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Review Paper

Hyperaccumulation: A Phytoremediation approach for pollution control

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Abstract

The problem of environmental pollution has achieved unprecedented approach throughout the world. Soil pollution has accelerated drastically since the beginning of industrial revolution, especially the accumulation of heavy metal. Human activities are the main source of heavy metal accumulation in soil on a global scale (eg 5.6-38 x 10^6 kg Cd /yr) and comes mainly from industrial activities like mining, smelting, refining and manufacturing processes. The toxic substances are released into the environment and cause variety of toxic effects on living organism and their food chain. In areas with high anthropogenic pressures, heavy metals such as Cd, Cu, Pd, Cr, Zn and Ni are important environmental pollutants. They are also present in significant amount in sewage sludge and reach the soil where they become part of the lifecycle. This review explores phyto-remediation as a potential remediation strategy which is eco-friendly and low tech alternative to more active and intrusive remedial methods.

Keywords: Phytoremediation, Environmental pollution, Metal toxicity, Heavy metal accumulation.

Introduction

Source of metal pollution: Various sources of metal pollution include the industrial effluents, fuel production, mining, smelting, military operations, and small scale industries such as cable coating, battery production, metal product manufacturing, coal combustion, brick kilns and use of agricultural chemicals[1]. Mercury is a liquid volatile metal used in production of fungicides, paints, cosmetics, paper pulp etc. Lead compounds are added to gasoline as anti knocking agents and are emitted from the automobile exhausts as volatile lead halides. Zinc is mainly released from steel, copper and lead refineries; open hearth furnace emits 20-25 g Zn/hr on refining the galvanized iron scrap. Cd is mainly released from the industries engaged in extraction, refining, electroplating and welding, Cd containing products as well as from pesticide and fertilizer industries. One of the potential sources of soil contamination is the improper disposal of municipal wastes. These wastes are either dumped on the roadside releasing toxic gases or used as landfills, while sewage is used for irrigation purpose. These wastes are also progenitors of carcinogens and many other toxic metals. Water contaminated by sewage and industrial effluents are some other potential sources of heavy metals and adds to the contaminated soil and vegetables[2]. Excessive application of banned pesticides, fungicides and fertilizers also contribute to metal pollution[3].

Implications of Heavy Metal toxicity: Passow[4] defined heavy metals as those metals having specific density greater than five & include about thirty eight elements. They have atomic weights ranging from 63.54 to 200.59. They have variety of roles in the biological system ranging from regulator of biological processes to being important structural components of proteins. However when present in large quantities they are toxic to most plant species[5]. Heavy metals cause toxicity around the world and are a global environmental problem. Mining of precious metals such as coal and other commodities adds to the economy of most countries. Brazil, China, India, Peruand many other developing countries contribute a large proportion of world's mining products. Of the total world production of iron ore, 21% is produced by China, 19% by Brazil and 7% by India. Heavy metals form a heterogeneous group where toxicity varies by metal and concentration based upon their chemical properties and biological function. Hg, Cd, Ni, Pb, Cu, Zn, Cr and Co are highly toxic both as element and soluble salt, when present even in traces they pose a serious environmental threat. Bioaccumulation of heavy metals in food chain has serious implications on health, common route of exposure being through ingestion of both contaminated food and water sources[6]. Soil fertility is drastically reduced by the accumulation of heavy metals due to destruction of its beneficial microbial flora. Toxicity responses may also be defined by a number of physical factors which govern both availability and relative toxicity of metal contaminants such as pH, clay content, organic metal content and nutritional status[7].

Natural and human activities over the year have contributed towards continuous build up of toxic metals in soil and water bodies. Constant anthropogenic release of the toxic metal in the environment has further aggravated the problem. Excessive accumulation of the metal in the soil is equally toxic to the plant and human beings. Unlike organic compounds, metals can not be degraded and their cleanup usually requires removal from the sites. Heavy metal toxicity can damage the cell structure as a result of induced oxidative stress caused by reactive oxygen species and inhibit saveral enzymatic activities in the plants. It also causes indirect toxic effect by replacing the essential nutrients at the cation exchange sites in the plant[8-9]. Cu accounts for several physiological processes in the plants such as phosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production and disease resistance. Cu toxicity results to suppressed root growth and leaf chlorosis in plant[10]. Using cell culture techniques, Cu tolerance traits were identified in cultures derived from mature sycamore trees growing in woodland and subjected to aerial deposition of Cu[11]. Phytotoxic concentrations of Cu and Cd have been reported in surface soil layers. In cell suspension cultures originating from contaminated sites, growth was inhibited at 12 to 15 mg/l Cu, however, this trait was absent in cultures derived from non-tolerant seedlings at the metal contaminated sites. Repeated exposure to high Cu concentration induced this trait in cultures derived from non-contaminated sites. Phenotypic variation induced by environmental stress play a key role in the survival of individual as a result of random mutations, tolerant genotypes evolved that could withstand metal toxicity.

