8

Phytoremediation of Metals and Radionuclides

Susan Eapen, Shraddha Singh and S.F. D'Souza

Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, INDIA, Email: eapenhome@yahoo.com

1. Introduction

The air, water and soil have been contaminated as a result of industrial revolution and increased urbanization of the landscape. Excavation and deposition of contaminated soil in depositories are of common occurrence and physico-chemical methods are normally used for the remediation of contaminants. Recently, bioremediation - the use of biological agents for remediation of soils and solutions has received a lot of attention (Suresh and Ravishankar 2004). In our laboratory, a variety of biological systems of microbes and plant organs are being investigated for the treatment of heavy metal and radionuclide waste (Bhainsa and D'Souza 1999; Sar and D'Souza 2001 2002; Melo and D'Souza 2003; Eapen et al. 2003). Phytoremediation - the use of plants for environmental clean-up, offers an attractive, environmental friendly and cost-effective approach to remediate metal and radionuclide polluted solutions and soil (Entry et al. 1997, Zhu and Shaw 2000) (Table 1). Plants have constitutive (present in most phenotypes) and adaptive (present only in tolerant phenotypes) mechanisms for accumulation or tolerance of high contaminant concentration in their rhizosphere. A phytoremediation system capitalizes on the synergistic relationship among plants, micro-organisms, water and soil that have evolved naturally in wetlands and upland sites over millions of years. This approach makes use of the plant's ability to extract, concentrate and metabolize materials from air, water and soil (Salt et al. 1995). Plants can be described as solar-driven pumping stations (Cunningham et al. 1995) and possess homeostatic mechanisms to maintain the correct concentrations of essential metal ions in different cellular compartments and to minimize the damage from exposure to non-essential metal ions.

Phytoremediation is an umbrella term which covers several plant-based approaches for cleaning up contaminated environments and includes phytoextraction, the accumulation of high concentrations of metals in plant biomass; rhizofiltration, removal of contaminants from aqueous wastestreams by adsorption into plant roots; phytovolatalization, which includes volatilization into the air through plants, phytodetoxification, which involves the ability of plants to change the chemical species to a less toxic form and phytostabilization, where plants immobilize contaminants chemically and physically at the site, thereby preventing their movement to the surrounding areas.

Table 1. Advantages and disadvantages of Phytoremediation

Cost
- Low capital and operational costs
- Metal recycling in case of phytoextraction
Performance
- Not capable of 100% reduction
- Low concentration of waste- it is very effective
- May not be applicable to all types of waste
- Only applicable to surface soil
Others
- Aesthetically pleasing
- Environmentally non-destructive
- Public acceptance

2. Metals in Soils

Enhanced anthropogenic activities and increased industrialization like mining, smelting, electroplating and agriculture have contributed to an increase in the deposition of undesirable concentrations of metals such as Cd, Cr, Cu, Ni, Pb and Zn in the soil and water (Singh et al. 2004). Metal concentrations in soil range from < 1mg/kg to as high as 100,000 mg/kg, depending on the material and deposition event. The risk and the regulatory limits for each metal varies (Table 2). Solubility of metal is dependent on soil characterstics and is strongly influenced by pH of the soil and degree of complexation with soluble ligands (Norvell 1984). Different metals in soil can exist as discrete particles or be associated with different soil components like exchangeable ions sorbed onto inorganic select phase surfaces, non-exchangeable ions sorbed onto inorganic solid phase surfaces, insoluble inorganic metal compounds (oxides, hydroxides, phosphates, or carbonates), metal complexed with soluble or insoluble inorganic material and metals bound in silicate materials.

Metal uptake is an essential component of the plant nutrition. Metals, which are taken up by plants are those which exist as soluble components in the soil solution or are easily desorbed or solubilized by root exudates. Only a small portion of the total metal content in the soil is normally taken up by plants. For effective phytoextraction, it is essential to have abundant source of soluble metal and conditions of soil can be altered to increase metal solubility and availability. By decreasing the pH below 5.5, metal availability for plant roots can be enhanced. However, growth of plants at low pH may be inhibited because of increased Al solubility and subsequent toxicity. Lead in soil is normally unavailable for plant uptake and solubilization through addition of chelating agents like EDTA complexes the free metal ion in the solution, allowing further dissolution of the sorbed or precipitated phases until an equilibrium between complexed metal, free metal and insoluble phases occurs (Norwell 1991).

