

PHYTOREMEDIATION OF HEAVY METALS CONTAMINATED SOILS

Ankush Sheoran^{1*} and Sunita Sheoran²¹Department of Chemistry, Panjab University, Chandigarh²Department of Soil Science, College of Agriculture, CCS Haryana Agricultural University, HisarEmail: sheoransunita27@gmail.com

Received-06.10.2017, Revised-22.10.2017

Abstract: Soils may be contaminated by the accumulation of heavy metals and metalloids through the emissions from rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation etc. Excessive accumulation of heavy metals can have deleterious effects on soil fertility and productivity, disrupts ecosystem functioning and can lead to serious health risks to animals and human beings. Many methods of preventing or removing these pollutants from soils are identified, however, most of these conventional remedial processes are expensive and adversely affect the soil fertility and productivity. Therefore, phytoremediation which uses higher plants to reduce contaminant levels in soil is an eco-friendly and cost effective technology. The objective of this review is to discuss the different mechanisms of phytoremediation, their potentials, limitations, and techniques to enhance the phytoremediation efficiency.

Keywords: Phytoextraction, Hyperaccumulator, Ecofriendly, Cost effective, Chelates, Microbes

INTRODUCTION

Heady metal is defined as d-block element of periodic table which may be transition metal, metalloid, lanthanoids or actinoids, having metallic properties, atomic weight more than calcium, density $>5 \text{ Mg/m}^3$, have great capacity to form complexes with organic matter and often associated with contamination and potential toxicity (Duffus, 2002). As per the role of heavy metals in living systems, these are classified into two groups: essential and non-essential. Some metals like Mn, Fe, Ni, Cu and Zn are essential for plant growth, development and physiological functions at low concentration that is why known as essential heavy metals (Gohre and Paszkowski, 2006). Most of heavy metals are cofactors of enzymes. Some of them are involved in important processes such as photosynthesis (Mn, Cu), DNA transcription (Zn), hydrolysis of urea into carbon dioxide and ammonia (Ni), legume nodulation and nitrogen fixation (Co, Zn, Co), flowering and seed production (Cu, Zn), especially when their availability is very low. Second groups *i.e.* non-essential heavy metals includes elements like Cd, Pb, Hg and As which are not required by living organisms or plants for any physiological functions (Peng *et al.*, 2009). Heavy metals are highly carcinogenic, teratogenic and mutagenic even at low or trace concentration. Phytotoxicity is mainly associated with accumulation of non-essential heavy metals which generally have very low toxicity thresholds (Clemens, 2006). The major hazardous heavy metals concerned with environmental and health issues are As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb (Basta *et al.*, 2005, Wright, 2007 and Gosh, 2010). Among these, Cd and Pb are the most dangerous metals for human health (Sekara *et al.*, 2005). Heavy metals at higher

concentrations may disturb the normal physiology and biochemistry of living systems, can block the essential functional groups, displace other metal ions, or modify the active configuration of biological molecules. Threshold toxic limits of heavy metals in soil and plants and their associated health risk to humans are given in table 1.

Heavy metals may exist in colloidal, ionic, particulate and dissolved phase. They also have high affinity for humic substances, organo-clays and oxides coated with organic matter (MacCarthy, 2001 and DeVolder *et al.*, 2003). The soluble forms of heavy metals may be ions or organo-metallic chelates or complexes and their solubility or retention is controlled by pH (Ross *et al.*, 2003), amount of metal, cation exchange capacity, organic carbon content (DeVolder *et al.*, 2003), oxidation state of metals and the redox potential of the system. Among these factors, soil pH is predominant and solubility of heavy metals is increased with decrease in pH.

Sources of heavy metals in soil

Heavy metal can be introduced into any system from natural or anthropogenic sources. Naturally heavy metals are released into the soil, sediments or aqueous system through chemical as well as physical weathering of igneous, metamorphic rocks and soil. They are also generated from volcanic activities, wind erosion, forest smoke fire and fossil fuels. These metals may also be derived from remobilization from natural soils due to the changes in local redox conditions and the corrosion of subsurface engineering structures due to prolonged submergence under acidic groundwater. Whereas on other hand, anthropogenic sources such as mines, foundries, smelting of ores, electroplating, gas exhaust, coal burning power plants etc. also releases heavy metals and imposes a harmful threat to humans as well as environment. Among these

*Corresponding Author

factors, mining contribute the maximum in contamination of soil followed by agriculture and wastewater. The most hazardous trace-elements such as As, Cd, Co, Cu, Mn, Pb and Zn were found, mainly around industry and mining areas and poses risk to human and ecological health. The materials generated from various industries such as textile, tanning, petrochemicals from accidental oil spills or utilization of petroleum-based products, and pharmaceutical facilities are highly variable in composition (Sumner, 2000 and DeVolder *et al.*, 2003). Industrial activity has led to very high heavy metal concentrations on the environment, which are in general 100–1000 fold higher than those in the Earth's crust, and in turn, living organisms can be exposed to even higher levels. Second major source of heavy metal contamination is related to agricultural issues that include (i) uses of agrochemicals such as fertilizers, pesticides, fungicides, (ii) application of manures and bio-solids (iii) utilization of waste water for crop production. The fertilizers which are used to supply micronutrients contain trace amounts of heavy metals (e.g., Cd and Pb) as impurities and continued application of these fertilizers may significantly increase the content of heavy metals in the soil. Other than micronutrient fertilizers, certain phosphatic fertilizers also supply Cd, Pb and other potentially toxic elements such as F, Hg and As to the soil (Raven *et al.*, 1998). Besides fertilizers, commonly used pesticides or fungicides for insect-pest or disease control of field crops also contain heavy metals like Cu, Hg, Mn, Pb, Zn etc. (Jones and Jarvis, 1981). Bordeaux mixture, a Cu-containing fungicide, is most widely used for field crops while for fruit orchard, lead arsenate is fairly extensively used to control parasitic insects. Arsenic containing compounds are also used to control cattle ticks and pests in banana. The application of various bio-solids (e.g. livestock manures, composts, and municipal sewage sludge) in the fields to maintain soil fertility and productivity inadvertently lead to the accumulation of heavy metals such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb etc. in the soil (Basta *et al.*, 2005). In the pig and poultry industry, Cu and Zn are added to diets as growth promoters and As is a part of poultry health products, so manures produced from animals on such diets contain high concentrations of As, Cu, and Zn and repeated application of such manures cause considerable buildup of these metals in the soil and may have the potential to cause contamination of the soil (Sumner, 2000). Most commonly found heavy metals in bio-solids include Pb, Ni, Cd, Cr, Cu, and Zn. Heavy metal concentrations in bio-solids are determined by the nature and intensity of industrial activity and type of process employed during the bio-solids treatment and production. Several studies suggest that agriculture based on waste water irrigation accounts for 50 percent of vegetables

supply to the urban areas (Bjuhr, 2007). Although, it is considered that waste water contains relatively low concentrations of heavy metals, however, long-term utilization of such waste water for irrigation purpose may lead to accumulation of heavy metals in the soil. **Remedial measures of heavy metals contaminated soils**