Cadmium is not an essential element for plant growth, metabolism but can be highly phytotoxic leading to even plant death. Cd inhibit the DNA mediated transformation in microorganisms, intereferes in the symbiosis between microbes and plants, effects many enzyme activities and increase plant's predisposition to fungal invasion[12].Cd contamination causes Itai-itai disease in rice. Its accumulation in liver and kidney causes hypertension, emphysema, and cancer in humans. Lead(Pb) is a non-essential element in plants but may become toxic to many organisms even in traces. Pb caused phytotoxic effects such as chlorosis, necrosis, stunted growth of root/shoot and less biomass production in sunflower and tobacco[13]. Intake of the lead contaminated food damages RBC resulting in anemia, infection of liver and kidney in humans.

Quantification of plant metal tolerance

Most of the work on identifying and quantifying tolerance in short lived herbaceous plant species has been based on the use of root growth technique developed[14]. It involves comparative measurements of rate of root growth of the test plants in control and metal containing solutions. Originally developed for grasses but has been applied on plants with diverse range of root morphology such as *Plantagolanceolata, Leucanthemumvulgare, Mimulusguttatus* and *Armeria maritima*[15]. The tolerance index(TI)can be calculated based upon the relative root growth rate measurements as: i. Tolerance Index (%) = Root growth in solution containing metal /Root growth in solution without metal x 100, ii. The use of range in metal concentrations allows the use of regression or probit analysis which is often preferred method for analysis. Though this technique is satisfactory for distinguishing large tolerance differences, it suffers from serious flaw when more subtle differences are being explored[16]. iii. Alternative method includes use of pollen germination and tissue culture techniques. Both pollen tube growth and germination are effected by metal toxicity. The stimulation and inhibition of the pollen characteristics is also determined by the pollen species, pollutant concentration and relative humidity. The response of pollen to pollutants provide parallel expression of tolerance to the parent plant. The parallel expression of metal tolerance in pollen and sporophytes of *silenedioca* and *Mimulusguttatus* has been determined[17]. The effects of toxicity of various metals on red pine (*Pinusresinosa*) pollen germination and germ tube elongation has been studies[18]. The decreasing order of toxicity to pollen germination was Cd>Cu>Hg>Pb>Zn>Ba. Tissue culture technique offers a method for determining indices of metal tolerance representative of the whole plant[19]. Plant tissue culture technique has been widely used to identify range of traits that are often maintained following the regeneration of whole plants[20]. Tissue culture technique has been used for the selection of tolerant plants for salt stress, Aluminium tolerance and heavy metals[21-22]. Cu and Cd tolerance traits have been identified in cell cultures derived from mature sycamore trees subjected to episodic metal pollution from nearby refinery[23].

Table-1: A preview of the advantages and disadvantages of Phytoremediation.

Advantages	Disadvantages
It is amendable to a variety of organic and inorganic compounds.	It is restricted to sites with shallow contamination within rooting zone of the remediative plants.
Its In Situ / Ex Situ application is possible with effluent and soil substrate respectively.	It may take up to several years to remediate a contaminated site.
It decreases the amount of soil disturbance during. *In Situ* applications as compared. To the conventional methods.	It is restricted to the sites with low concentration of contaminants.
It reduces the amount of waste produced (up to 95%) and can be further utilized as bio-ore of heavy metals.	The biomass of the harvested plants from phytoextraction act as a hazardous waste hence should be properly disposed.
In Situ applications of phytoremediation decreases the spread of contaminant via air and water.	Climatic conditions are a limiting Factor
It does not require expensive equipment or highly specialized personnel.	Phytoremidiation involving the introduction of non-native species may affect biodiversity
During the large scale phytoremedial applications, the potential energy stored can be utilized to generate thermal energy.	Consumption and utilization of contaminated plant biomass is a cause of concern.