Table 2. Regulatory guidelines for metals and radionuclides

Element	Concentration range (μg/kg)	Regulatory limit (mg/kg)
Metals		
Lead	1000-6,900,000	600
Cadmium	100-345,000	100
Arsenic	100-102,000	20
Chromium	5.1-3,950,000	100
Mercury	0.1-1,800,000	270
Copper	30-550,000	600
Zinc	150-5,000,000	1,500
Radionuclides		
Uranium	0.2-16,000 (μg/g)	
Cesium	0.2-46,900 (μg/g)	
Plutonium	0.00011-3,500,000 pci/kg	
Strontium	0.03-540,000 pci/kg	

Plant species differ in their ability to accumulate metals from contaminated soils and some plant species have an inherent ability to accumulate high levels of toxic metals (Sinha et al. 2002). Plants are called as hyperaccumulators when they can accumulate more than 0.1% Pb, Co, Cr or more than 1% Mn, Ni or Zn in plant shoots when grown in their natural habitats (Brooks et al. 1979, 1980, Baker and Brooks 1989). More than 400 plant species are so far known to be hyperaccumulators of metals, belonging to Euphorbiaceae, Brassicaceae, Asteraceae and Rubiaceae (Table 3).

Different species of *Alyssum*, such as *A. bertolonii*, *A. murale* and *Thlaspi goesingense* and *Hybanthus floribundus* are known to take up high levels of Ni (Minguzzi and Vergnano 1948, Doksopulo 1961, Severne and Brooks 1972), while *Viola sp.*, *Thlaspi caerulescens* and *T. rotundifolium* are recognized as accumulators of zinc (Rascio 1977, Barry and Clark 1978). *Thlaspi caerulenscens* has been also found to accumulate high concentrations of Cd. Similarly, *Crotolaria cobalticola* accumulated high concentrations of Co from cobalt rich soils of Zaire (Brooks et al. 1980). High concentration of Cr was detected in the leaves of *Diccoma nicolifera* and *Sutera fodina* growing near a chrome mine in Zimbabwe (Wild 1974). *Astragalus* species were found to accumulate high concentrations of selenium (Christopher et al. 2003) and chinese brake fern *Pteris vittata* is known to take up high concentrations of arsenic (Ma et al. 2001). However, many of these hyperaccumulator plants show slow growth rate and low biomass and hence cannot be used for commercial phytoextraction.

Table 3. Selected examples of hyperaccumulators of different metals

	Concentration (mg/kg)
A. Nickel	
Berkheya codii (Asteraceae)	11,600
Pentacalia spp. (Asteraceae)	16,600
Senecia spp. (Asteraceae)	11,000
Alyssium spp. (Brassicaceae)	1280-29,400
Bornmuellera spp. (Brassicaceae)	11,400-31,200
Thlaspi spp. (Brassicaceae)	2000-31,000
Psychotria coronata (Rubiaceae)	25,540
B. Zinc	
Thlaspicaerulescence (Brassicaceae)	43,710
Thlaspi rotundifolium (Brassicaceae)	18,500
Dichopetalum gelonioides (Brassicaceae)	30,000
C. Cadmium	
Thlaspi caerulescens (Brassicaceae)	2,130
D. Lead	
Minuartia verna (Caryophyllaceae)	20,000
Agrostis tenuis (Poaceae)	13,490
Festuca ovina (Poaceae)	1,750
E. Cobalt	
Haumaniastum robertii (Lamiaceae)	10,232
Aeollanthus subacaulis (Lamiaceae)	4,300
Crotolaria cobalticola (Fabaceae)	30,100
F. Copper	
Ipomoea alpina (Convolvulaceae)	12,300
Aeollanthus subacaulis	13,700
G. Manganese	
Maystenus bureaviana (Celastraceae)	19,230
Maystenus sebertiana (Celastraceae)	22,500
Macadania Neurophylla (Proteaceae)	55,200
H. Selenium	
Astragalus racemosus (Leguminosae)	1,49,200
Lecithis ollaria (Lecithidiaceae)	18,200

3. Radionuclides

Radioactive contamination of the environment can be due to emissions and accidental spills from operations typical of nuclear fuel cycle like mining

(^{220}Rn), milling (^{238}U, ^{230}Th, ^{226}Ra, ^{310}Pb) and fall out from nuclear testing (^{131}I, ^{90}Sr, ^{137}Cs, Pu) and accidents like Chernobyl disaster in Ukraine in 1986. Naturally occurring radionuclides, such as U, Rn, Ra and Th, may be brought to the surface of the Earth by extraction processes such as oil drilling. Problems associated with remediation of soil, ground water and wastewater with radionuclides are similar to those with metals. However, one of the important factors is the radioactive decay component in the selection of appropriate technology. Selection of suitable technology for the remediation of soil and aqueous streams contaminated with radionuclides is based on the environmental chemistry of each element, type of deposition and the rate of radioactive decay. A variety of physico-chemical methods for treatment of radionuclide contamination include removal of top soil, soil washing, leaching with chelating agents, flocculation and reverse osmosis-ultrafiltration. Recently, there has been a spark of interest in the biological methods for radionuclide removal. Phytoremediation, a novel plant-based technology, is being tested for a variety of radioactive contaminated sites, especially for treatment of low level radionuclides in large areas.