Remediation refers to any process or technique with the help of which concentrations of contaminants are reduced below the threshold levels so that associated health hazard can be eliminated (Martin and Ruby, 2004). Due to immutable nature of heavy metals, metal-contaminated soils are notoriously hard to remediate. For remediation of heavy metal-contaminated soils, selection of appropriate remedial process depends on physical and chemical form of the contaminants of concern, soil properties and site conditions. Among the best demonstrated available technologies for remediation of heavy metals contaminated soils, immobilization, soil washing and phytoremediation are most frequently used. Each of these remediation technologies has its specific benefits and limitations.

1. Immobilization: Immobilization refers to the process in which heavy metals remains in the soil itself but reduce the toxicity by decreasing its bioavailability through in situ immobilisation processes (Diels *et al.*, 2002). Immobilization uses organic and inorganic amendments to reduce the bioavailability and toxicity of heavy metals. These immobilizing amendments alter the physicochemical states of metal and transform them into more geochemically stable forms via sorption, ion exchange, precipitation, redox reactions and complexation phenomena (Hashimoto *et al.*, 2009 and Wang *et al.*, 2009). The mostly commonly used inorganic amendments include clay, cement, fly ash, blast furnace slag, calcium carbonate, Fe/Mn oxides, charcoal, zeolites, minerals, phosphates (Ling *et al.*, 2007 and Fawzy, 2008) whereas organic amendments include bitumen, organic composts, manures and microbes (Farrell, 2010) or a combination of organic-inorganic amendments may be used. Immobilization mainly includes stabilization or vitrification process. Vitrification is defined as high-temperature treatment of contaminated soil that volatilize/destroy volatile metals. However, because of very complexed nature of soil, immobilization is not so successful.

2. Soil Washing: It is defined as a volume reduction process in which contaminants are washed off from the soil with the help of aqueous solutions or chemicals. Washing solution may be water or aqueous solution of acids, alkali, solvents or surfactants (Chen and Hong, 1995 and Wuana *et al.*, 2008). Organic acids such as oxalic, citric, formic, acetic, malic, succinic, lactic and fumaric acids also dissolve the metal and make it available to biota (Labanowski *et al.*, 2008). Soil washing can be performed in situ or ex situ depending on nature of

soil and contaminant to be removed and extent of contamination. Soil washing is an efficient remedial process and eliminates the contaminants for longer time period. However, the most important problem is associated with disposal of hazardous waste solution. Secondly, it is high cost process that is economically infeasible. Also, the hazardous acids if persist in soil for longer time, they may cause adverse effects on plant growth and soil fertility and subsequently causes negative impacts on the ecosystem.

3. Phytoremediation The term phytoremediation was coined by Ilya Raskin in 1994, it consists of Greek word "phyto" means "plant", and the Latin suffix "remedium" means "able to cure" or "restore" (Cunningham *et al.*, 1996). So, phytoremediation is defined as an *in situ* remedial process that uses plants to remove, transfer, stabilize, and destroy contaminants in soil, sediments or aqueous system. It is also termed as green remediation, botanoremediation, agroremediation, or vegetative remediation. Efficiency of phytoremediation depends on the nature of contaminant to be removed, its bioavailability and soil properties (Cunningham and Ow, 1996). Phytoremediation is an emerging non-destructive, aesthetically pleasing and cost effective strategy to clean up the contaminated soil. Additionally, it is socially acceptable technology. In contrast to its many positive aspects, limitations of phytoremediation includes: (i) all type of plants are

not suitable for removal of all type of contaminants, in tern, its success is limited by growing habit of plants in specific environmental and soil conditions it is dependent on the growing conditions required by the plant, (ii) it is a slow process and takes longer time than other technologies (iii) success is dependent on the tolerance of the plant to the pollutant. Most of the conventional remedial technologies are expensive and inhibit the soil fertility, hence, in order to deal with these contaminants in an eco-friendly manner, phytoremediation is most suitable option.

Different mechanisms of phytoremediation

Phytoremediation is a broad term that has been used since 90's to describe the use of plants to remediate the contaminated media (USEPA, 2000). Phytoremediation includes six main mechanisms namely Phytostabilisation, Phytoextraction, Rizofiltration, Phytodegradation, Rizodegradation and Phytovolatilisation (Figure 1). Use of the mechanism depends on the nature of the contaminant to be dealt with and soil conditions. Out of these six mechanisms of phytoremediation, for remediation of heavy metal contaminated soils, potentially used technologies are phytoextraction (phytoaccumulation), phytostabilization, and phytofiltration (Garbisu and Alkorta, 2001). Further among these three, phytoextraction is most primarily used (USEPA, 2000).

