediation. More information about plant community that can grow on metal enriched soil is essential to determine their potential for mine reclamation/remediation and for biological exploration [12].

Corresponding Author: Subodh Kumar Maiti, Department of Environmental Sc. & Engg/ Centre of Mining Environment, Indian School of Mines, Dhanbad-826004 (Jharkhand), India. E-mail: subodh_maiti@yahoo.com.

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 [13]. 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.

Phytoextraction: This process reduces soil metal concentrations by cultivating plants with a high capacity for metal accumulation in shoots [14]. 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 [15]. 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 [16]. 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 [17].

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 (^{7}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])	Phenolicsreleasers (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

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 [18]. 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 [19].

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.* [20] 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 [21].

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 [22].

Rhizodegradation: Rhizodegradation is the breakdown of 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 [23]. 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 [24].

Phytorestoration: It involves the complete remediation of contaminated soils to fully functioning soils [25]. 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.

HYPERACCUMULATOR AND HYPERTOLERANCE

Some plants which grow on metalliferous soils have developed the ability to accumulate massive amount of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Table 2) [26, 27]. Chaney [28] 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. By definition, a “hyperaccumulator” must accumulate at least 100 mg g^{-1} (0.01% dry wt.) of Cd, As and some other trace metals, 1000 mg g^{-1} (0.1% dry wt.) of Co, Cu, Cr, Ni and Pb and 10,000 mg g^{-1} (1 % dry wt.) of Mn and Ni [29]. Brassicaceae had the highest number taxa *i.e.* 11 genera and 87 species that are established for hyperaccumulation of metals. In Brassicaceae Ni hyperaccumulation is reported in 7 genera and 72 species while Zn in 3 genera and 20 species [30]. Hypertolerance to metals is the main characteristic for plant being classified as hyperaccumulators; the key features appear to be vacuolar compartmentalisation and chelation, which allow accumulation of metals [31, 32].

Table 2: Concentration of heavy metals in soils and plants [26]

Elements	Normal range in soil (ppm)	Critical soil total conc[a] (ppm)	Normal range in plants (ppm)	Critical conc. in plants[b] (ppm)
As	0.1 - 40	20 - 50	0.02 - 7	5 - 20
Cd	0.01 - 2	3 - 8	0.1 - 2.4	5 - 30
Co	0.5 - 65	25 - 50	0.02 - 1	15 - 50
Cr	5 - 1500	75 - 100	0.03 - 14	5 - 30
Cu	2 - 250	60 - 125	5 - 20	2 - 100
Hg	0.01 - 0.5	0.3 - 5	0.005 - 0.17	1 - 3
Mn	20 - 10000	1500 - 3000	20 - 1000	300 - 500
Mo	0.1 - 40	2 - 10	0.03 - 5	10 - 50
Ni	2 - 750	100	0.02 - 5	10 - 100
Pb	2 - 300	100 - 400	0.2 - 20	30 - 300
Se	0.1 - 5	5 - 10	0.001 - 2	5 - 30
Zn	1 - 900	70 - 400	1-400	100 - 400

[a]The critical soil concentration in the range of values above which toxicity is considered to be possible

[b]The critical concentration in plants is the level above which toxicity effects are likely to occur

Among the mechanisms directly involved in detoxification of heavy metals, the following may be responsible: chelation, transport and sequestration [33]. Selective breeding, adding soil amendments to increase metal uptake and transfer of hyperaccumulator genes into genetically modified crop plants are all real possibilities and huge advances in these areas have been made in the last few years [34, 35]. In addition, synthetic metal chelates have been used to artificially induce hyperaccumulation of toxic metals, as for example Pb from the soil into plant shoots [36].

PHYTOAVAILABILITY OF METALS

Single Extraction Methods: It is a common conception nowadays that the total concentrations of metals in soils are not a good indicator of phytoavailability, or a good tool for potential risk assessment, due to the different and complex distribution patterns of metals among various chemical species or solid phases [37]. The most widely used methods for the evaluation of the availability of metals in soils are single extraction [38, 39] and sequential extraction methods [40]. However, the sequential extraction method is rather laborious and time consuming. Among *single extraction methods*, $CaCl_2$, DTPA, EDTA and CH_3COOH were the most widely used extractants [41, 42, 43, 44, 45, 46, 47]. Single extraction methods used by different researchers are shown in Table 3.

DTPA (containing 0.01 M $CaCl_2$, pH 7.3 and 0.1 M triethanolamine) is suitable for calcareous soils, as it is buffered at a pH 7.3 and therefore prevents $CaCO_3$ from dissolution and release of occluded metals especially Cd^{2+} and Zn^{2+}. Triethaloamine (TEA), which is protonated at pH 7.3 and could exchange with cations from the exchange sites as suggested by Lindsay and Norvel [38]. $CaCl_2$ is the primary component of soil background electrolytes. The exchangeable cations may be displaced by the basic cations commonly present in extraction solution (Ca^{2+}). Heavy metals, which are originally absorbed by soils, will be competed by calcium ions and be replaced from the binding sites [44]. EDTA is a very good chelating agent, which can solubilise carbonate-occluded metals from soil [43]. Acetic acid comprises the largest component of total organic acids in rhizosphere soil of plant [48]. And using organic acids to extract bioavailable metals from soils has been widely accepted because organic acid can dissolve the particulate-bound metals into soil solution and exudates of plant roots [49]. The extraction with water is to simulate the metal distribution equilibrium of metals in soil pore water [44].

It has long been recognized that the soluble, exchangeable and loosely adsorbed metals are quite labile and hence more available for plants [50]. Therefore, in order to assess the environmental risk and the phytoavailability of metals, efforts should be concentrated on the measurements of these available fractions.

Sequential Extraction: In sequential extraction procedure exchangeable metals, bound to carbonates or specifically adsorbed portions, bound to Fe/Mn oxides, bound to organic matter and sulphides and residual are quantified [40]. The reagents used for sequential extractions are depicted in Table 4.

