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REVIEW ARTICLE

PHYTOREMEDIATION OF HEAVY METALS - PROGRESSES AND PERSPECTIVES

[*,1]Manimekalai, D., [1]Dr. Padmavathy, P., [2]Dr. Rani, V., [1]Dr. Aanand, S. and [1]Divya, M.

[1]Department of Fisheries Environment, Fisheries College and Research Institute, Thoothukudi – 628 008
[2]Department of Coastal Aquaculture, Fisheries College and Research Institute, Tharuvaikulam

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ABSTRACT

Heavy metals are metallic elements which have a high atomic weight and a density much greater (at least 5 times) than water. There are more than 20 heavy metals, but four are of particular concern to human health: lead (Pb), cadmium (Cd), mercury (Hg), and inorganic arsenic (As). Phytoremediation, an eco-friendly technology which is both ecologically and economically viable is an attractive alternative to the current clean-up methods that are very expensive. This technology involves efficient use of aquatic plants to remove, detoxify or immobilize heavy metals. It is best applied at sites with shallow contamination of organic, nutrient or metal pollutants that are amenable to one of the five applications; phytotransformation, rhizosphere bioremediation, phytostabilization, phytoextraction and rhizofiltration. Phytoremediation of heavy metals and its effect on plants have been compiled to provide a wide applicability of phytoremediation.

INTRODUCTION

Phytoremediation is a word formed from the Greek prefix "phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore (Cunningham *et al*., 1997). The term actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Ensley, 2000). Research using semi-aquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era (Salt *et al*., 1995a; Timofeev-Resovsky *et al*., 1962). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Baker and Brooks, 1989; Baker *et al*., 1991; Reeves and Brooks, 1983). Chaney (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Comis, 1996; Cunningham *et al*., 1995; Ebbs *et al*., 1997). By definition, a hyperaccumulator must accumulate at least 1000 μg Ag^{-1} of Co, Cu, Cr, Pb, or Ni, or 10,000 μgAg^{-1} (i.e. 1%) of Mn or Zn in the dry matter (Reeves and Baker, 2000; Wantanabe, 1997). Some plants tolerate and accumulate high concentrations of metal in their tissue but not at the level required to be called hyper accumulators. These plants are often called moderate metal-accumulators, or just moderate accumulators (Kumar *et al*., 1995). Phytoremediation has also been called green remediation, botano-remediation, agro remediation and vegetative remediation. The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time (Mudgal *et al.,* 2010).

Corresponding author: Manimekalai, D.
Department of Fisheries Environment, Fisheries College and Research Institute, Thoothukudi – 628 008.

Process of phytoremediation

Phytoextraction

Phytoextraction refers to the ability of plants to remove metals and other compounds from the subsurface and translocate them to the leaves or other plant tissues. The plants may then need to be harvested and removed from the site. Even if the harvested plants must be land filled, the mass disposed of is much smaller than the original mass of contaminated soil (EPA, 2000).

Phytovolatilization

Phytovolatilization also involves contaminants being taken up into the body of the plant, but then the contaminant, a volatile degradation product is transpired with water vapor from leaves (EPA, 2000). Phytovolatilization may also entail the diffusion of contaminants from the stems or other plant parts that the contaminant travels through before reaching the leaves (McCutcheon, 2003).

Phytodegradation

When the phytodegradation mechanism is at work, contaminants are broken down after they have been taken up by the plant. It has been observed to remediate some organic contaminants, such as chlorinated solvents, herbicides and it can address contaminants in soil, sediments, or groundwater (EPA, 2000).

Rhizodegradation

Rhizodegradation refers to the breakdown of contaminants within the plant root zone, or rhizosphere. Rhizodegradation is believed to be carried out by bacteria or other microorganisms whose numbers typically flourish in the rhizosphere (McCutcheon, 2003). Microorganisms may be so prevalent in the rhizosphere because the plant exudes sugars, amino acids, enzymes, and other compounds that can stimulate bacterial growth. The roots also provide additional surface area for microbes to grow on and a pathway for oxygen transfer from the environment.

Rhizofiltration

It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate and contaminants from polluted aqueous sources in their roots (Jadia and Fulekar, 2009). Terrestrial plants are more preferred because they have a fibrous and much longer root system, increasing amount of root area that effectively removed the potentially toxic metals (Nandakumar *et al.,* 1995).

Phytostabilization

Phytostabilization takes advantage of the changes that the presence of the plant induces in soil chemistry and environment. These changes in soil chemistry may induce adsorption of contaminants onto the plant roots or soil or cause metals precipitation onto the plant root. The physical presence of the plants may also reduce contaminant mobility by reducing the potential for water and wind erosion.