Phytoremediation is not commercially used for decontamination of radioactive sites. However, it has been successfully tested for remediation of uranium from wastewater in Ashtabula site and Fernald site, both at Ohio, USA. Remediation of ^{137}Cs from soil at Brookhaven National lab, NY and ^{90}Sr and ^{137}Cs from a pond near Chernobyl, Ukraine, through plants has also been studied. While the technology can be used for removal of groundwater and surface water contamination, radionuclides from soils are more difficult to be decontaminated. Specific amendments and treatment of the soil may increase the rate of transfer of radionuclide in to the plant available forms.

^{137}Cesium (half life 32 years) is one of the most important constituents of fallouts and is also a consequence of spills and accidents. Cesium binds tightly to soils and in the soil after Chernobyl accident, 60-90% of ^{137}Cs was found to be unavailable for plant uptake. Beet (*Beta vulgaris*), quinoa (*Chenopodium quinoa*), red pigweed (*Amaranthus retroflexus*) and russian thistle (*Salsola kali*) are known to remove ^{137}Cs (Arthur 1982; Broadley and Willey 1997). Water hyacinth (*Eichornia crassipes*) was found to take up ^{137}Cs and a 60-fold increase in medium activity resulted in a 17-fold increase in accumulation levels (Jayaraman and Prabhakar 1982). Monterey pine and Pondorosa pine seedlings grown on spiked medium were shown to take up 6-8% of ^{137}Cs in 4 weeks (Entry et al. 1993). Dushenkov et al. (1999) found a drastic reduction in ^{137}Cs in solutions in which sunflower plants were grown hydroponically. ^{137}Cs could also be taken up by the leaf surface and transported to roots and subsequently to the soil (Zehnder 1995). Studies in the ponds near the vicinity of Chenobyl, Ukraine, showed that sunflower plants grown hydroponically in the pond could take up 90% of ^{137}Cs (from 80Bq/L ^{137}Cs) in 12 days. It was estimated that 55 kg of dry sunflower biomass could remove the entire radioactivity in the pond in the Chernobyl having 9.2×10^{6} Bq ^{137}Cs and

$1.4x10^8$ Bq ^{90}Sr (Dushenkov et al. 1999). *Amaranthus retroflexus* was shown to accumulate high concentrations of ^{137}Cs from soil in experiment conducted at Brookhaven National Laboratories (BNL), NY. Cornish et al. (1997) conducted field trials at BNL soil and found that Indian mustard and corn could remove high amounts of ^{137}Cs. Studies at Argonne National Lab (ANL), West site in Idaho showed that ^{137}Cs removal using phytoremediation may take upto 4-7 years for complete removal. Idaho National and environmental laboratory used *Kochia scoparia* plants for soil contaminated with ^{137}Cs and the harvested plant matter was treated and disposed off at disposal facilities (http://www.incl.gov/facilities/ant-w-status.shtml). Field and bench studies on phytoremediation of Cs are shown in the Table 4.

Table 4. Studies on phytoremediation of cesium

Radionuclides	Sites	Type of study	Reference
^{137}Cs	Brookhaven, National lab N.Y.-soil	Bench, greenhouse, field	Cornish et al. 1997; Lasat et al. 1997
^{137}Cs	Argonne National lab, soil	Bench, greenhouse, field	Idaho Dept. of Health and Welfare 1998
^{137}Cs and ^{90}Sr	Chernobyl Ukraine, surface water	Greenhouse, field	Dushenkov et al. 1999