Table 3: Single extraction methods as indicator of metal phytoavailability

Type of samples [reference]	Extractant used	Metals analyzed
Contaminated soil from vegetable yard [44]	0.005 mol L^{-1} DTPA solution	Cr, Ni, Zn, Cu, Cd, Pb, La, Ce, Pr and Nd
	0.01 mol L^{-1} $CaCl_2$ solution	
	0.11 mol L^{-1} CH_3COOH solution	
	Deionized water	
Lead/zinc mine tailings [45]	0.005 mol L^{-1} DTPA solution Distilled water	Zn, Pb, Cu and Cd
Agricultural soil [43]	0.05 mol L^{-1} EDTA solution 0.005 mol L^{-1} DTPA solution	Cd, Cr, Cu, Ni, Pb and Zn
Opencast mine tailings [46]	Acidified 0.1 mol L^{-1} $CaCl_2$ solution	
	0.005 mol L^{-1} DTPA solution	Cd, Cr, Cu, Ni, Pb and Zn.
Lead/zinc mine tailings [55]	0.005 mol L^{-1} DTPA solution	Pb, Zn and Cu
Bauxite mine tailings [56]	0.005 mol L^{-1} DTPA solution	Mn, Zn, Cu and Pb

Table 4: Tessire's scheme for sequential extraction of metals [40]

Metal fractions	Reagents used
Exchangeable	$MgCl_2$ 1 mol L^{-1} at pH 7
Carbonatic	CH_3COONa 1 mol L^{-1}/ HOAc at pH 5
Oxides Fe/Mn	$NH_2OH.HCl$ 0.04 mol L^{-1} in 25% HOAc
Organic matter and sulphidic	H_2O_2 8.8 mol L^{-1}/ HNO_3 and NH_4OAC 0.8 mol L^{-1}
Residual	$HF/HClO_4$

TOTAL METAL ANALYSIS IN MINE WASTES AND PLANTS

In order to determine metals in soil/mine spoil, it is first necessary to bring them into solution. Extraction methods have been well documented and involve fusion or acid dissolution; the later type of technique has several advantages. Mineral acid can be obtained in a sufficiently pure form that their use does not introduce any appreciable impurities. Acid decomposition methods, unlike fusion technique, do not allow large amounts of salts to be introduced into the solution: a high salt content can cause instability and lead to high instruments background readings. In addition, fusion techniques are restricted to the determination of the total metal content of silicates only. On the otherhand, the concentration of acids can be varied by dilution and therefore selective dissolution of several components of soil/mine spoil can be affected.

Five mineral acids, namely hydrochloric (HCl), nitric (HNO_3), sulphuric (H_2SO_4), perchloric ($HClO_4$) and hydrofluoric (HF) acids, have been very widely used. For the simultaneous extraction of a large number of metals, H_2SO_4 has the one notable property of dissolving silica. Thus it can be used in conjugation with HNO_3, HCl or $HClO_4$ for the total decomposition of silicates. Some times HF is also used in conjugation with HNO_3, $HClO_4$ [44, 51, 52] or HCl [46, 47, 53,] for the same purpose. The HNO_3 is also used separately [54] or with either HCl or $HClO_4$ [45]. Such methods provide a high degree of metal extractability but do not dissolve silicates completely; they destroy organic matter, dissolve all precipitated and adsorbed metals and leach out a certain amount of the metal from the silicate lattice. HNO_3 serves only as a safety measure if large amounts of organic matter are present. The amount of metal extracted by $HClO_4$ depends on the type of mineral and organic matter content. For many type of samples, this acid is suitable for the total metal extraction. There was one unsatisfactory recovery with $HClO_4$, this being that of chromium (Cr). The low boiling point of chromyl chloride (CrO_2Cl_2), 116°C, compared with about 200°C for $HClO_4$, probably results in volatilisation losses. With HNO_3 or aqua regia, these losses do not occur because the boiling points of HNO_3 and HCl acids are lower. Aqua regia and nitric acid are weaker extracting agents than $HClO_4$. Aqua regia is a stronger oxidising and extracting agent than nitric acid as a result of the presence of nascent chlorine. HNO_3, aqua regia and $HClO_4$ have their strongest leaching effect when they are boiling. $HClO_4$, especially, is a strong leaching, dehydrating and oxidising agent only when it is hot and concentrated.

HNO_3 is mostly used for metal extraction from plant samples [43, 55]. Sometimes binary acid mixture of HNO_3 and $HClO_4$ [44, 45, 47, 54, 56] or tertiary acid mixtures (HF, HNO_3 and $HClO_4$) are also used for the same purpose [44, 51, 53, 57]. Different acid or acid mixtures used by various researchers for plant analysis are shown in Table 5.

Table 5: Digestion method for total metal analysis in plants and contaminated soil/mine spoil/tailings samples

Type of samples [reference]	Acid/oxidizing agent used	Metal analyzed
Metal contaminated soil Leaves and twigs [54]	HNO_3 HNO_3 and $HClO_4$	Cu, Zn and Cd
Contaminated superficial soil [53]	Aqua regia-HF	Cd, Cr, Cu,Fe,Mn, Ni, Pb and Zn
Grass samples [53]	HNO_3, HF and $HClO_4$	
Plant samples (stem, root, flowers) collected from Cu mine spoil [57]	HNO_3, HF and $HClO_4$ (5: 1: 1)	Cr, Ni, Co, Cu and Zn
Soil from vegetable yard Plant samples [44]	HNO_3, HF and $HClO_4$ (3: 1: 1)	Cr, Ni, Zn, Cu, Cd, Pb, La, Ce,
	Conc. HNO_3 and $HClO_4$ (1: 1)	Pr and Nd
Lead/zinc mine tailings Plant root and shoot [43]	HNO_3 and $HClO_4$ (5: 1)	Zn, Pb, Cu and Cd
Opencast mine tailings [46]	HNO_3, HCl and HF (1: 3: 3)	Cd, Cr, Cu, Ni, Pb and Zn
Lead/zinc mine tailings [52]	HNO_3 and $HClO_4$ (5: 1)	Pb, Zn, Cu and Cd
Plants grown on Lead/zinc mine tailings [43]	HNO_3	Cd, Cr, Cu, Ni, Pb and Zn