Mechanism of phytoremediation of heavy metals

The metal must mobilise into the soil solution, for the plants to accumulate metals from soil. The bioavailability of metals is increased in soil through several means. One way plants achieve it by secreting phytosidophores into the rhizosphere to chelate and solublise metals that are soil bound. Both acidification of the rhizosphere and exudation of carboxylates are considered potential targets for enhancing metal accumulation. Following mobilization, a metal has to be captured by root cells. Metals are first bound by the cell wall. It is an ion exchanger of comparatively low affinity and low selectivity. Transport systems and intracellular high-affinity binding sites then mediate and drive uptake across the plasma membrane. Uptake of metal ions is likely to take place through secondary transporters such as channel proteins and/or H^+ coupled carrier proteins. The membrane potential that is negative on the inside of the plasma membrane and might exceed –200 mV in root epidermal cells provides a strong driving force for the uptake of cations through secondary transporters. Once inside the plant, most metals are too insoluble to move freely in the vascular system, so they usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intra cellular) compartments (Raskin *et al.,* 1997). Unless the metal ion is transported as a non-cationic metal chelate, apoplastic transport is further limited by the high cation exchange capacity of cell walls (Raskin *et al.,* 1997). The apoplast continum of the root epidermis and cortex ias readily permeable for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow and diffuse without having to cross a membrane. The cell walls of the endodermal cell layer act as a barrier for apoplastic diffusion into the vascular system. In general, solutes have to be taken up into the root symplasm before they can enter the xylem (Tester and Leigh, 2001). Subsequent to metal uptake into the root symplasm, three processes govern the movement of metals from the root into the xylem. Sequestration of metals inside root cells, symplastic transport into the stele and release into the xylem. The transport of ions into the xylem is generally a tightly controlled process mediated by membrane transport proteins. Symplastic transport of heavy metals probably takes place in the xylem after they cross the casparian strip. It is more regulated due to the selectively permeable plasma membrane of the cells that control access to the symplast by specific or generic metal ion carriers or channels (Gaymard, 1998). Symplastic transport requires that metal ions move across the plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane). This membrane potential provides a strong electrochemical gradient for the inward movement of metal ions. Most metal ions enter plant cells by an energy dependent saturable process via specific or generic metal ion carriers or channels (Bubb and Lester, 1991). The vacuole is an important component of the metal ion storage where they are often chelated either by organic acid or phytochelatins. Insoluble precipitates may form under certain conditions. Precipitation compartmentalisation and chelating are the most likely major events that take place in resisting the damaging effects of metals (Cunningham *et al.,* 1995). Transporters mediate uptake into the symplast, and distribution within the leaf occurs via the apoplast or the symplast (Karley *et al.,* 2000). Plants transpire water to move nutrients from the soil solution to leaves and stems, where photosynthesis occurs. Willows, hybrid poplar are also good phytoremediators, because they take up and process large volumes of soil water.

Phytoremediation of Heavy Metals in Soil

Heavy metal contamination of soil is still an unsolved problem. Heavy metal compounds in soil are very hazardous pollutants for the following reasons:

- Non-biodegradable,
- Extremely toxic at low concentrations, and
- Chances of mobilization under changing physical-chemical conditions.

Selection of a remediation technique for a site contaminated with metals is complex, time consuming and site specific. Some factors that influence selection of a suitable procedure are size, location and history of site, accessibility to the site, effectiveness of treatment options, soil and contaminant characteristics, availability of technical and financial resources, and degree of contamination (McIntyre, 2003). Phytoremediation is an emerging technology which can be effectively used for the remediation of metal contaminated sites. The bioavailability of metals to plants is affected by different factors such as soil and plant characteristics, and various environmental factors. The main soil characteristics include pH, presence of hydrous oxides of iron and manganese, organic matter content, clay content, phosphate content, redox potential, soil particle size (surface area of soil particles), and cation exchange capacity. Climatic conditions, irrigation, and soil fertilizing practices are examples of environmental factors. The species of plant, character of plant tissue, and age of vegetation also affect metal uptake (McIntyre, 2003).