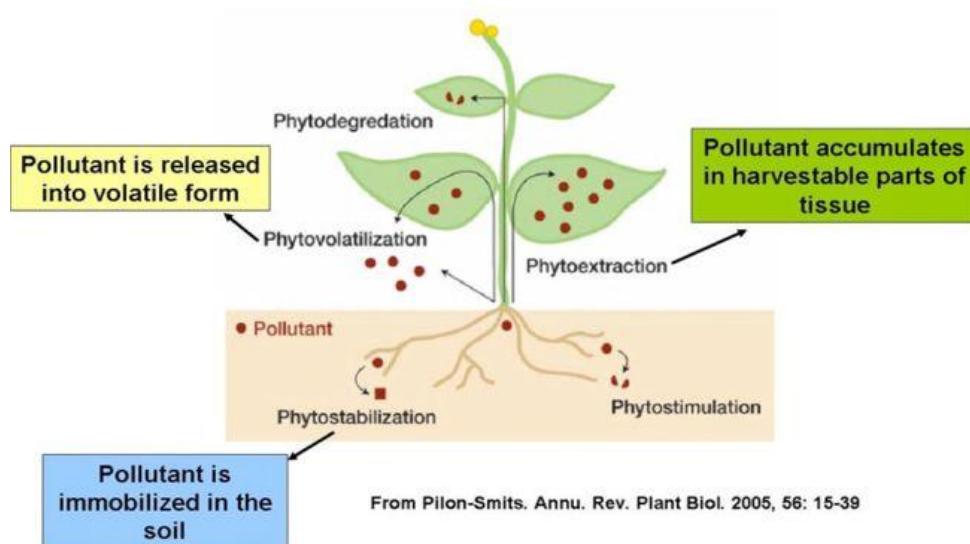


Figure 1: Different mechanisms of phytoremediation

Phytoextraction: It is defined as the process in which plants uptake the metal contaminants by roots from soil and then accumulate the contaminant into the above ground parts such as shoots, leaves etc. That is why it is also termed as phytoaccumulation. The discovery of hyperaccumulator plants lead to the idea of using plants for remediation of metal contaminated soils (Raskin and Ensley, 2000). A hyperaccumulator is defined as the plant species that

have potential to accumulate high concentrations of metals in their foliage and often it is endemic (Raskin *et al.*, 1997 and Brooks, 1998). Generally, it is believed that hyperaccumulator is capable of accumulating the metal about 100 times higher as compared to non-accumulating plants (McGrath *et al.*, 2002). About 400 plants species have been discovered and identified as hyperaccumulators and these plant species differ widely in their potential of

phytoextraction for different heavy metals (Table 2). Majority of hyperaccumulator plants reported belong to the families like Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae and Eupobiaceae. Among these reported families, Brassicaceae had the largest number of taxa viz. 11 genera and 87 species. Metals such as Ni, Zn, Cu are best studied for phytoextraction because these metals are preferred by majority of plants for uptake and accumulation. Hyperaccumulator plants used for phytoextraction purpose should have the following characteristics: (i) should extract high concentration of heavy metals from contaminated soil (ii) have capacity to translocate the extracted metals to the above ground biomass (iii) should have fast growing habit and produce large quantity of plant biomass and (iv) have capacity to tolerate high levels of metal (Brennan and Shelley, 1999 and Garbisu *et al.*, 2002). Plants suitable for phytoextraction also have been characterized by shoot-to-root metal concentration ratio (also termed as Translocation factor) of >1 (McGrath *et al.*, 2002, Yoon *et al.*, 2006). Ali *et al.*, 2012 observed that root bioconcentration factor of *Trifolium alexandrinum* for Zn, Pb, Cu and Cd were 4.242, 1.544, 1.071 and 0.604, respectively. The phytoextraction is fairly inexpensive technology as compared to conventional methods. Major limitation associated with phytoextraction is disposal of harvested above ground biomass. Volume reduction of contaminated material can be achieved by ashing or composting (Garbisu *et al.*, 2002). Harvested biomass can also be used for land filling or may be used for metal recovery (Salt *et al.*, 1998 and Koppolu and Clements, 2003).

Phytovolatilization: Phytovolatilization process uses the naturally occurring or genetically modified plants to absorb metals from the soil/sediments/water, transforming them into less toxic, volatile gaseous into plants and releasing them into atmosphere (USEPA, 2000). This process is applicable only for those heavy metals which can exist as gaseous forms in environment such as As, Hg, and Se may exist as gaseous species in environment. Remediation of heavy metals contaminated soils through phytovolatilization has been observed in several studies. Unlike phytoextraction, problem of disposal of harvested biomass is not associated with phytovolatilization. However, it is a promising technology for remediation of volatile heavy metals, but it is likely to be recycled through precipitation and re-deposited back into lakes and oceans (USEPA, 2000).

Phytostabilization: This mechanism of phytoremediation uses plant species to stabilize the heavy metals in soil through absorption and accumulation by plant roots (Vamerali *et al.*, 2009). It is also termed as in-place inactivation. Phytostabilization may also occur through

precipitation or complexation of heavy metals within rhizosphere by exudates secreted by plant roots. The most important task is to find out suitable plant species (Rizzi *et al.*, 2004 and Mendez and Maier, 2008). This technique is useful for the remedial of Pb, As, Cd, Cr, Cu and Zn contaminated soils. However, this technology reduces the entry of toxic metals by decreasing their uptake by plants, but its drawback is that contaminants still remain in soil.

Phytodegradation: Phytodegradation is defined as breakdown of toxic compounds into simpler molecules by plant metabolism after the toxic metals has been uptaken by the plants and translocated to above ground tissues (Trap *et al.*, 2005). This is also termed as phytotransformation.

Rhizofiltration: Rhizofiltration is defined as adsorption and precipitation of toxic metals onto plant roots and roots are harvested after they become saturated with contaminants. Plants utilized may be terrestrial or aquatic in nature, however, because of having fibrous and longer roots, terrestrial plants are preferred (Raskin and Ensley, 2000). It is applicable for aqueous system where toxic elements are present in groundwater, irrigation water or wastewater. Metals suitable for rhizofiltration include Pb, Cd, Cu, Ni, Zn, and Cr etc. (USEPA, 2000). Potential of several plant species such as sunflower, Indian mustard, tobacco, rye, spinach, and corn have been studied for removal of lead from water.

Rhizodegradation: Rhizodegradation is defined as the breakdown of toxic metals/contaminants into less toxic forms within the rhizosphere. This degradation or break down can be carried out by microbes present in rhizosphere or by exudates (includes sugars, amino acids or amino sugars etc.) secreted by plant roots. This process is also known as phytostimulation.

Ways to improve efficiency of phytoremediation
Most of metals are immobile in nature so phytoremediation rate and efficiency are limited by solubility and diffusion of metals to root surface. There are a number of ways to enhance the efficiency of phytoremediation which includes uses of microbes, chelates, organic manures and compost, other chemicals like fertilizers, fungicides, genetic modified plants etc.