EXTRACTABLE HEAVY METAL FRACTION IN MINE WASTES

In metal enriched mine waste different forms of metals are extracted using aqua regia, DTPA, acetic acid, EDTA, $CaCl_2$ and water to establish a correlation between concentration of metals in plants and bioavailable fraction of metals in mine waste. A study was carried out to investigate the trace metal (Fe, Cu, Ni, Mn, Zn, Pb and Cd) contents and its accumulation in natural plants thriving on abandoned Cu-tailings pond of Rakha mines, Ghatsila, Jharkhand. The maximum concentration of Fe, Cu and Ni were found very high reaching 113000, 2380 and 1086 mg/kg respectively. In most of the samples total Cu, Ni and Cd concentration exceeded the toxicity threshold as defined by Kabata-Pendias and Pendias [58]. Fe was the most abundant metal in the CH_3COOH extract followed by Cu, Ni, Mn, Zn, Pb and Cd. EDTA, DTPA and $CaCl_2$ extractable Cu in the tailings was found higher than respective Fe concentrations [47].

Total, DTPA-extractable and total dissolved contents of Cd, Cr, Cu, Ni, Pb and Zn were determined in minesoils of two mining areas, Touro Copper mine and Meirama lignite mines located in Galicia, Spain. The total dissolved heavy metal (< 2 mg/kg) and DTPA-extractable contents were low in all the soils except the Cu-dissolved content in soils from Cu mine spoils. The Cu, Cr and Zn total content in Cu mine soil was reported higher than lignite mine soil but DTPA-extractable Zn and Cr were found higher in the latter. The proportion of DTPA-extractable Cd in lignite minesoil was 11.48% although the total content was low (< 5 mg/kg) and in Cu minesoil it was absent. Dissolved Cd was not found in any minesoils. The relation established between the soil organic matter content and the humified organic matter, with the total, DTPA-extractable Cu content, indicate that the humified organic matter was the fraction involved in the formation of soluble complexes and in the electrostatic adsorption of Cu. This demonstrates that capacity of the organic matter to establish not only soluble complexes but also insoluble compounds with Cu. A positive correlation was also found between the DTPA-extractable Cu and Fe and Mn oxides content, which probably indicate that the Cu^{2+} can be partly adsorbed by oxides. The origin of the Zn, Ni and Cr contents of minesoils was confirmed by means of the established positive correlation between the total content of these metals which indicates that these metals came from the minerals of the parent matter of Cu minesoils (Chalcopyrite, amphibolites and limonite) [46].

The concentrations of different forms of heavy metals (Fe, Cu, Mn, Zn, Ni, Co and Pb) were determined in an iron-ore-tailing and compared with those of the natural vegetation colonizing on the dump. Tailings had neutral pH (6.14) and low electrical conductivity (55.9 μS/cm). Four forms of metals, total, bioavailable, acid extractable and water-soluble were studied. Iron was the most abundant metals in all forms and the relative abundance of metals were as follows: Fe> Mn> Zn> Cu> Ni> Pb> Co. The average concentration of total Fe was 41670 mg/kg, total Mn 86 mg/kg, total Cu 23 mg/kg, total Zn 30 mg/kg and Pb, Ni, Co were found in traces. The fraction of bioavailable to total metal, for Fe was (0.03%), Mn (6.0%), Zn (3.8%) and Cu (1.8%). The concentrations of acid extractable fractions were as follows: Fe- 215 $mgkg^{-1}$, Mn - 25 mg/kg, Zn - 2.40 mg/kg, Cu - 1.68 mg/kg, Ni - 1.10 mg/kg, Pb- 0.62 mg/kg and Co in traces. In water extract solution, only Fe (0.4 mg/kg) was present [59].

Freitas ***et al.*** [60] reported high levels of As, Cu, Pb and Zn in the degraded soil of Sao Domingos mine in the southeast Portugal. Copper concentration in soils could reach 1829 mg/kg as a result of the former smelting activities. Maximum concentration of As in soils was very high, reaching 1291 mg/kg. The concentration of Pb in soil was also very high, 2693.7 mg/kg as the average value registered. The average Zn concentration in soil was of 218.2 mg/kg but it could reach 713.7 mg/kg, a level that can be extremely toxic for plants. Co and Cr concentrations in soils were normally low, ranging from 20.1-54.3 mg/kg and 5.1-84.6 mg/kg for Co and Cr respectively. Ni and Ag were also low, varying from 27.2-52.9 for Ni and 2.5-16.6 mg/kg for Ag.

Amount extracted from agricultural and geochemically polluted soil of Ireland by EDTA or DTPA varied by a factor of 5-10 between highest and lowest values for each element. EDTA was twice as effective an extractant as was DTPA for nearly all elements. Extractability followed the sequence Cd> Cu = Pb> Ni = Zn> Cr. Metal extracted by DTPA was very strongly correlated with amount extracted by EDTA for all elements. Regression analysis were also performed for amount of trace elements extracted and proportion of trace element extracted against soil parameters pH, organic carbon, silt and clay content and total Fe, Al and Mn. Only one element was involved in significant relationships. Zn extracted by EDTA and DTPA was related ($p \leq 0.001$) to organic carbon ($r^2 = 0.85$ and 0.70 respectively) and the proportion of total Zn that was extracted was similarly related. Total Zn showed no significant relationship to organic carbon [43].

Wang *et al.* [44] studied the trace elements concentration in a metal contaminated calcareous soil in Northern China and investigated the phytoavailability of metals in the vegetables. They found that amount of total trace elements in soils varied widely for different metals such as Cr 9.0-160, Ni 6.4-46.4, Zn 7.9-205, Cu 6.7-234, Cd 0.0538-4.08, Pb 20.2-210, La 1.8-49.6, Ce 3.15-107, Pr 0.391-15.2 and Nd 1.54-45.4 ng/g. They used four frequently used extractants like, $CaCl_2$, DTPA, CH_3COOH and water and compared the phytoavailability of trace metals amongst the extractants. The concentrations of metals extracted by these four extractants ranged from 3.42 - 815, 1.51 - 6965, 0.732 - 24473, 0.688 - 7863, 0.246 - 685, 1.99 - 5337 ng/g for Cr, Ni, Zn, Cu, Cd, Pb and REEs respectively.