Uranium is a naturally occurring radionuclide and consists of ^{234}U, ^{235}U and ^{238}U and is a key element of the nuclear fuel cycle. Nuclear reactor operations, weapons research, nuclear fuel productions and waste reprocessing have resulted in uranium concentration in surface soils and groundwater. Under acidic conditions, uranyl (UO_2^{2+}) is the prominent U species, while hydroxide complexes such as UO_2OH^+, $UO_2(OH)_2^{2+}$ and phosphate complexes form under natural conditions (Langmuir 1978). Uranyl (UO_2^{2+}) cation is taken up more readily by plants compared to carbonate and U complexes (Ebbs et al. 1998). Cornish et al. (1995) conducted experiments to phytoremediate U from soil at the Fernald site in Ohio and at a uranium waste dumps in Montana, USA. Chelating agents like citric acid, and other organic acids that are present in the root exudates of plants have been shown to help in the uptake of uranium. Huang et al. (1998) found that addition of 20 m mol/ kg citric acid increased the uptake of U and its accumulation in shoots in *Brassica* species and *Amaranth.* Ebbs et al. (1998) observed that tepary bean and beet showed the greatest accumulation of uranium and addition of citric acid increased U accumulation by a factor of 14. A commercial scale pilot rhizofiltration system set up at Ashtabula site (Dushenkov et al. 1997) containing wastewater (20-870 µg/L), considerably reduced the U concentration in wastewaters with 95% being removed in 24 h. The bench and field studies on rhizofiltration of uranium is given in Table 5.

Table 5. Studies on remediation of Uranium

Site	Type of study	Reference
Ashtabula OH, wastewater	Pilot Rhizofiltration	Dushenkov et al. 1997
Ashtabula OH, soil	Bench Phytoextraction	Huang et al. 1998
Ferland, OH, soil	Green house	Cornish et al. 1995

Strontium 90 (^{90}Sr) – a fission product with a half life of 28 years is very mobile and is available to plant uptake. Water hyacinth could take up ^{90}Sr depending on the pH (highest at 9 and lowest at 4) with 80-90% activity confined to the roots (Jayaraman and Prabhakar 1982). Dushenkov et al. (1999) found that hydroponically grown sunflower reduced Sr concentrations from 200 to 35 µg/l within 48 h and it was further reduced to 1µg/l. Plants such as *Salsola kali* (Blanchfield and Hoffman 1984) and Atriplex (Wallace and Romney 1972), are known to accumulate ^{90}Sr substantially. Monterey pine and Pondorosa pine seedlings also accumulated high concentrations of ^{90}Sr (Entry et al. 1993), when grown on artificially contaminated medium. Studies by Phytotech Inc and International Institute of Cell Biology, Kiev, showed that sunflower plants could effectively remove strontium from ponds at Chernobyl with bioaccumulation concentration of 600 for both shoots and roots. However, very little information is available on the removal of Sr from soil of the site.

Plutonium isotopes are present in the environment as a consequence of nuclear weapons testing, fuel reprocessing facilities and accidental releases and include $^{239\text{-}240}Pu$, ^{241}Pu and ^{238}Pu. North Atlantic Sargassum was shown to have a high affinity for plutonium with a concentration factor of 21,000 over the marine water (Noshkin 1972). Plutonium uptake by plants appears to vary with plant species, tissue, age and soil characterstics (Garland et al. 1987).

Tritium (half life 12.3 years) occurs naturally when cosmic radiation reacts with gases in the upper atmosphere. Natural tritium combines with oxygen to form water and reaches earth's surface as rain. Tritium also results as a component of nuclear weapons, reactors and nuclear test explosions and contaminates groundwater. Tritium, since it is directly incorporated into water, is taken up by plants which later on release trace amounts of tritium through foliage. Tritium incorporated in water is used by plants for transpiration (IAEA 1981). The tritium phytoremediation project using trees has effectively reduced tritium concentration in waste discharges at Argonne National Laboratory site in Illinois, U.S. However, modeling studies are needed to assess the hazard posed by tritium.

4. Phytoextraction

Phytoextraction refers to the use of metal accumulating plants that translocate and concentrate chemical elements from the soil to roots and finally in the above ground shoots and leaves. Phytoextraction exploits vascular plant's natural ability to take up a variety of chemical elements through the root system, deliver these elements to the vascular tissues and to transport and compartmentalize in different organs. Above-ground biomass loaded with metals/radionuclides is harvested, processed for volume reduction and further element concentrations and safely recycled to reclaim metals of economic importance or disposed off as waste in the case of radionuclides. Phytoextraction offers cost advantages over alternative schemes of soil excavation and treatment or disposal. Major limiting factor for phytoextraction are lower metal availability in soil and poor metal translocation from root to shoots. Application of soil amendments could eliminate the limiting steps in metal phytoextraction. Addition of soil amendments increased the metal availability in solutions more than 10-fold for ^{137}Cs and 100-fold for Pb and U (Huang et al. 1997 1998). In order to use this practically, it is essential to have vigorously growing plant (>3 tons dry matter/ha-yr) which cause easily harvested and that accumulates large concentrations of metal in the harvestable portions (> 1000mg/kg metal). This technique has been effectively used by Phytotech Inc. (USA) for removal of Pb and Cd from contaminated soil. Excessive selenium in agricultural soils is also successfully remediated by plants using this technology (Banuelos 1993).