Chelating agents: Chelating agents increase metal bioavailability and plant uptake and increases efficiency of phytoextraction. Chelating agents may be organic and inorganic although inorganic are most commonly used (Quartacci *et al.*, 2006). Various inorganic chelates used are ethylene diamine tetraacetate (EDTA), ethylene diamine disuccinate (EDDS), nitrilo triacetate (NTA), di ethylene triamine pentaacetic acid (DTPA) and cyclohexane diamino tetraacetic acid (CDTA) (Ramprakash *et al.*, 2009). Among these, EDTA is most frequently used to enhance the uptake of several heavy metals (Huang *et al.*, 1997). Other than these inorganic chemicals, low molecular weight organic acids like

acetic, citric, oxalic, fumaric and succinic acids may also be used to improve efficiency of phytoremediation (Chen *et al.*, 2003 and Wenger *et al.*, 2003). Singh *et al.*, 2013 reported that all the chelating agents increased Ni desorption from Ni spiked soil, however, order of effectiveness of chelating agents for desorption in Ni was followed as : NTA>CDTA>DTPA>CA. They also reported that desorption was highest in first extraction followed by second, third and fourth successive extraction. The increased uptake might have been due to increased availability of Ni in soils due to addition of chelating agents resulting in its higher accumulation in roots and shoots and higher dry matter yields of both the components of *B. juncea* (Ishikawa *et al.*, 2006). Amongst the commercial crops grown in this region *B. juncea* has been reported to produce high biomass and accumulate significant amount of heavy metals. Ramprakash *et al.*, 2013 reported that mean uptake of Cr by shoot increased from 303.25 $\mu\text{g pot}^{-1}$ in control to 389.57, 696.17, 868.19 and 427.43 $\mu\text{g pot}^{-1}$ due to application of CDTA, CA, NTA, FYM, respectively (Table 3). EDDS is structural isomer to EDTA, however, its efficiency to improve is mainly associated with Cu or Zn or Pb (Tandy *et al.*, 2004). Application of microbial inoculants in combination with chelating agents further improves the efficiency of phytoextraction as reported by Panwar *et al.*, 2011. They conducted an experiment with *Brassica juncea* grown on a spiked soil with EDTA, farmyard manure, vermicompost and microbial inoculants (*Azotobacter* and *Pseudomonas*) and it was observed that application of microbial inoculants, EDTA, FYM and vermicompost significantly increased Cd uptake and highest uptake was recorded with vermicompost treatment (Table 4).

Genetic engineering/ modified plants: With the help of genetic engineering technique, transgenic plants are developed which have manipulated capacity to uptake, accumulate and can tolerate high concentration of pollutant. Genes which are involved in metabolism and detoxification of pollutants are identified and manipulated thus enhanced phytoextraction is achieved in several studies (Meda *et al.*, 2007 and Reisinger *et al.*, 2008). Genetic engineering also improved phytovolatilization potential of Indian mustard as reported by Banuelos *et al.*, 2005. They tested three transgenic lines of Indian mustard, over expressing the genes encoding enzymes adenosine triphosphate sulfurylase (APS), γ -glutamyl cysteine synthetase (ECS) and glutathione synthetase (GS), for their potential to remove Se from contaminated sediment. The APS, ECS and GS transgenic plants accumulated 4.3, 2.8 and 2.3 fold more Se in their leaves than wild type, respectively (Figure 2). Improved ability of APS Indian mustard may be due to the reason that APS plants over express ATP sulfurylase, a rate-limiting step in the conversion of selenate to selenite. This enables APS plants to rapidly convert selenate via selenite to

organic-Se forms, while wild type plants accumulate mostly selenate. Secondly, APS plants may accumulate high concentrations of Se in shoots by accumulating more Se in nontoxic organic Se forms such as non protein amino acid methyl seleno cysteine (Met-SeCys). ECS and GS would have increased Se uptake and assimilation through increased activity of sulfate permease and ATP sulfurylase.

Humic Acids: Best part of organic matter which has significance importance in agriculture is humus, which is well decomposed part by action of microbes. Humic substances (humic acid+fulvic acid+humin) maintain soil fertility and productivity by improving soil chemical, physical and biological properties such as retention capacity, porosity, aggregate stability, cation exchange capacity etc. Other than these functions, humic acid typically contains of heterocyclic compounds with carboxylic, phenolic, alcoholic and carbonyl functional groups and this characteristic play a vital role in enhancing nutrient uptake and also heavy metals by plants. This is attributed to the reason that humic acids being acidic in nature increases solubility and bioavailability of heavy metals (Bianchi *et al.*, 2008). That is why humic acids can be used to improve phytoremediation as alternatives to inorganic chelating agents. In contrast to this, humic acids can also decreases the mobility of some toxic metals and results in phytoaccumulation (Halim *et al.*, 2003). Therefore, contradictory results have been reported related to mechanism of humic acids in improving phytoremediation.

Mycorrhizae Fungi: Role of mycorrhizae is well known in increasing nutrient uptake by exploiting more volume of soil. This positive effect is also applicable for improving phytoextraction by increasing uptake of heavy metals also (Giasson *et al.*, 2005). However, high concentration of toxic metals can also adversely affect the growth of mycorrhizae fungi.

Bacteria: Addition of bacteria significantly improves the microbial biomass in the rhizosphere and might help to increase As uptake and accumulation (Table 5). The possible explanation was that the application of arsenate reducing bacteria improved the rhizosphere microbial environment, and increased the number and the mycelium of microbes as well as enhanced the biomass of the plant root systems, which might help to take up As, hold soil As, and prevent As losing (Yang *et al.*, 2012).

Chemical fertilizers: Chemical fertilizers can improve phytoextraction process by improving plant growth. Mandal *et al.*, 2012 phytoextracted the soil with *Pteris vittata* grown for two cycles and fertilized with di-ammonium phosphate (DAP) and SSP. However, DAP was found more effective over SSP in stripping more arsenic by *Pteris vittata* resulting in lesser arsenic accumulation in rice crop (Figure 3). Phosphate may compete with arsenic for

plant uptake (Cao *et al.*, 2003). On the other hand, phosphate addition as an essential fertilizer for plant development, would enhance arsenic release from soil through competitive exchange (Smith *et al.*, 2002).

Possible utilization of biomass after phytoextraction: A serious challenge for the commercialization of phytoextraction has been the disposal of contaminated plant biomass especially in the case of repeated cropping where large tonnages of biomass may be produced. The biomass has to be stored, disposed of or utilized in an appropriate manner so as not to pose any environmental risk (Blaylock and Huang, 2000). The major constituents of biomass material are lignin, hemicellulose, cellulose, minerals, and ash. It possesses high moisture and volatile matter, low bulk density, and

calorific value (Ghosh and Singh, 2005). Controlled combustion and gasification of biomass can yield a mixture of producer gas and/or pyro-gas which leads to the generation of thermal and electrical energy. Composting and compacting can be employed as volume reduction approaches to biomass reuse (Raskin *et al.*, 1997 and Garbisu and Alkorta, 2001). Ashing of biomass can produce bio-ores especially after the phytomining of precious metals. Heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, and Zn are plant essential metals, and most plants have the ability to accumulate them (Jadia and Fulekar, 2009). The high concentrations of these metals in the harvested biomass can be “diluted” to acceptable concentrations by combining the biomass with clean biomass in formulations of fertilizer and fodder.