Lechang Pb/Zn mine (Nothern Guangdong, China) contained high levels of total and DTPA extractable Pb (4164 and 331 mg/kg) and Zn (4377 and 187 mg/kg) and low levels of micronutrients (N, P, K) and organic materials. The total and extractable concentrations of Pb and Zn greatly exceeded the background values of normal soil (Pb 22.5, Zn 29.0 mg/kg) and nutrient contents and organic matter were much lower than normal soil [45].

The Fankou and Lechang Pb/Zn mine tailings of China, contained elevated concentrations of total and DTPA extractable Pb, Zn and Cu. Lechang tailings contained the highest total Cu (198 mg/kg), Zn (7607 mg/kg) and Fankou tailing contained the highest total Pb (5686 mg/kg). DTPA extractable Pb (219-269 mg/kg), Zn (249-326 mg/kg) and Cu (6.95-10 mg/kg) concentrations in tailings were similar ($P>0.05$) among the tailing samples and significantly higher than those of normal soil (Pb 6.82, Zn 3.79, Cu 1.10 mg/kg) [55].

Chemical characterisation of the Vigonzano (Northern Apenines, Italy) copper mine spoil revealed high concentrations of Fe, Mg, Co, Cu, Ni and S, some of them related to sulphide mineralisation (Fe, Cu, S), the others to serpentines rock abundant in the mine spoil material. Water soluble concentrations of metals indicate that Cu was the element with the largest solution concentration in the mine spoils material. Cr concentrations were low, in most cases below analytical detection limit. Water leaching test on mine spoil material pointed out the following order of extraction Zn ≥ Cu> Ni> Fe ≥ Cr [57].

Alvarez *et al.* [61] studied accumulation of heavy metals in plants and reported wide variation of metals growing on the dump of abandoned mine in Galicia (NW Spain). The concentration of heavy metals in plants varied widely between 150-900 mg Fe/kg, 84-2069 mg Mn/kg, 20.5-106 mg Cu/kg and between 35-717 mg Zn/kg. They observed opposite trend for Fe and Mn concentrations, suggesting a possible interaction between these two elements. In general terms, the species with the highest contents of Cu were those with the highest concentrations of Fe and the lowest of Mn.

Heavy metal contents in natural soil, contaminated soil near by a Cu-smelter unit and tailings from abandoned Cu-tailings ponds are presented in Table 6 [47, 62].

METAL ACCUMULATION IN PLANTS GROWING ON METAL ENRICHED SOIL

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 and herbaceous legumes can be used to as pioneer species to solve the problem of nitrogen deficiencies in mining wastelands because of their N_2 fixing ability [63].

Table 6: Metal concentration (mg/kg) in natural soil, contaminated soil and Cu-Tailings

Elements	Natural soil[a]		Soil near by Cu-smelter unit[b]		Cu-Tailings[b]	
	Total	DTPA	Total	DTPA	Total	DTPA
Fe	20509.0	234.00	23300.0	49.40	59700.0	40.00
Cu	34.8	0.51	2472.0	947.00	1027.0	21.30
Ni	15.0	0.11	105.0	1.60	339.0	1.95
Mn	353.3	31.80	447.0	32.00	144.0	1.87
Zn	117.3	0.65	242.0	50.00	67.1	0.63
Pb	18.5	2.30	128.0	3.00	29.0	1.98
Cd	2.0	<0.30s	3.1	0.22	6.9	0.37

[a]Maiti [62]; [b]Das and Maiti [47]

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 coal mine restoration in a dry tropical environment was described by Singh *et. al.* [64]. 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* showed 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.

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 [65]. 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.

The plant *Silene armeria* (Caryophyllaceae), *Salix* sp. (Salicaceae) and *Populas nigra* (Salicaceae) were sampled at four stages from Vigonzano mining area (Italy) to evaluate seasonal variations in metal concentration. The study indicated that metal concentration increases with plant ageing, the highest concentration was observed in plant leaves. *Silene armeria* was widespread in the mine spoil area and was actually able to tolerate high metal concentrations (upto 504 μg/g Cu, 174 μg/g Zn, 127 μg/g Ni and 138 μg/g Cr), extremely high when compared to those of a *S. armeria* population from an unpolluted site. The variation of BAC (Biological Accumulation Coefficient) for the plants growing on the Vigonzano mine spoil area indicates that Zn was the element mostly absorbed by the plants. An absorbance sequence Zn> Co> Cu> Ni> Fe> Cr was found for plant growing on the mine soil area indicating the importance of soil solution composition in plant absorption [57].

Selection of plant materials is an important factor for successful field phytoremediation. Zhuang *et al.* [66] 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 kg^{-1} Cd and *S. alfredii* accumulated 6,279 mg kg^{-1} Zn (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.* [67] 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 analysis of field samples infers that accumulation of heavy metals in shoots (As, Cu and Zn) is a function of extractable metal concentrations in the soils. The different plant species collected from the severely polluted soils exhibited large differences in accumulation of heavy metals in shoots. 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. 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). *Paspalum racemosum* also accumulated considerable concentrations of Cu and Zn. 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.* [68] conducted plot scale study with the aim to identify pioneering species that naturally colonize Fe tailings and heavy metals accumulation on them. Total, bioavailable, acid extractable and water-soluble fractions of metals in Fe tailings 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 and 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 [59] 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 [69] carried out 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* accumulated highest Cu concentration in the order of >1000 mg kg^{-1} dry weight (DW). Metals accumulated by *A. baccifera* were mostly concentrated 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 [70].