Remediation measures

Soil remediation can be stated as return of the soil to its ecologically stable conditions together with the plant communities it supports prior to disturbance[24]. To address the growing need for the control of environmental pollution many remediation strategies have been developed to treat the soil leachate, waste water, and ground water contaminated with various pollutants including the in-situ and ex-situ methods[25]. It can be achieved by using biological, physical and chemical technologies in conjunction, to reduce contamination to safe and acceptable level. Conventional remedial techniques such as soil flushing, solidification/stabilization, vertification, thermal desorption, encapsulation can be used for highly contaminated soil but are not applicable to larger areas. Moreover these methods require high energy input and expensive machinery[26], destroy soil structure and reduce soil fertility and thus have negative impacts on the ecosystem[27]. There are generally four major soil remediation methods:

Physical remediation: These include cement kiln, air stripping, thermal desorption and incineration.

Chemical remediation: It consists of oxidation-reduction, precipitation, encapsulation and solvent extraction.

Bioremediation: It includes natural attenuation, land farming, bio-piling, bio augmentation, bioreactor and bioventing as some of the methods for remediation.

Phytoremediation: Derived from Greek word *Phyto* meaning plant and Latin suffix *remedial* which means curing or restoring. *Phytoremediation* is an environmental friendly, safe and cheap technique of using plants (including trees and grasses) to remove, destroy or sequester hazardous contaminants from various media such as air water and soil[27].

All the above mentioned remediation techniques are less amenable to environment extremes than the conventional remediation methods and are also costly[10]. As Heavy metals are not subjected to degradation and bioremediation has limited potential to remediate metal polluted environment. The use of natural materials to remediate the contaminated water and soil has been investigated for the past 35 years. Over the past decades there has been increased interest for development of plant based remediation technologies which are not only less costly, have high impact and are environment friendly, a concept called *Phytoremediation*[28]. This technique basically involves absorption of metal elements from soil by the plant roots and their transportation to the above ground plant parts such as shoot where they tend to accumulate. After sufficient metal accumulation has taken place, above ground plant parts are harvested resulting in permanent removal of metals from site[29].Phytoremediation is a novel approach that offers ecological benefits and is also cost effective. However, it requires defined strategy, expert project designers with field experience that choose the proper species and cultivar for particular metal and region. The plant selected for the technique should have sufficient capacity for metal absorption, its accumulation and strength to decrease the treatment time. Over 400 hyperaccumulator plants have been reported and include members of Brassicaceae, Caryophyllaceae, Cyperaceae, Asteraceae, Fabaceae, Flacourtiaceae, Laminaceae, Poaceae, Violaceae, and Euphorbiaceae[27]. Brassicaceae is an important hyperaccumulator group. These hyperaccumulators are highly metal selective, produce large amount of biomass and can be grown in natural habitats[30].

Table-2: Some of the hyperaccumulator plants suitable for phytoremediation.

Scientific Name	Common Name	Origin & Characteristics	Accumulated Elements
Azollafiliculoides Bacopamonnieri, Eichorniacrassipes	Water fern Water hyssop Water hyacinth	Africa, floating India, emergent species Pantropical/subtropical, troublesome weed	CuA, NiA, PbA, MnA HgA, CuH, CrH, PbA, CdH CdH, CrA, ZnA, HgH, PbH, CuA
Hydrillaverticillata	Hydrilla	Southern Asia, but spreading as a troublesome weed in warmer states of America	CdH, CrA, HgH, PbH
Salviniamolesta	Water fern	India	CrH, NiH, PbH, and ZnA
Brassica juncea	Indian mustard	Cultivated	PbH, PbP, ZnH, NiH, CuH, CrA, CdA, UrA
Helianthus annus	Sunflower	Cultivated	PbH,UrH, SrH, CsH, CrA, CdA, Cua, MnA, NiA and ZnA
Thlaspicaerulescens	Alpine pennycress	Europe	ZnH, CdH, CoH, CuH, NiH and CrA
Lemna minor	Duckweed	Native to north America but widespread	PbH, CdH, CuH and ZnA

* H: Hyper-accumulator, A: Accumulator.

Genetic engineering for improved phytoremediation

Phytoremediation has been applied to number of contaminants including heavy metals, radionuclides, petroleum hydrocarbons, organophosphate insecticides, chlorinated solvents explosives and surfactants[31]. The capacity of metal accumulation and tolerance can be enhanced by over expressing natural or modified genes encoding antioxidant enzymes or those that are involved in the biosynthesis of glutathione and phytochelatins. The development of transgenic poplar with 'Mer A' gene having capacity to volatilize mercury and transgenic mustard with an ability to accumulate selenium and boron[32]. Other processes that can be targeted for improved phytoremediation is the over expression of genes for metal transport membrane protein, metal chelator molecules of various types, metal modifying enzymes involved in the repair of metal damage (e.g oxidative stress) and several regulatory proteins. In addition to the mining of genome sequences in Arabidopsis thalliana, rice, poplar, papaya along with availability of the new genomic techniques, it should lead to the identification of novel genes important for pollution remediation.