Table 1. Toxic limits of heavy metals in soil and plant and their toxic responses to humans

Heavy metal	Toxic limit in plant (mg kg ⁻¹)	Toxic limit in soil (mg kg ⁻¹)	Toxic response in human
Pb	30-300	600	Irreversible neurological damage, renal disease, reproductive toxicity
Cd	5-30	100	Stomach irritations, lung damage, cancer, bone defects
As	>2.6	20	Cancer, Cardiovascular, gastrointestinal, hepatic and renal disease, DNA damage
Cr	3-30	100	Genotoxic carcinogens, lung cancer, muscle cramps
Hg	0.1-10	270	Brain damage, birth defects, sensory impairment, hearing loss
Cu	100-200	600	Inhibition of dihydrophilhydratase, accumulation in liver and kidney
Zn	100-400	1500	Inhibition of copper absorption, nausea, loss of appetite, abdominal cramps

Source: Salt *et al.*, 1995

Table 2. Concentrations of heavy metals in field crops obtained from field experiments

Species	Metal concentrations (mg/kg)							
	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
<i>Brassica carinata</i>	12	12	-	9.8	37	7.6	50	1650
<i>Brassica juncea</i>	30	10	-	5.2	71	-	55	2029
<i>Brassica napus</i>	5.8	11	-	9	40	7	39	1400
<i>Festuca spp.</i>	-	-	-	-	106	-	-	90
<i>Glycine max</i>	230	2.4	-	-	440	-	72	430
<i>Helianthus annus</i>	20	0.64	0.71	-	70	-	5	150
<i>Hordeum vulgare</i>	20	0.44	-	-	16	-	27	334
<i>Medicago sativa</i>	85	53	-	-	77	-	2177	-
<i>Oryza sativa</i>	-	-	-	-	34	-	6	90
<i>Phaseolus vulgaris</i>	-	53	-	-	2230	-	1000	1440
<i>Pisum sativum</i>	-	-	-	-	-	-	1390	-
<i>Raphanus sativus</i>	-	9.4	-	5	34	6.5	28	1450
<i>Sorghum bicolor</i>	240	3.7	1.8	-	540	-	100	580
<i>Triticum secalotriticum</i>	21	1.9	-	-	27.5	-	37	588
<i>Zea mays</i>	30	20	-	-	1220	-	257	1200

Source :Vamerali *et al.*, 2010

Table 3. Effect of chelating agents and sewage sludge on Cr uptake ($\mu\text{g pot}^{-1}$) by roots and shoots of subsequent *Brassica juncea* crop in Cr contaminated soil

Treatment	50DAS		80DAS		Mean
	Without SS	With SS	Without SS	With SS	
Root					
Cr ₂₀	34.31	53.91	128.52	154.43	92.79
Cr ₂₀ + CDTA	41.17	56.26	108.92	136.69	85.76
Cr ₂₀ + CA	98.68	133.81	201.84	232.73	166.77
Cr ₂₀ + DTPA	34.46	48.28	82.14	100.06	66.23
Cr ₂₀ + NTA	125.72	151.55	288.49	318.95	221.18
Cr ₂₀ + FYM	72.87	92.99	161.19	194.57	130.41
Mean	67.87	89.47	161.85	189.57	
CD (0.05) Soil=6.96, Time=6.96, Chelating agent= NS, S×T=9.84, S×CA=17.05, T×CA=NS, S×T×CA=NS					
Shoot					
Cr ₂₀	51.59	124.61	469.35	567.46	303.25
Cr ₂₀ + CDTA	85.40	154.07	560.01	758.78	389.57
Cr ₂₀ + CA	335.41	530.77	881.29	1039.33	696.17
Cr ₂₀ + DTPA	59.36	99.43	453.53	581.73	298.51
Cr ₂₀ + NTA	411.44	646.53	1125.69	1289.10	868.19
Cr ₂₀ + FYM	194.26	273.84	547.09	694.54	427.43
Mean	189.57	304.88	672.83	821.82	
CD (0.05) Soil=38.7, Time=38.7, Chelating agent= NS, S×T=54.73, S×CA=94.8, T×CA=NS, S×T×CA=NS					

Source : Ramprakash *et al.*, 2013**Table 4.** Cadmium uptake ($\mu\text{g pot}^{-1}$) of shoots and roots of Indian mustard as influenced by different chelating agents and bio-inoculants in Cd-enriched soil

Treatments	Control	Cd ₁₀₀	Cd ₁₀₀ +FYM	Cd ₁₀₀ +VC	Cd ₁₀₀ +EDTA	Mean
Shoots						
(-) Microbial inoculants	126.6	871.6	1381.6	2238.6	2139.0	1351.5
(+)Microbial inoculants	132.0	993.1	1485.7	2265.7	2251.2	1425.6
Mean	129.3	932.4	1433.6	2252.2	2195.1	
CD (5%) Microbial inoculants (M)=11.99, Cd=18.95, Interaction of M×Cd=26.81						
Roots						
(-) Microbial inoculants	36.7	233.3	395.3	477.9	417.6	312.2
(+)Microbial inoculants	47.0	250.6	413.0	499.7	455.0	333.0
Mean	41.8	241.9	404.2	488.8	436.3	
CD (5%) Microbial inoculants (M)=4.06, Cd=6.42, Interaction of M×Cd=9.08						

Source : Panwar *et al.*, 2011**Table 5.** Effectiveness of arsenate reducing bacteria to enhance arsenic removal from polluted soils by *Pteris Vittata*

Arsenate reducing bacteria	As conc. (mg/kg)	As uptake (mg/m^2)
Control	615.74 c	30.47 c
Ts 1	704.32 b	53.39 b
Ts 33	886.47 a	65.04 a
Ts 37	652.55 bc	48.33 b
Ts 41	835.56 a	48.98 b
PSQ 22	698.47 b	44.04 b