In a study conducted by Blaylock *et al.* [71] 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 percent. In addition, the target soil concentration of 400 mg/kg was achieved in approximately 72 percent 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 copper, lead and zinc 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) [72].

The bioremediation of Cu tailings was carried out using grasses (*Cymbopogon citratus* and *Vetiveria zizanioides)* and effects of chicken manure and soil-manure mixtures as amendments on growth and accumulation of metals were studied by Das and Maiti [73]. Application of manure and soil-manure mixtures resulted in significant increase in pH, EC, OC, CEC and nutritional status of Cu tailings. The environmentally available and DTPA extractable Cu and Ni concentration reduced in amended tailings, while Mn and Zn content increased significantly. Plants grown on amended tailings accumulated lesser Cu and Ni but higher Mn and Zn. Plant biomass increased proportionally to manure and soil-manure mixtures application rates. *C. citratus* produced more biomass than *V. zizanioides* in either of the amended tailings. From the pot experiment, it was suggested that application of chicken manure @ 5% (w/w) and in combination with *C. citratus*, could be a viable option for phytostabilization of toxic tailings.

Yang *et al.* [45] 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.

Shu *et al.* [74] 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 domestic refuse and complex fertilizer. The results indicated that *V. zizanioides* was a typical heavy metal excluder, because the lowest concentrations of metals found in shoots among the four plant species tested. 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. Although the metal (Pb, Zn and Cu) concentrations in shoots and roots of *V. zizanioides* were the lowest, the total amount 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.* [75] studied the effect of the use of maize (*Zea mays*) and poplar (*Populus sp.*) in chelant-enhanced phytoextraction of lead from contaminated soils. They concluded that EDTA (ethylenediamine-tetraacetic acid) was more efficient than EDDS (ethylenediaminedisuccinic acid) in desorbing and complexing Pb from soils, removing as much as 60% of Pb. Maize exhibited better results than poplar at more acidic (pH-4) and more contaminated agricultural soil (upto 1360 mgPb kg^{-1}) originating from the smelting area. On the other hand, poplars proved to be more efficient when grown on the near-neutral (pH-6) and less contaminated agricultural soil (upto 200 mg Pb kg^{-1}) originating from the mining area.

Zhuang *et al.* [76] conducted a field trial to evaluate the phytoextraction efficiencies of three plants and the effects of EDTA and ammonium addition [$(NH_4)_2SO_4$ and NH_4NO_3] 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. Results 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.

A significant correlation was observed between the $CaCl_2$ extractable metals in soil solution and Chinese cabbage (*Apium graveolens* L. ssp. *perkinensis)* grown on metal contaminated soil of northern China. An empirical model was developed to express the combined effect of soil properties on the phytoavailability [44].

$$\log [M_{root}] = a + b \log [M_{soil}] + c\,OM + d\,pH + e\,CEC + f \log [M_{extra}]$$

Where, $[M_{root}]$ and $[M_{soil}]$ = total metal concentration in plant roots and soils respectively,

$[M_{extra}]$ = metal concentration in the extractable soil fraction,

a-f = coefficients determined with statistical regression.

The stepwise multiple regression analysis demonstrated that the phytoavailability of trace elements strongly correlated with the extractable fraction by $CaCl_2$, total metal concentration in soils and soil pH, OM, CEC. That model can describe 75-95% of the variability of metal uptake and r^2 values ranged from 0.741 to 0.954, which were much better than the single correlation analysis. For cerely (*Apium graveolens* L. ssp. *chinensis*) and cole (*Brassica campestris*), a strong correlation was observed for Cr, Ni, Zn, Cu, Cd, La, Ce, Pr and Nd whereas spinach and Chinese cabbage, however, a positive correlation was only observed for Fe and Zn metals, respectively [44].

TECHNOLOGY DEVELOPMENT

The development of commercial phytoextraction technologies require plants that produce high biomass and that accumulate high metal concentration 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*) [77]. In an effort to correct for small size of hyperaccumulator plants, Brewer *et al*. [78] 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 [79].

Transgenic plants, which detoxify/accumulate cadmium, lead, mercury, arsenic and selenium have been developed. The most spectacular application of biotechnology for environmental restoration has been the bioengineering of plants capable of volatilizing mercury 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 [48]. Hg reductase has also been successfully transferred to *Brassica,* tobacco and yellow poplar trees [21].

Although some information regarding genes controlling the synthesis of peptides that sequester metals, like phytochelatins (*e.g.* the *Arabidopsis cad1* gene [80]), genes encoding transport proteins, such as the *Arabidopsis IRT1* gene that encodes a protein that regulates the uptake of iron and other metals [81] or genes encoding enzymes that change the oxidation state of heavy metals, like the bacterial *merA* gene encoding mercuric oxide reductase [82] are currently being used to improve metal hyperaccumulation in plants, further identification of plant genes encoding metal-ion/metal complex transporters and their molecular components could be of immense use for bioremediation studies. Further manipulations of these genes would prove useful to determine plant metal hypertolerance and hyperaccumulation. The specifically select several more plant species fit for phytoremediation studies.

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 [83]. Results of this evaluation are shown in Table 7.

The biggest advantage of using plants for bio remediation process is the utilization of their inherent traits and benefits like high biomass, extensive root systems, ability to withstand heavy metal stress, *etc.* Plant-facilitated bioremediation is aesthetically pleasing and makes the environment green and clean. As the entire process is solar energy driven, no artificial source of energy is required to drive the bioremediation process,

Table 7: Current research status, readiness for commercialization and regulatory acceptance of phytoremediation for several metal and metalloid contaminants[83]

	Contaminant							
Metal	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

making it cost-effective and environmental friendly [83]. Plants offer a permanent, *in situ*, non-intrusive, self sustaining method of removal of soil contaminants and reduce erosion. Few concerns regarding the phytoremediation technology are the slow speed of the process when compared to mechanical methods. Plants can take many growing seasons to clean up a site due to slow growth pertaining to climatic restrictions and species variations.