Successful phytoextraction of radionuclides depends on the bioavailability of radionuclides in soil, the rate of uptake by the plant roots and efficiency of radionuclide transport through the vascular system. However, not every site is conducive to phytoremediation as a result of excessively high contaminant concentration, which may be unsuitable for the plant growth. Only phytoextraction of ^{137}Cs, ^{90}Sr and ^{235,238}U is approaching field application (Dushenkov et al. 1999, Huang et al. 1998), being an element specific and site specific technology. It is possible to formulate a general approach to develop a phytoextraction process for radionuclides, even though numerous challenges have to be overcome to ensure a substantial flux of radionuclide from soil to the aboveground biomass. The radionuclide uptake by plant roots need not necessarily result in translocation to shoots. The majority of ^{137}Cs taken up by plants tends to be localized in the roots (Clint and Dighton 1992). Ebbs et al. (1998) demonstrated in hydroponic U uptake studies at pH 5, that the uranyl (UO_2^{2+}) cations were more readily taken up and translocated by plants than hydroxyl (pH 6) and carbonate (pH 8) U complexes. Formation of stable U-phosphate complexes in roots may prevent U translocation to aboveground plant parts. In contrast to Cs and U, almost 80% of ^{90}Sr taken up the plant, is usually localized in the shoots.

Radionuclides such as ^{90}Sr, ^{95}Nb, ^{99}Tc, ^{106}Ru, ^{144}Ce, ^{226,228}Ra, $^{239\text{-}240}$Pu, ^{241}Am, ^{228,230,232}Th, ^{244}Cm and ^{237}Np, were tested for phytoremediation (Dushenkov 2003). A pilot scale phytoextraction project was conducted in the Chernobyl Exclusion Zone (Dushenkov et al. 1999). Three sequential mustard crops were used to obtain noticeable decrease in ^{137}Cs activity that was reduced from an average of 2558 Bq/kg to an average of 2239 Bq/kg. In one growing season, areas having ^{137}Cs levels>3000 Bq/kg decreased from 29.4% of the total plot area before treatment to 7.7% after treatment. After the final harvest of the phytoremediation crop, areas having ^{137}Cs levels<2000 Bq/kg increased to 33.3% compared to 27.4% before treatment. Some of the plants, which can be used for phytoextraction are listed in Table 6.

Table 6. Plants with potential for the phytoextraction of various metals and radionuclides

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

5. Rhizofiltration

Rhizofiltration is the use of plant roots to sorb, concentrate or precipitate metal contaminants from solutions. The ideal plant for rhizofiltration should have the capacity to remove maximum amount of toxic metal from contaminated streams coupled with easy handling. An ideal plant used for rhizofiltration should produce significant amount of root biomass with large surface area when grown hydroponically, should be able to take up high concentration of toxic metal and tolerate high amount of toxic metal in roots. Nutrients can be supplied to the plant through artificial soil mixture kept on the top of the hydroponic system (feeder layer). Indian mustard plants were capable of removing Pb from aqueous solutions in the range of 4 to 500 mg/l (Dushenkov et al. 1995). The roots of Indian mustard could effectively remove Cd, Cr, Cu, Ni and Zn. Sunflower plants, tested in the batch experiments in a growth chamber significantly, reduced the concentrations of Cd, Cr, Cu, Mn, Ni and Pb within an hour of treatment. Most cationic species of toxic metals were removed from solutions at least initially and more rapidly in comparison with anionic ones.

Rhizofiltration has been successfully employed by Phytotech Inc. using sunflower at a US Dept of energy (DOE) pilot project with uranium wastes at Ashtabula, Ohio and water from a pond near Chernobyl nuclear plant in Ukraine. In batch experiments with hydroponically grown sunflower plants (Dushenkov et al. 1997), it was shown that concentrations of Cs, U and Sr in contaminated water were significantly reduced within a few hours. Uranium concentration was reduced 10 fold in 1 h while Cs concentration showed a decrease after 6 h and within 24 h, almost all the Cs was removed. Strontium concentration was reduced to 35μg/l within 48 h and at the end of 4 days, it was further reduced to 1μg/l. Sunflower roots concentrated uranium from solution by upto 10,000 fold. Rhizofiltration is proved to be a feasible approach for removing radionuclides from aqueous streams. However, it requires optimization and economic evaluation against conventional technologies.