Source : Yang *et al.*, 2012

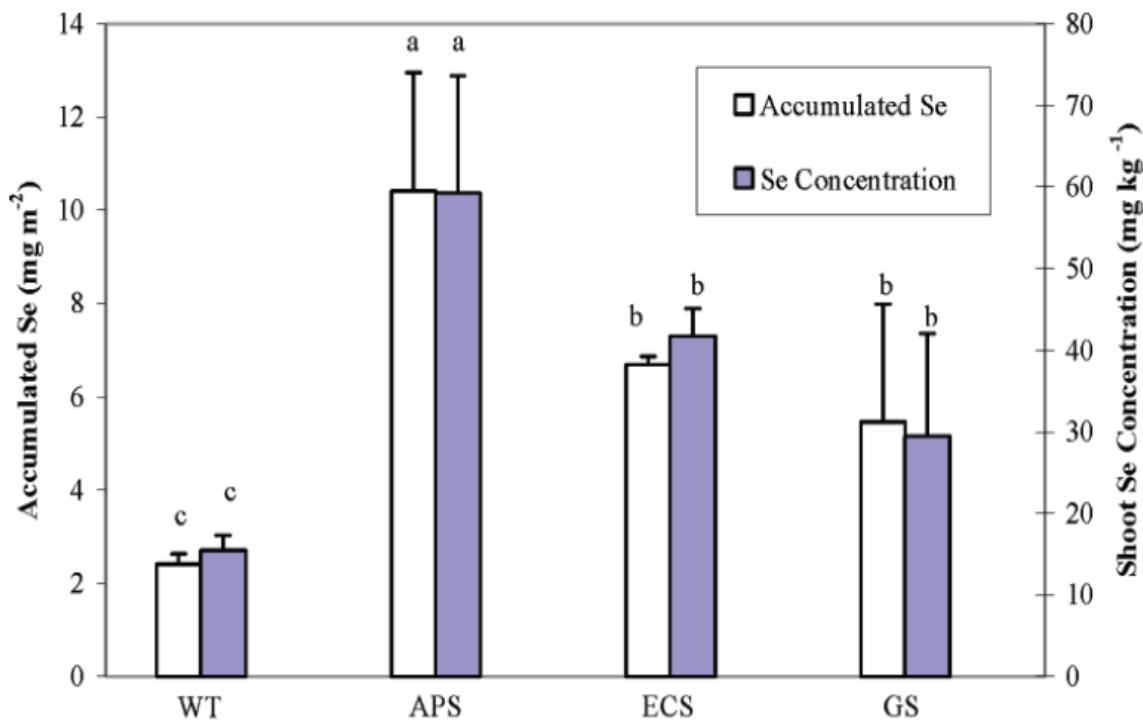


Figure 2: Field trials of transgenic Indian mustard for phytoremediation of selenium contaminated sediment
(Source : Banuelos *et al.*, 2005)

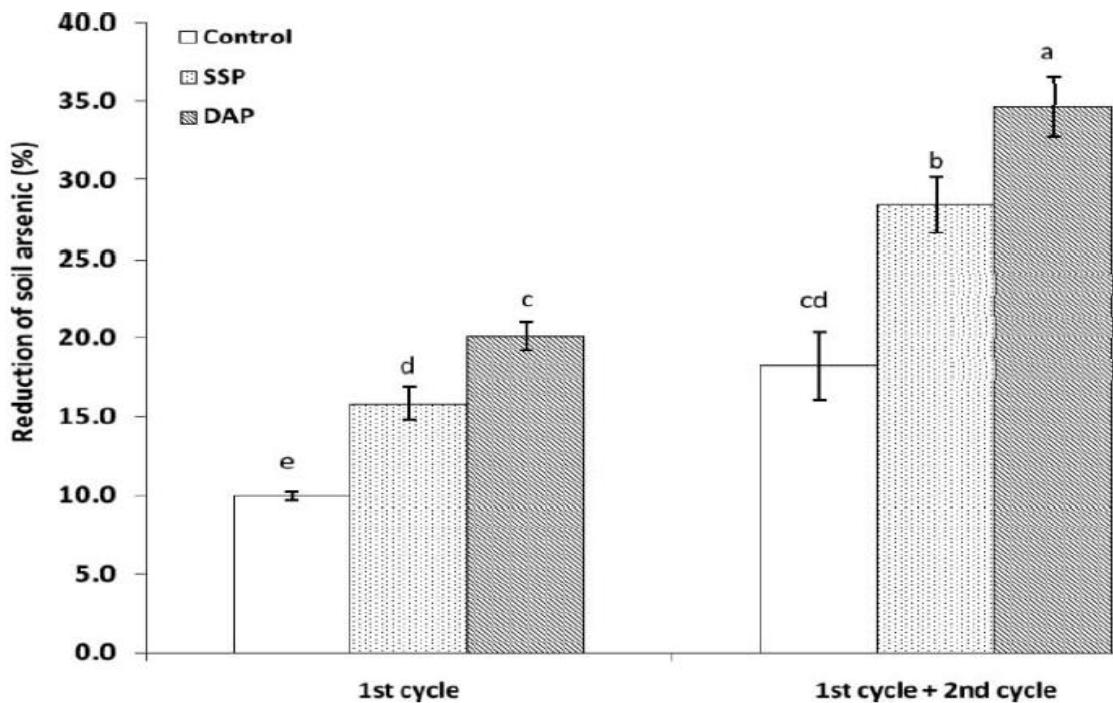


Figure 3: Phytoextraction of soil with *Pteris vittata* grown for two cycles and fertilized with DAP and SSP.
(Source : Yang *et al.*, 2012)

CONCLUSION

As highlighted above, there are several ways in which plants are used to clean up or remediate contaminated sites. The success of phytoremediation

at a given site cannot always be attributed to just one of these mechanisms because a combination of mechanisms may be at work. Phytoremediation is a low cost, solar energy driven and natural cleanup technique, which are most useful at sites with

shallow, low levels of contamination. In addition to this, it is easy to implement and maintain, does not require the use of expensive equipment or highly specialized personnel and is environmentally friendly and aesthetically pleasing to the public. Although it is an easy and cost effective process yet to become a commercially available technology in many parts of the world especially the developing countries like India.

REFERENCES

Ali, H., Naseer, M. and Sajad, M.A. (2012). Phytoremediation of heavy metals by *Trifolium alexandrinum*. International Journal of Environmental Sciences, 2(3):1459-1469.

Banuelos, G., Terry, N., Leduc, D. L., Elizabeth, A.H., Smits, P. and Mackey, B. (2005). Field trials of transgenic Indian mustard plants shows enhanced phytoremediation of selenium contaminated sediment. Environ Science and Technology, 39(6):1771-78.