CONCLUSION

High metal concentrations and soil acidity represent primary limiting factors for plant growth on mine waste. Metals like Cu, Zn, Ni, Pb, Cr, As, Cd, Co cause toxicity problems in mine waste due to the presence of sulphide ores, which lowers the pH of the substrate due its oxidation. Thus evaluation of toxic metal content in metal enriched soil in terms of total, bioavailable, exchangeable or soluble fraction is a good tool for potential risk assessment, due the different and complex distribution patterns of metals among various chemical species or solid phases. Plant species found in metal enriched soil take up metals and eventually accumulate them. The bioavailability index are calculated by considering both bioavailable metals fractions (DTPA) as well as total metal contents with respects to the total metal accumulation in plant tissues.

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., *C. citratus, Sesbania* sp were found to be most promising for bioremediation of tailings. Adding organic amendment facilitates the effective establishment and colonisation of pioneer species.

Thus, identification and thorough analysis of plant species grown on metals contaminated soil is an effective method for selecting potential plants to be used for reclamation purpose. Adding organic amendment is essential to facilitate the effective establishment and colonisation of these pioneer plants. They can eventually modify the habitat render it more suitable for subsequent plant communities. Planting of different grass species, rotating with legumes and native species will be able to reclaim soil fertility and accelerate ecological succession. 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.

REFERENCES

1. Davies, B.E., 1980. Trace element pollution. In Applied Soil Trace Elements, Ed., B.E. Davies. Wiley New York, pp: 287-351.
2. Davies, B.E., 1983. Heavy metal contamination from base metal mining and smelting: implication for man and his environment. In Applied Environmental Geochemistry, Ed., I. Thornton. Academic Press, London, pp: 425-462.
3. Wang, W.S., 2003. Relationship between the extractable metals from soils and metals taken up by maize roots and shoots. Chemosphere, 53: 525-530.
4. Williamson, A. and M.S. Johnson, 1981. Reclamation of metalliferous mine wastes. In: Effect of heavy metal pollution on plants. Vol. 2, Metals in the environment. Eds., NW Lepp. Applied Science Publishers, London, pp: 185-212.
5. Pichtel, J. and C.A. Salt, 1998. Vegetative growth and trace metal accumulation on metaliferous wastes. J. Environ. Qual., 27: 618-642.
6. Sposito, G., 1983. The chemical forms of trace metals in soils. In Applied Environmental Geochemistry, Ed., I. Thornton, Academic Press, London, pp: 121-170.
7. Kabata-Pendias, A. and K. Pendias, 1992. Trace elements in Soils and Plants (2nd Ed). CRC press, Ann Arbor.

8. Adriano, D.C., 1986. Trace elements in the Terrestrial Environment. Springer, New York, pp: 533.
9. Baker, A.J.M., 1981. Accumulators and excluders: strategies in the response of plants to trace metals. J. Plant Nutr., 3: 643-654.
10. Blaylock, M.J. and J.W. Huang, 2000. Phytoextraction of metals. In Phytoremediation of toxic metals: Using plants to clean up the environment. Eds., I. Rakshin and B.D Ensley. John Wiley and Sons Inc., New York, pp: 314.
11. Khan, A.G., 2001. Relationship between chromium biomagnification ratio, accumulation factor and mycorrhizae in plants growing on tannery effluent-polluted soil. Environ. Int., 26: 417-423.
12. Brooks, R.R., 1983. Biological methods of prospecting for minerals. Wiley-Interscience, New York, pp: 313.
13. Cunningham, S.D. and D.W. Ow, 1996. Promises and prospects of phytoremediation. Plant Physiol., 110: 715-719.
14. Barceló, J. and C. Poschenrieder, 2003. Phytoremediation: principles and perspectives. Contributions to Sci., 2(3): 333-334.
15. Salt, D.E., R.D. Smith and I. Raskin, 1998. Phytoremediation. Ann. Rev. Plant Mol. Biol., 49: 643-668.
16. Dushenkov, V., P.B.A. Nanda Kumar, H. Motto and I. Rakin, 1995. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ. Sci. Technol., 29: 1239-1245.
17. Raskin, I. and B.D. Ensley, 2000. Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. John Wiley & Sons, Inc., New York, pp: 50-70.
18. Ghosh, M. and S.P. Singh, 2005. A review on phytoremediation of heavy metals and utilization of it's by products. Applied Ecology and Environmental Res., 3(1): 1-18.
19. Berti, W.R. and S.D. Cunningham, 2000. Phytostabilizaton of metals. In Phytoremediation of toxic metals: Using plants to clean up the environment, Eds., I. Raskin and B. Ensley. Wiley Interscience, New York, pp: 71-88.
20. Neumann, P.M., M.P. De Souza, I.J. Pickering and N. Terry, 2003. Rapid microbial metabolism of selenate to volatile dimethylselenide. Plant Cell Environ., 26: 897-905.
21. Meager, R.B., C.L. Rugh, M.K. Kandasamy, G. Gragson and N.J. Wang, 2000. Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In Phytoremediation of Contaminated Soils and Waters, Eds., N. Terry and G. Bañuelos, CRC Press LLC, Boca Raton, FL, USA, pp: 201-219.
22. Newman, L., S. Strand, N. Choe, J. Duffy, G. Ekuan, M. Ruszaj, B. Shurtleff, J. Wilmoth, P. Heilman and M. Gordon, 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. Environ Sci. Tech., 31: 1062-1067.
23. Kirk, I., I. Klironomos, H. Lee and J.T. Trevors, 2005. The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ. Pollut., 133: 455-465.
24. Jordahl, J.L., L. Foster, J.L.Schnoor and P.J.J. Alvarez, 1997. Effect of hybrid poplar trees on microbial populations important to hazardous waste bioremediation. Environ. Toxicol. Chem., 16(6): 1318-1321.
25. Bradshaw, A., 1997. Restoration of mined lands - using natural processes. Ecol. Eng., 8: 255-269.
26. Alloway, B.J., 1990. Heavy metals in soil. Blackie and Son Ltd., London, pp: 1-339.
27. Baker, A.J.M. and R.R. Brooks, 1989. Terrestrial higher plants which hyperaccumulate metallic elements– a review of their distribution, ecology and phytochemistry. Biorecovery, 1: 81-126.
28. Chaney, R.L., 1983. Plant uptake of inorganic waste constituents. In Land Treatment of Hazardous Wastes, Eds., James F. Parr, Paul B. Marsh and Joanne M. Kla. Noyes Data Corp., Park Ridge, N.J., pp: 50-76.
29. Reeves, R.D. and A.J.M. Baker, 2000. Metal-accumulating plants. In: Phytoremediation of toxic metals: using plants to clean-up the environment. Eds. Raskin, I. and B.D.Ensley. New York, John Wiley and Sons, pp: 193-230.
30. Reeves, R.D., A.J.M. Baker, A. Borhidi and Berazain, 1999. Nickel hyperaccumulation in the serpentine flora of Cuba. Annals of Botany, 83(1): 29-38.
31. Vogeli-Lane, R. and G.J. Wagner, 1990. Subcellular localization of cadmium and cadmium binding peptides to tobaco leaves: implication of a transport functions for cadmium binding peptides, Plant Physiol., 92: 1086-1093.