Grouping of phytoremediation: Phytoremediation of the contaminants is grouped into four major sub-groups.

Phytoextraction: It utilizes plants to remove the soil contaminants and transport them to the above ground parts. It is the most promising technique for phytoremediation[33]. It offers following significant advantages i. It is applicable to larger areas. ii. It is aesthetically acceptable iii. Plant biomass produced can be converted into raw material for furniture making, fiber production, power generation etc iv. It requires low capital investments and operating cost, v. It is least harmful as its utilizes naturally occuring organisms and preserve the natural state of environment.

Phytovolatilization: It utilises plants to volatilize the soil contaminants into the atmosphere.

Rhizofiltration: It involves the use plant roots for the absorption of contaminants from waste water and aqueous waste streams.

Phytostabilization: It involves the use of plants to reduce the mobility and bio-availability of pollutants in the environment thus preventing their migration to the ground water or food chain.

Phytotransformation: The degradation of the complex organic molecules to simple molecules and their integration into plant tissues.

Phytostimulation: It requires the stimulation of microbial and fungal degradation by release of exudates/enzymes into the root zone.

Dendroremediation: It involves the evaporation of water by use of trees to extract pollutants from soil.

Hydraulic control: It involves the use of plant canopies for regulating the water table and soil field capacity.

Phytoremediation is a natural and eco-friendly technology, is cost effective, diversity enhancing, and involves energy derivation from sunlight besides helps in retaining the fertility of soil[10]. However, this novel technology has certain disadvantages too enlisted below: i. It is limited to the surface area and depth occupied by the root. ii. The survival of the plant is regulated by the toxicity of the contaminated land and general condition of the soil. iii. It requires necessary demand for nutritional material, specific climatic conditions as well as proper soil characteristics to sustain the normal plant growth. iv. Slow growth and low biomass accumulation require a long term commitment. v. There is possibility of transfer of bio-accumulated contaminants into the food chain from the primary level consumer upwards.

Factors regulating phytoremediation efficiency

Increasing the uptake of Heavy metals: Metal hyperaccumulators are the plant species that accumulate 100 fold higher metals than the non-accumulator plant species[34]. Hyper-tolerance, the ability of the plants to tolerate high level of elements in its root and shoot, is the key property that makes hyperaccumulation possible. Such hyperaccumulation results from vacuolar compartmentation and chelation[35]. The most important application of the chelating reagent is related to phytoremediation of less biodegradable heavy metals such as lead[36]. However, the bioavailabilty of heavy metals can also be increased by decreasing the soil pH[37]. There was increase in the accumulation of Cd in transgenic tobacco as pH decreases[38] while the concentration of Ni in *Allyssum lesbiacum* paralleled with the increase in pH. Another approach is to increase the electrode potential (Eh) which increases the availability of heavy metals in the soil solution[39] however, the adjustment of Eh is generally complicated[40].

Decrease in the phytoremediation period: Another approach is to increase the growth of plants which consequently decrease the phytoremediation cycle by providing specific demand of the respective plant species[41] or transfer of the seedlings to field in order to decrease the duration of the phytoremediation cycle. However, this technique has its own limitation as the sowing of the seedlings over a vast area of the contaminated land is quite cumbersome.

Increasing the growth of plants: Plant biomass especially that of the shoot plays an important role in metal removal. This process can be stimulated by any physical method (such as use of fertilizers) physico-chemical (as adgustment of the soil pH) which improves the efficiency of phytoremediation. The increase in biomass and accumulation of arsenic in silverback fern (*Petyrogemma calomelanos*) by application of phosphorus was reported[42]. There was increase in biomass and heavy metal tolerance when Pb, Cd and Zn contaminated soils were treated with fertilizers[43]. However, there was reduction in the accumulation of Zn in soil treated with manure in case of *Solanum nigrum*[44].