Basta, N. T., Ryan, J. A. and Chaney, R. L. (2005). Trace element chemistry in residual-treated soil: key concepts and metal bioavailability. Journal of Environmental Quality, 34(1):49-63.

Bianchi, M.J., Masciandaro, G., Giraldi, D., Ceccanti, B. and Iannelli, R. (2008). Enhanced heavy metal phytoextraction from marine dredged sediments comparing conventional chelating agents (citric acid and EDTA) with humic substances. Water Air Soil Pollution, 193:323-333.

Blaylock, M.J. and Huang, J.W. (2000). Phytoextraction of metals. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. – John Wiley and Sons, New York, pp. 53-70.

Brennan, M. A. and Shelley, M. L. (1999). A model of the uptake, translocation, and accumulation of lead (Pb) by maize for the purpose of phytoextraction. Ecological Engineering, 12:271-297.

Brooks, R.R., Chambers, M.F., Nicks, L.J. and Robinson, B.H. (1998). Phytomining. Trends in Plant and Science, 1: 359-362.

Cao, X., Ma, L.Q. and Shiralipour, A. (2003). Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator *Pteris vittata* L. Environmental Pollution, 126:157-167.

Chen T. C. and Hong, A. (1995). Chelating extraction of lead and copper from an authentic contaminated soil using N-(2-acetamido)iminodiacetic acid and S-carboxymethyl-Lcysteine. Journal of Hazardous Materials, 41(2-3):147-160.

Chen, Y.X., Lin, Y.M., He, Y.F., Zhen, S.J., Yu, Y.L., Tian, G.M. and Wong, M.H. (2003). The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere, 50:807-811.

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie, 88(11):1707-1719.

Cunningham, S. D. and Ow, D. W. (1996). Promises and prospects of phytoremediation. Plant Physiology, 110(3):715-719.

Cunningham, S. D., Anderson, T. A., Schwab, P. A, and Hsu, F. C. (1996). Phytoremediation of soils contaminated with organic pollutants. Advances in Agronomy, 56: 55-114.

DeVolder, P. S., Brown, S. L., Hesterberg, D. and Pandya, K. (2003). Metal bioavailability and speciation in a wetland tailings repository amended with biosolids compost, wood ash, and sulfate. Journal of Environmental Quality, 32(3):851-864.

Diels, N., van der Lelie, D. and Bastiaens, L. (2002). New developments in treatment of heavy metal contaminated soils. Reviews in Environmental Science and Biotechnology, 1:75-82.

Duffus, J.H. (2002). Heavy metals—A meaningless term? Pure and Applied Chemistry, 74:793-807.

Farrell, M., Perkins, W. T., Hobbs, P. J., Griffith, G. W. and Jones, D. L. (2010). Migration of heavy metals in soil as influenced by compost amendments. Environmental Pollution, 158(1): 55-64.

Fawzy, E. M. (2008). Soil remediation using in situ immobilization techniques. Chemistry and Ecology, 24(2):147-156.

Garbisu, C. and Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technology, 77(3):229-236.

Garbisu, C. and Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technology, 77: 229-236.

Garbisu, C., Hernandez-Allica, J., Barrutia, O., Alkorta, I. and Becerril, J.M. (2002) Phytoremediation: A technology using green plants to remove contaminants from polluted areas. Reviews on Environmental Health, 17: 75-90.

Ghosh, M. and Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of its byproducts. Applied Ecology and Environmental Research, 3(1):1-18.

Giasson, P., Jaouich, A., Gagne, S. and Moutoglis, P. (2005). Arbuscular mycorrhizal fungi involvement in zinc and cadmium speciation change and phytoaccumulation. Remediation Journal, 15:75-81.

Gohre, V. and Paszkowski, U. (2006). Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta, 223:1115-1122.

Gosh, S. (2010). Wetland macrophytes as toxic metal accumulators. International Journal of Environmental Sciences, 1 (4):523-528.

Halim, M., Conte, P. and Piccolo, A. (2003). Potential availability of heavy metals to phytoextraction from contaminated soils induced by

exogenous humic substances. *Chemosphere*, 52:265-275.

Hashimoto, Y., Matsufuru, H., Takaoka, M., Tanida, H. and Sato, T. (2009). Impacts of chemical amendment and plant growth on lead speciation and enzyme activities in a shooting range soil: an X-ray absorption fine structure investigation. *Journal of Environmental Quality*, 38(4):1420-1428.

Huang, J.W., Chen, J., Berti, W. R. and Cunningham, S.D. (1997). Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology*, 31:800-805.

Ishikawa, S., Noriharu, A.E., Masaharu, M.I. and Tadao, W. (2006). Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination. Possibility of using other plant species for Cd-phytoextraction. *Soil Science and Plant Nutrition*, 52:32.

Jadia, C. D. and Fulekar, M. H. (2009). Phytoremediation of heavy metals: recent techniques. *African Journal of Biotechnology*, 8(6):921-928.

Koppolu, L. and Clements, L.D. (2003). Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part I: Preparation of synthetic hyperaccumulator biomass. *Biomass and Bioenergy*, 24: 69-79.

Labanowski, J., Monna, F. and Bermond A. (2008). Kinetic extractions to assess mobilization of Zn, Pb, Cu, and Cd in a metal-contaminated soil: EDTA vs. citrate. *Environmental Pollution*, 152(3):693-701.

Ling, W., Shen, Q., Gao, Y., Gu, X. and Yang, Z. (2007). Use of bentonite to control the release of copper from contaminated soils. *Australian Journal of Soil Research*, 45(8):618-623.

MacCarthy, P. (2001). The principles of humic substances. *Soil Science*, 166:738-751

Mandal, A., Purakayastha, T. J., Patra A. K. and Sanyal, S. K. (2012). Phytoremediation of Arsenic Contaminated Soil by *Pteris Vittata* L. II. Effect on Arsenic Uptake and Rice Yield. *International Journal of Phytoremediation*, 14(6):621-628.

Martin, T. A. and Ruby, M. V. (2004). Review of in situ remediation technologies for lead, zinc and cadmium in soil. *Remediation*, 14(3):35-53.

McGrath, S.P., Zhao, F.J. and Lombi, E. (2002). Phytoremediation of metals, metalloids, and radionuclides. *Advances in Agronomy*, 75: 1-56.