The metal uptake by a plant is depends on the concentration of soluble and bio available fraction of metals in the soil solution. The bioavailable fraction of metal in the soil can be determined by the Potential Bioavailable Sequential Extraction (PBASE) procedure (Basta and Gradwohl, 2000). Even though chemical extraction won't extract metal from the soil in a manner identical to that of a plant root system, it can be used as a reliable method for assessing the bioavailability of metals bound to soil particles (Basta and Gradwohl, 2000). In a polluted soil, the concentration of bioavailable pollutants tends to reduce over time due to physical, chemical and biological processes. Because of this reason, aged soils are more difficult to phytoremediate (Pilon-Smits, 2005). It is known that to enhance metal solubility, plants either excrete organic ligands or lower the soil pH in the rhizosphere. To improve metal solubility in the soil solution, synthetic chelates such as ethylene diamine tetra acetic acid (EDTA), nitrilotriacetic acid (NTA), pyridine-2-6- dicarboxylic acid (PDA), citric acid, nitric acid, hydrochloric acid and fluorosilicic acid can be used in phytoremediation studies (Romkens *et al*., 2002). The addition of excess chelating agents may increase the chances of leaching the metals from the soil to groundwater (Romkens *et al*., 2002). If the metal concentration in the soil is near to the phytotoxic levels, then addition of lime or organic matter reduces the metal solubility (Pilon-Smits, 2005).

Phytoremediation of toxic Metals

A major disadvantage of phytoremediation is that high concentrations of heavy metals or certain combinations of heavy metals may adversely affects plant growth and biomass production by disrupting the physiology and morphology of plants. Some plant species have the ability to grow and develop in metalliferous (metal rich soils) soils such as near to mining sites. Such plants can be utilized to clean up heavy metal polluted sites. General effects of various metals in plant are (Gardea-Torresdey *et al*., 2005):

Cadmium is used to decreases seed germination, lipid content and plant growth, but induce the production of phytochelatins. Phytochelatin is a metal binding peptide and has an important role in cadmium detoxification in plants. Chromium Causes decrease in enzyme activity and plant growth, and produces membrane damage, chlorosis and root damage. Copper can be disrupts photosynthesis, plant growth and reproductive processes, and decreases thylakoid surface area. Mercury helps to accumulate phenol, but decreases the photosynthetic-activity, water uptake and antioxidant enzymes. Nickel is used to reduce seed germination, protein production, chlorophyll and enzyme production, and accumulation of dry mass, but increases the amount of free amino acids. Lead reduces chlorophyll production and plant growth, but increases superoxide dismutase (metal containing antioxidant enzyme). Zinc reduces nickel toxicity and seed germination, but increases plant growth and ATP/chlorophyll ratio at moderate concentrations (Gardea-Torresdey *et al.*, 2005).

Remediation Measures

Soil remediation is defined by Allen (1988) as the return of soil to a condition of ecological stability together with the establishment of plant communities it supported to conditions prior to disturbance. Conventional technologies involve the removal of metals from polluted soils by transportation to laboratories, soil washing with chemicals to remove metals, and finally replacing the soil at its original location or disposing of it as hazardous waste (Francis *et al*., 1999). This decontamination strategy is an ex situ approach and can be very expensive and damaging to the soil structure and ecology (Salt *et al*., 1995a). Immobilization of heavy metals through the addition of lime (Krebs *et al*., 1999),phosphate (Ebbs *et al*., 1998) and calcium carbonate (CaCO3) (Chen *et al*., 2000) have been suggested as remediation techniques. These remediation technologies have the advantage of immediately reducing the risk factors arising from metal contamination, but may only be considered temporary alternatives because the metals have not been removed from the soil environment. In response to a growing need to address environmental contamination, many remediation technologies have been developed to treat soil, leachate, wastewater, and ground-water contaminated by various pollutants, including in situ and ex situ methods (Aboulroos *et al*., 2006). A particular contaminated site may require a combination of procedures to allow the optimum remediation for the prevailing conditions. Biological, physical, and chemical technologies may be used in conjunction with one another to reduce the contamination to a safe and acceptable level. Conventional methods to remediate metal-contaminated soils (soil flushing, solidification/ stabilization, vitrification, thermal desorption, encapsulation) (Bio-Wise, 2003) can be used at highly contaminated sites but are not applicable to large areas. These remediation methods require high energy input and expensive machinery (Schnoor, 1997). At the same time they destroy soil structure and decrease soil productivity (Leumann *et al*., 1995).

Conclusion

Phytoremediation is a relatively new technology that offers clear advantages over traditional methods for site cleanup. Research related to this relatively new technology needs to be promoted and emphasized and expanded in developing countries since it is low cost. In situ, solar driven technology makes use of vascular plants to accumulate and translocate metals from roots to shoots. Harvesting the plant shoots can permanently remove these contaminants from the soil. Phytoremediation does not have the destructive impact on soil fertility and structure that some more vigorous conventional technologies have such as acid extraction and soil washing.

This technology can be applied "in situ" to remediate shallow soil, ground water and surface water bodies. Also, phytoremediation has been perceived to be a more environmentally-friendly "green" and low tech alternative to more active and intrusive remedial methods.

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