32. Salt, D., 2002. Molecular physiology of metal hyperaccumulation in plants. 9th New Phytologist Symposium. Heavy metals and plants. Philadelphia.
33. Cobbett, C., 2002. Heavy metal detoxification mechanisms in plants. 9th New Phytologist Symposium. Heavy metals and plants. Philadelphia.
34. Chaney, R.L., J.S. Angle, Y.M. Li and A.J.M. Baker, 1999. Method for photomining of nickel, cobalt and other metals from soil. US Patent 5: 711-784.
35. Salt, D.E., M. Blaylock, P.B.A. Nanda Kumar, V. Dushenkov, B.D. Ensley and I. Raskin, 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnol., 13: 468-474.
36. Huang, J.W., J.J. Chen, W.R. Berti and S.D. Cunningham, 1997. Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol., 31: 800-805.
37. Chen, B., X.Q. Shan and J. Qian, 1996. Bioavailability index for quantitative evaluation of plant availability of extractable soil trace elements. Plant Soil, 186: 275-283.
38. Lindsay, W.L. and W.A. Norvel, 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil. Sci. Soc. Am. J., 42: 421-428.
39. Leschber, R., R.D. Davis and P. Lhermite, 1985. Chemical methods for assessing bio-available metals in sludges and soil. Elsevier Applied Science Publishers, London.
40. Tessier, A., P.G.C. Cambell and M.X. Bisson, 1979. Sequential extraction procedures for the speciation of particulate trace metals. Anal. Chem., 51: 844-851.
41. Novozamsky, I., Th.M. Lexmond and V.J.G. Houba, 1993. A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. Int. J. Environ. Anal. Chem., 51: 47-58.
42. Houba, V.J.G., Th. M. Lexmond, I. Novozamsky and J.J. Van Der Lee, 1996. State of the art and future developments in soil analysis for bioavailability assessment. Sci. of the Total Environ., 178: 21-28.
43. McGrath, D., 1996. Application of single and sequential extraction procedures to polluted and unpolluted soils. Science of the Total Environment, 178: 37-44.
44. Wang, X.P., X.Q. Shan, S.Z. Zhang and B.A. Wen, 2004. A model for evaluation of the phytoavailability of trace elements to vegetables under the field conditions. Chemosphere, 55: 811-822.
45. Yang, B., W. Shu, Z. Ye, C. Lan and M. Wong, 2003. Growth and Metal Accumulation in Vetiver and two Sesbania Species on Lead/Zinc Mine Tailings. Chemosphere, 52: 1593-1600.
46. Vega, F.A., E.F. Covelo, M.L. Andrade and P. Marcel, 2004. Relationship between heavy metals content and soil properties in minesoils. Analytica Chimica Acta, 524(1/2): 141-150.
47. Das, M. and S.K. Maiti, 2008. Metal accumulation in naturally colonizing vegetation in an abandoned Cu-tailings pond of Rakha mines, East Singhbhum, Jharkhand, India. Land contamination and Reclamation, 16(2): 135-153.
48. Bazirmakenga, R., R.R. Siomard and G.D. Leroux, 1995. Determination of organic acids in soil extracts by ion chromatography. Soil. Biol. Biochem., 27: 349-356.
49. Cieslinske, G., K.C.J. Van Rees, A.M. Szmigielska, G.S.R. Krishnamurti and P.M. Huang, 1998. Low molecular weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. Plant and Soil, 203: 109-117.
50. Kabata-Pendias, A., 1993. Behavioural properties of trace metals in soils. Appl. Geochem., 2: 3-9.
51. Shan, X.Q., Z.W. Wang, W.S. Wang, S.Z. Zhang and B. Wen, 2003. Labile rhizosphere soil solution fraction for prediction of bioavailability of heavy metals and rare earth elements to plants. Anal Bioanal Chem., 375: 400-407.
52. Shu, W.S., Z.H. Ye, C.Y. Lan, Z.Q. Zhang and M.H. Wong, 2001. Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. Environment International, 26: 389-394.
53. Maiz, I., I. Arambarri, R. Garcia and E. Millán, 2000. Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. Environmental Pollution, 110: 3-9.
54. Rosseli, W., C. Keller and K. Boschi, 2003. Phytoextraction capacity of trees growing on a metal contaminated soil. Plant and Soil, 256: 265-272.
55. Shu, W.S., Z.H. Ye, C.Y. Lan, Z.Q. Zhang and M.H. Wong, 2002. Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. Environmental Pollution, 120: 445-453.
56. Brofas, G., P. Michopoulos and D. Alifragis, 2000. Sewage sludge as an amendment for Calcareous Bauxite Mine Spoils Reclamation. J. Environmental Quality, 29(3): 811-816.