Meda, A.R., Scheuermann, E.B., Prechsl, U.E., Erenoglu, B., Schaaf, G., Hayen, H., Weber, G. and von Wieren, N. (2007). Iron acquisition by phytosiderophores contributes to cadmium tolerance. *Plant Physiology*, 143:1761-1773.

Mendez, M. O. and Maier, R. M. (2008). Phytostabilization of mine tailings in arid and semiarid environments-an emerging remediation technology. *Environmental Health Perspectives*, 116:278-283.

Panwar, B.S., Kadar, I., Biro, B., Rajkai-Vegh, K., Ragayi, P., Rekasi, M. and Marton, L. (2011). Phytoremediation: enhanced cadmium (cd) accumulation by organic manuring, EDTA and microbial inoculants (*Azotobacter* sp., *Pseudomonas* sp.) in Indian mustard (*Brassica juncea* L.). *Acta Agronomica Hungarica*, 59(2):101-107.

Peng, K.J., Luo, C.L., Chen, Y.H., Wang, G.P., Li, X.D. and Shen, Z.G. (2009). Cadmium and other metal uptake by *Lobelia chinensis* and *Solanum nigrum* from contaminated soils. *Bulletin of Environmental Contamination and Toxicology*, 83:260-264.

Quartacci, M.F., Argilla, A., Baker, A.J.M. and Navari-Izzo, F. (2006). Phytoextraction of metals from a multiply contaminated soil by Indian mustard. *Chemosphere*, 63:918-925.

Ramprakash, Kumari, S., Sangwan, A., Rajpaul and Kumar, S. (2013). Phytoextraction of chromium from contaminated soil by *Brassica juncea* as influenced by chelating agents. *Asian Journal of Chemistry*, 25(10):5357-5359.

Raskin, I. and Ensley, B. D. (2000). Recent developments for in situ treatment of metal contaminated soils. In: *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*. John Wiley & Sons Inc., New York.

Raskin, I., Smith R.D. and Salt, D.E. (1997): Phytoremediation of metals: Using plants to remove pollutants from the environment. *Current Opinion in Biotechnology*, 8(2): 221-226.

Raven, P. H., Berg, L. R. and Johnson, G. B. (1998). *Environment*, Saunders College Publishing, 2nd edition. New York, NY, USA.

Reisinger, S., Schiavon, M., Terry, N. and Pilon-Smits, E.A.H. (2008). Heavy metal tolerance and accumulation in Indian mustard (*Brassica juncea* L.) expressing bacterial gamma-glutamyl cysteine synthetase or glutathione synthetase. *International Journal of Phytoremediation*, 10:440-454.

Rizzi, L., Petruzelli, G., Poggio, G. and VignaGuidi, G. (2004). Soil physical changes and plant availability of Zn and Pb in a treatability test of phytostabilization. *Chemosphere*, 57:1039-1046.

Ross, A., Suther and Filip, M.G. (2003). Tack, Fractionation of Cu, Pb and Zn in certified reference soils SRM 2710 and SRM 2711 using the optimized BCR sequential extraction procedure. *Advances in Environmental Research*, 8:37-50.

Ramprakash, Singh, S.K., Singh, A. (2009). Effect of chelating agents on distribution of lead in soil after harvest of *Brassica juncea*. *Environment and Ecology*, 27 (3):981-984.

Salt, D.E., Blaylock, M., Kumar, P.B.A.N., Dushenkov, V., Ensley, B.D., Chet, I. and Raskin, I. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology*, 13: 468-475.

Salt, D.E., Smith, R.D. and Raskin, I. (1998). Phytoremediation. *Annual Review of Plant*

Physiology and Plant Molecular Biology, 49: 643–668.

Sekara, A., Poniedzialek, M., Ciura, J. and Jedrszczyk, E. (2005). Cadmium and lead accumulation and distribution in the organs of nine crops: Implications for phytoremediation. Polish Journal of Environmental Studies, 14:509–516.

Singh, S.K., Ramprakash, Kumari, S. and Duhan, B.S. (2013). Phytoextraction of Ni from contaminated soil by *Brassica juncea* as influenced by chelating agents. Annals of Biology 29(1):15–18.

Smith, A.H., Lopipero, P.A., Bates, M.N. and Steinmaus, C.M. (2002). Public health arsenic epidemiology and drinking water standards. Science, 296:2145–2146.

Sumner, M. E. (2000). Beneficial use of effluents, wastes, and biosolids, Communications in Soil Science and Plant Analysis, 31(11–14):1701–1715.

Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R. and Nowack, B. (2004). Extraction of heavy metals from soils using biodegradable chelating agents. Environmental Science and Technology, 38:937–944.

Trap, S., Kohler, A., Larsen, L. C., Zambrano, K. C. and Karlson, U. (2005). Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. Journal of Soils and Sediments, 1: 71–76.

United States Environmental Protection Agency (USEPA). (2000). *Introduction to Phytoremediation*. EPA 600/R-99/107, U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

Vamerali, T., Bandiera, M., Coletto, L., Zanetti, F., Dickinson, N.M. and Mosca, G. (2009). Phytoremediation trials on metal and arsenic-contaminated pyrite wastes (Torviscosa, Italy). Environmental Pollution, 157:887–894.

Wang, L. Q., Luo, L., Ma, Y. B., Wei, D. P. and Hua, L. (2009). In situ immobilization remediation of heavy metals-contaminated soils: a review. Chinese Journal of Applied Ecology, 20(5):1214–1222.

Wenger, K., Gupta, S.K., Furrer, G. and Schulin, R. (2003). The role of nitrilotriacetate in copper uptake by tobacco. Journal of Environmental Quality, 32:1669–1676.

Wright, R.T. (2007). Environmental Science: Toward a Sustainable Future. 9th Ed. Prentice Hall of India, New Delhi.

Wuana, R. A., Okieimen, F. E. and Ikyereve, R. E. (2008). Removal of lead and copper from contaminated kaolin and bulk clay soils using acids and chelating agents. Journal of Chemical Society of Nigeria, 33(1):213–219.

Yang, Q., Tu, S., Wang, G., Liao, X. and Yan, X. (2012). Effectiveness of Applying Arsenate Reducing Bacteria to Enhance Arsenic Removal From Polluted Soils by *Pteris Vittata* L. International Journal of Phytoremediation, 14(1): 89–99.

Yoon, J., Cao, X. and Zhou, O. (2006). Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. Science Total Environment, 368:456–464.