6. Phytostabilization

Phytostabilization is stabilizing process for contaminated soils and sediments in place using vegetation, thus preventing the migration of toxic metals. This is applicable for metal contaminants of waste sites where the best option is to immobilize them *in situ*. Low level of radionuclides also can be maintained this way. Metal cations are most tightly bound and form strong complexes with -H groups on the surface of minerals and hydrous oxides in waste materials. Metals can also bind to the organic material. Addition of manure, digested sewage sludge, straw etc. to inorganic waste sites may help in binding of metals. Supplementation of lime (CaO) and limestone ($CaCO_3$) may help in neutralizing acid soils so as to help in binding of cationic metals with inorganic wastes. Anions such as arsenate and chromate can form surface complexes on hydrous oxides.

Unlike plants chosen for phytoextraction, candidate plants for phytostabilization should be poor translocators of metal contaminants to above ground tissues of plants. The plants should be capable of tolerating high level of metal contaminants and should have efficient growth with dense root system and canopies. Plants which are most suitable for soil conservation are suitable for phytostabilization. Mine tailing at Superfund site in South Dakota with upto 1000 mg/kg of arsenic and also lower concentrations of cadmium and smelter in Kansas with 200,000 mg/kg of zinc could be phytostabilized by decreasing vertical migration of leachate to groundwater using hybrid poplar trees (Hse 1996).

Phytostabilization is particularly suitable for radionuclide-contaminated sites, where one of the alternatives is to hold contaminants in place to prevent secondary contamination and exposure. Capturing radionuclides *in situ* is often the best alternative at sites with low contamination levels or vast contaminated areas where a large scale removal action or other *in situ* remediation is not feasible. This can result in a considerable risk reduction, especially if radionuclides with relatively short half –lives are involved. Plant roots also help to minimize water percolation through soil, thus reducing radionuclide leaching. Phytostabilization may be useful in controlling tailings in uranium mining areas. However, phytostabilization does not remove the radioactivity from the site which has the potential risk of radiation exposure to wild life and humans.

7. Phytovolatilization

Phytovolatilization exploits a plant's ability to transpire large amounts of water and is currently used for ^{3}H remediation. Phytoremediation of ^{3}H through irrigation of forest area has been investigated at Savannah River Site (SRS) for consideration as part of a system to reduce the discharge of ^{3}H from the Burial Ground Complex southwest plume. This system is a combination of hydraulic control and enhanced evapotranspiration. Tritium contaminated water is collected, moved to a location upgradient of the discharge point and used to irrigate plants.

8. Design of Phytoremediation System

Design of a phytoremediation system will depend on the various parameters, such as the type of contaminant, concentration, clean up required, condition of the site and selection of plant. Phytoextraction has a different design requirement compared to phytostabilization. Most important parameters will include selection of suitable plants, planting density and pattern, contaminant uptake, clean up time required, ground water capture zone and transpiration rate.

Plants generally used for phytoextraction include sunflower and Indian mustard for lead and sunflower and aquatic plants for radionuclides . Recovery of metals from vegetation will depend on recovery from the ash or use of wet extraction techniques. If the metal is for disposal, they will have to be concentrated into a much smaller volume for ultimate disposal/ storage. Aquatic plants include emergent, submerged and floating species. It is easier to harvest emergent populations, while submerged species have more biomass in contact with the solution. Some of the plants generally used for phytostabilization, phytoextraction and rhizofiltration are given in Table 7 and the critical success factors are included in Table 8.

Table 7. Phytoremediation applications for metals and radionuclides

Application	Media	Contaminants	Plants/Character
Phytostabilization	Sediments, Soil	Pb, Cd, Zn, As, Cu, Cr, Se, U	-Trees which transpire large amounts of water for hydraulic control -Grasses with fibrous roots to stabilize soil erosion -Dense root systems needed to sorb/bind
Phytoextraction	Sediments, Soil	Pb, Cd, Zn, Ni, Cu, EDTA addition for Pb, Citric acid addition for U	-Sunflower -Indian mustard -Rapeseed -Amaranthus -Chenopodium
Rhizofiltration	Groundwater, Wastewater, Created wetland	Pb, Cd, Zn, Ni, Cu, ^{137}Cs, ^{90}Sr, U	-Sunflower -Indian mustard -Aquatic plants-Emergent- water hyacianth, Duckweed Submerged plants-Hydrilla,

Table 8. Critical success factors for Phytoremediation

Phytoremediation process	Critical factors	Conditions for success	Basis for success	Data required	Type of plants
Phytostabi-lization	Immobili-zation Hydraulic control Soil stabilization	Good roots & biomass Immobile chemicals	Roots hold soil Immobilize metals	Fate and toxicity	Trees, Grasses, Legumes
Phytoextra-ction	High biomass Accumulati on in harvestable portion of plants	> 3 tons dry matter/acre/year > 1000 mg/kg of metal	Vigorous growth	Fate and toxicity	Terrestrial plants Aquatic plants
Rhizofiltration	Sorption/filt ration by roots	Plant densities 200-1000 gm/m^2	Roots sorb and immobilize contaminants	Fate and toxicity	Aquatic plants -Submerged -Emergent