Phytoremediation case studies

Phytoextraction: The extraction of metals by plant roots and their translocation to shoot, which are then harvested to remove the contaminants from the soil. It has been reported that the cost involved in phytoremediation would be more than ten times less per hectare relative to the conventional methods of remediation[45]. Phytoextraction coefficient which is the ratio of metal concentration found within the surface biomass of plant over metal concentration in soil is 1.7 for *Brasssica juncea*. Brassica species are known to accumulate excess of 500 ppm of the metals without showing any visual signs of metal toxicity. Chelate assisted extraction involves two basic processes (a) release of the bound metals in the soil solution and their uptake combined with their transport to the harvestable shoot. The efficiency of phytochelation can be increased by using the synthetic chelators having high efficiency of chelation to the metal of interest for eg EDTA for Pb, EGTA for Cd and Citrate for Uranium.

Since roots occupy very small portion of the soil volume majority of the chelate would be far from the uptake site[46]. Though the use of chelate increases the plant uptake and the translocation of the toxic metals to the shoot, it might increase the risk of toxic metal leaching out to the neighbouring sites thus posing the threat of release of the toxic metals into the food chain. At the same time the use of synthetic chelators in the long run may also destroy the soil structure, microbiota, soil fertility and may destabilize the natural ecosystem.

Rhizofiltration: The use of plants both terrestrial and aquatic to absorb, concentrate and precipitate the contaminants from the polluted aqueous sources into their roots[47]. This technique is quite effective for Pb, Cd, Cu, Ni, Zn and Cr which are primarily retained within roots. Kharkanis[48] in his greenhouse experiment to study the remediation of aquatic environment using water hyacinth and pistia, reported that pistia has high capacity for metal uptake (such as Zn, Cr and Cu) than duckweed. Rhizofiltration of Zn and Cu in case of water hyacinth was less relative to pistia. It was reported that the roots of the many hydroponically grown terrestrial plants such as Indian mustard and sunflower effectively remove potentially toxic metals such as Cu, Cr, Cd, Ni, Pb and Zn from aqueous solutions. Some of advantages of this technique are (a) Uses both terrestrial and aquatic plants for in-situ and ex-situ applications. (b) Contaminants need not be transported to shoots. Terrestrial plants are preferred more because of their well developed fibrous root system having larger area for the absorption of the contaminants[49].

Phytovolatilization: It involves uptake and possible transformation of compound by the plant and subsequent release into the atmosphere. Poplar, Alfalfa, Black locust and Indian mustard are some of the plants efficient in phytovolitilization. This process is mainly used for the removal of Hg from the contaminated sites. Meagher[50] in his experiment using Tobacco and Arabidopsis, genetically modified these plants to include mercury reductase gene that can convert the ionic form to its less toxic metallic form and volatilize it. The transformed plants could volitilize ten times more Hg as compared to the non-transformed ones[51]. The advantage of this method is that the mercury ion which is potentially toxic is converted to less harmful elemental form. However, the likelihood of elemental form released into the atmosphere and to be recycled and redeposited back into lakes and oceans thus producing methyl mercury compounds using anaerobic bacteria offers a great disadvantage for this technique.

Phytostabilization: It involves the stabilization of the metal contaminated soil by the plant roots. Phytostabilization can occur through precipitation, sorption, metal valence reduction, and complexation. It is quite effective in the removal of Pb, As, Cd, Cr, Zn and Cu from the contaminated sites. Jadia and Fulekar[10] in a greenhouse experiment studied the potential of Sorghum to remediate heavy metal contaminated site and the vermin-compost was amended in the contaminated site as a natural fertilizer, found that there was increase in the uptake of heavy metals by the root of the plant at all the evaluated concentration of 5, 10, 20, 40 and 50 ppm. The order of uptake of heavy metal was Zn>Cu>Cd>>Ni>Pb. The long surface area of the sorghum roots and their intensive penetration in the soil reduced the leaching due to soil stabilization therefore immobilized and concentrated the heavy metals in the roots. The main advantage of this method is the non requirement of the hazardous material/ biomass disposal[51] and secondly the efficiency of the rapid immobilization needed to preserve surface and ground waters.

Conclusion

The contamination of the soil and water bodies with heavy metals poses a serious threat to the environment as well as health. Metals, their compounds as well as their inorganic forms are the main sources of contamination. The increase in heavy metal pollution has led researchers to focus on the fast, economical, safe and environmental friendly remediation technologies. Phytoremediation is a potential remediation strategy that can be used to decontaminate soils contaminated with inorganic contaminants. It has been perceived as an eco-friendly and low tech alternative to more active and intrusive remedial methods. Based upon various success stories pertaining to studies on phytoremediation in developed countries, researches pertaining to this emerging bioremediation technology should be encouraged and intensified in order to serve as a safe and cheap approach for the pollution control.

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