57. Dinelli, E. and L. Lombini, 1996. Metal distribution in plants growing on copper mine spoils in Northern Apennies, Italy: the evaluation of seasonal variations. Applied Geochemistry, 11: 375-385.
58. Kabata-Pendias, A. and K. Pendias, 1984. Trace elements in soils and plants. Boca Raton, Fl: CRC Press, Inc., pp: 365.
59. Maiti, S.K. and S. Nandhini, 2005. Bioremediation of Fe ore tailings and bioaccumulation of metals in the naturally ocurring vegetation: A case of Noamundi Fe-ore tailings, TISCO, Noamundi. Int. seminar on Mineral Processing Technology (MPT - 2005), January 6-8, 2005.
60. Freitas, H., M.N.V. Prasad and J. Pratas, 2004. Plant community tolerant to trace elements growing on the degraded soils of Sao Domingos mine in the south east of Portugal: enviromental implications. Enviroment International, 30: 65-72.
61. Álvarez, E., M.L. Fernández, C. Vaamonde and M.J. Fernández, 2003. Heavy metals in the dump of an abandoned mine in Galicia (N W Spain) and in the spontaneously occurring vegetation. The Science of the Total Environment, 313: 185-197.
62. Maiti, S.K., 2006. Properties of minesoil in KD-Heslong project, North Karanpura area, CCL and its effect on bioaccumulation of toxic metals in tree species. Minetech, 27(3-4): 41-53.
63. Lan, C.Y., W.S. Shu and M.H. Wong, 1997. Revegetation of Pb/Zn mine tailings: phytotoxicity of the tailings. In. Global environmental biotechnology, Ed., D.L. Wise, Elsevier Science, London, pp: 119-130.
64. Singh, A.N., A.S. Raghubanshi and J.S. Singh, 2004. Impact of native tree plantations on mine spoil in a dry tropical environment. Forest Ecology and Management, 187: 49-60.
65. Konstantinou, M. and D. Babalonas, 1996. Metal uptake by Caryophyllaceae species from metalliferous soils in northern Greece. Plant Systematics and Evolution, 203: 1-10.
66. Zhuang, P., Q.W. Yang, H.B. Wang and W.S. Shu, 2007. Phytoextraction of Heavy Metals by Eight Plant Species in the Field. Water, Air, & Soil Pollution, 184: 235-242.
67. Bech, J., C. Poschenrieder, J. Barcelo and A. Lansac, 2002. Plants from Mine Spoils in the South American Area as Potential Sources of Germplasm for Phytoremediation Technologies. Acta Biotechnologica, 22: 5-11.
68. Maiti, S.K., S. Nandhini and M. Das, 2005. Accumulation of metals by naturally growing herbaceous and tree species in iron ore tailings. Int. J. of Environmental Studies, 62(5): 593-603.
69. Das, M. and S.K. Maiti, 2007. Metal accumulation in *A. baccifera* growing naturally on abandoned copper tailings pond. Env. Monit. and Assess., 127: 119-125.
70. Das, M. and S.K. Maiti, 2007. Metal accumulation in 5 native plants growing on abandoned Cu- tailings ponds. Applied Ecology and Env Res., 5(1): 27-35.
71. Blaylock, M.J., M.P. Elless, Huang, W. Jianwei, S.M. Dushenkov, 1999. Phytoremediation of lead-contaminated soil at a New Jersey brownfield site. Remediation, 9(3): 93-101.
72. Smith, R.A.H. and A.D. Bradshaw, 1979. The use of metal tolerant plant populations for the reclamation of metalliferous wastes. J. Appl. Ecol., 16: 595-612.
73. Das, M. and S.K. Maiti, 2009. Growth of *Cymbopogon citratus* and *Vetiveria zizanioides* on Cu mine tailings amended with chicken manure and manure-soil mixtures: a pot scale study. Int. J. Phytoremediation., 11(8) 651-663.
74. Shu, W., Y. Zhao, B. Yang, H. Xia and C. Lan, 2004. Accumulation of heavy metals in four grasses grown on lead and zinc mine tailings. J. of Env. Sci., 16: 730-734.
75. Komárek, M., P. Tlustoš, J. Száková, V. Chrastný and V. Ettler, 2006. The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere, 67: 640-651.
76. Zhuang, P., Z.H. Ye, C.Y. Lan, Z.W. Xie and W.S. Shu, 2005. Chemically Assisted Phytoextraction of Heavy Metal Contaminated Soils using Three Plant Species. Plant and Soil, 276: 153-162.
77. Brown, S.L., R.L. Chaney, J.S. Angle, A.M. Baker, 1995. Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci. Am. J., 59: 125-133.
78. Brewer, E.P., J.A. Saunders, J.S. Angle, R.L. Chaney and M.S. McIntosh, 1997. Somatic hybridization between heavy metal-hyperaccumulating *Thlaspi caerulescens* and canola. Agron. Abstr., pp: 154.
79. Maiti, I.B., G.J. Wagner and A.G. Hunt, 1991. Light-inducible and tissue-specific expression of achimeric mouse metallothionein cDNA gene in tobacco. Plant Sci., 76: 99-107.

80. Howden, R., P.B. Goldsborough, C.R. Anderson and C.S. Cobbett, 1995. Cadmium sensitive, cad1 mutants of *Arabidopsis thaliana* are phytochelatin deficient. Plant Physiol., 107: 1059-1066.
81. Eide, D., M. Broderius, J. Fett and M.L. Guerinot, 1996. A novel iron regulated metal transporter from plants identified by functional expression in yeast. Proc. Nat. Acad. Sci. USA, 93: 5624-5628.
82. Rugh, C.L., H.D. Wilde, N.M. Stack, D.M. Thompson, A.O. Summers and R.B. Meagher, 1996. Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial merA gene. Proc Nat Acad Sci USA, 93: 3182-3187.
83. Lasat, M.M., 2000. Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. J. Hazardous Substances Res., 2(5): 1-25.
84. Bizily, S.P., C.L. Rugh, A.O. Summers and R.B. Meagher, 1999. Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials. Proc. Nat. Acad. Sci. USA, 96: 6808- 6813.