8.1 Laboratory to Pilot Scale Studies

The sequence of information needed typically range from hydroponic studies to small pot studies with soil from the site in a green house to plot studies (15x15m). Different concentrations of contaminants can be used for toxicity studies. In the last 5 years, about 20 projects, which include field applications of phytoremediation of radionuclides were initiated in USA, Belarus, Ukraine, UK, Yugoslavia, Czech Republic and China.

8.2 Plant Density and Pattern

Hybrid poplar-1000 to 2000 per acre are planted normally. Willow and cottonwood belonging to Salix family can also be used for this purpose. The average life time of hybrid poplar is about 30 years and every 4-6 years, the above ground biomass can be cut and removed and new shoots will grow from the cut stem.

8.3 Irrigation and Maintenance

Irrigation of the plants ensures a vigorous growth of the plant. Hydrologic modeling may be required to estimate the rate of percolation to groundwater under irrigated conditions. After initial irrigation, irrigation can be discontinued provided the area receives sufficient rains. Agronomic inputs such as addition of NPK, addition of soil conditioners like straw, manure etc should be taken into account. Costs of fertilizer, monitoring of vegetation mowing, pruning, harvesting and replanting should also be included. For phytostabilization, phosphate fertilizers or rock phosphate are effective in binding lead and zinc. In case of phytoextraction, chelates such as EDTA (0.5-10ug EDTA/kg soil) have been added in soils to ensure effective plant uptake (Raskin 1996).

8.4 Cost

Phytoremediation is very cost-effective in comparison with other technologies. It is aesthetically pleasing and public acceptance is high (Table 1). Although phytoremediation offers cost advantages, the time period required for clean up is important. Mathematical modeling and monitoring are necessary to demonstrate the effectiveness of the technology to regulatory agencies.

9. Challenges for Phytoremediation

As the technology of phytoremediation emerges, so do its challenges. The technology of phytoremediation is still in research and development phase and there are some technical barriers, which need to be addressed. Most heavy metal accumulating plants have a small biomass and are slow growing. To make phytoremediation a viable technology, there is a need to either find fast growing (as yet undiscovered) hyperaccumulators or engineer common plants with hyperaccumulator genes for higher metal accumulation. Conventional breeding and biotechnology have been used to correct these shortcomings by transferring desired traits from metal hyperaccumulator plants to selected high biomass producing non accumulator species. For phytoremediation to be possible, heavy metals must be within the plant's root zone, biologically adsorbed and bioavailable. Attempts are being made to maximize heavy metal concentrations in the plant tissues that grow fast and to isolate genes for metal uptake, which can be potentially transferred to other high yielding biomass plants.

9.1 Genetic Engineering of Plants for Metal Tolerance and Accumulation

Several genes are involved in metal uptake, translocation, sequestration and transfer of these genes into candidate plants will result in developing transgenic plants with enhanced ability for metal uptake/accumulation.

Transfer of metallothionin genes have been achieved in several plants. Transfer of human MT-2 gene to tobacco and oil seed rape resulted in plants with enhanced Cd tolerance (Pan et al. 1994). Enhanced Cu accumulation was obtained in *Arabidopsis thaliana* with a pea MT gene (Evans et al. 1992). Transfer of yeast CUP1 gene resulted in 16-fold higher accumulation of cadmium in cauliflower plants (Hasegawa et al. 1997). Similarly, ransfer of two genes for production of γ-glutamylcysteine synthase or glutathione synthase showed enhanced tolerance/accumulation of Cd (Zhu et al. 1999a,b). De la Fuenta et al. (1997) obtained plants with enhanced Al tolerance by overexpression of citrate synthase which resulted in enhanced production of metal chelator-citric acid. Introduction of metal transporter genes also enhances accumulation of metals in plants as in case of *A. thaliana* having Zn-transporter-ZAT gene from *T. goesingense* resulting in 2-fold accumulation of Zn in roots. Likewise, increased Fe tolerance was obtained by overexpression of At Nramp/gene (Curie et al. 2000).

Introduction of merA and merB genes resulted in transgenic *A. thaliana* plants which could phytovolatalize mercury (Bizily et al. 2002). Dhankher et al. (2002) also developed transgenic *Arabidopsis* plants which could take up arsenate by introducing arsenic reductase and γ-glutamyl cysteine synthetase genes. Transport of oxyanion arsenate to above ground, reduction to arsenite and sequestration to thiol peptide complexes by transfer of E. coli ars c and γ ECS gene has been reported. Overexpression of oxidative stress enzymes such as ACC aminase resulted in transgenic plants which accumulated a variety of metals (Ezaki et al. 2000). Selected examples of transgenic plants developed for phytoremediation are shown in Table 9.

Table 9. Selected examples of transgenic plants for phytoremediation

Gene transferred	Plant	Effect
MT-1 gene from human	Tobacco, Seed rape	Cd toletrance
CUP-1 gene from yeast	Cauliflower	Cd accumulation
γ-glutamyl cysteine synthetase gene from rice	Indian mustard	Cd acumulation
At MTP-1 from Thlaspi goesingense	Arabidopsis	Zn accumulation
Arsenate reductase γ-glutamyl cysteine synthetase from *E.coli*	Indian mustard	As tolerance
Mer A and Mer B gene	Arabidopsis, Yellow poplar	Phytovolatilization of Hg

9.2 Field Testing of Transgenics and Risk Assessment

Transgenic mustard overexpressing phytochelatins were used for greenhouse studies in Leadville, Colorado such plants were shown to accumulate significant levels of Zn and Cd (Bennett et al. 2003). Some of the possible risks associated with the transgenics are enhanced exposure risk to wild life and humans. Suitable fencing off of the area and use of non-palatable species will prevent grazing/ingestion by wild animals/birds. No transgenic has been commercially used currently for phytoremediation, although mercury volatilizing plants pose no risk (Lin et al. 2002). The risk of escape of genes from transgenic plants is also negligible (Meagher et al. 2000).

10. Companies Developing Phytoremediation

In the last few years, several commercial companies on phytoremediation have started springing up in US and Europe and is similar to microbial bioremediation industries as listed in Table 10.

Dedicated companies exclusively working on phytoremediation are developing plants for remediation of metals and radionuclides from soil and water. Phytotech Inc., for example, has used *Brassica* species to remove lead from soil and sunflower to remove uranium and cesium from aqueous waste streams while Phytoworks Inc. is focusing on remediation of organics and mercury by introducing transgenic plants which metabolize mercury. Another company, Earthcare Inc., is working on phytoremediation of organic contaminants using different plants. Similarly, phytokinetics is using grasses to stimulate rhizospheric biodegradation of organics. A number of large industrial companies, principally the oil ad chemical industry, are also conducting or supporting phytoremediation. Phytoremediation is expected to have a large market in future as reflected in Table 11 for USA alone.

Table 10. Companies conducting Phytoremediation

1. Applied Natural Science (USA)
2. Aquaphyte Remediation (Canada)
3. BioPlanta (Germany)
4. Consulagri (Italy)
5. Earthcare (USA)
6. Ecolotree (USA)
7. OEEL (UK)
8. Piccoplant (Germany)
9. Phytotech (USA)
10. PhytoWorks (USA)
11. Plantechno (Italy)
12. Slater (UK)
13. Thomas Consultants (USA)
14. Verdant Technologies (USA)
15. Viridian Resources (USA)

Table 11. US Phytoremediation markets (2005) in millions of US Dollars*

Metals from soil	70-100
Metals from groundwater	1-3
Metals from wastewater	1-2
Radionuclides	40-80
Organics from groundwater	35-70
Others	65-115
Total	214-370

***** Taken from Glass Associates Inc.

11. Regulatory Acceptance and Public Acceptance

Phytoremediation's ability to make further strides will depend on how quickly the regulators become convinced of the efficacy of the technology. The regulatory agencies by nature are conservative and tend to have more confidence in technologies longest known to them. The use of plants is generally considered to be aesthetically pleasing means of remediating contaminated sites and is preferable than excavation and other remedial activities, which may involve environmental disruption, noise and frequent worker activity.

12. Conclusion

Phytoremediation is an emerging technology for contaminated sites and is attractive due to its low cost, high public acceptance and environmental friendliness nature. It is not a panacea for all waste problems, but a supplement to the existing technologies. The technology has been demonstrated, but not yet commercially exploited. More research background for development of plant tailored for remediation needs use of genetic engineering. The concept of manipulating plant genes for toxic metal uptake is today a cutting edge research area. The likelihood of public acceptance of genetically engineered plants for phytoremediation will be welcomed, since it will clean up the environment of toxic metals. No doubt phytoremediation technology has attracted a great deal of attention in recent years and it is expected that phytoremediation will capture a significant share of the environmental market in the coming years.

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