International Research Journal of Environment Sciences ISSN 2319–1414
Vol. **2(1),** 74-78, January **(2013)** *Int. Res. J. Environment Sci.*

Mycorrhizae and Phytochelators as Remedy in Heavy Metal Contaminated Land Remediation

Hasan Saba, Prakash Jyoti and Singh Neha
Amity Institute of Biotechnology, Amity University, Viraj Khand-5, Gomtinagar, Lucknow, UP, INDIA

Available online at: www.isca.in
Received 05th November 2012, revised 2012, accepted 2012

Abstract

Phytoremediation is the direct use of living plants for in situ remediation of contaminated soil, sludges, sediments, and ground water through contaminant removal, degradation, or containment. Growing and, in some cases, harvesting plants on a contaminated site as a remediation method is an aesthetically pleasing, solar-energy driven, passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination. This technique can be used along with or, in some cases, in place of mechanical cleanup methods. Phytoremediation can be used to clean up metals, pesticides, solvents, explosives, crude oil, polycyclic aromatic hydrocarbons, and landfill leachates. This sustainable and inexpensive process is emerging as a viable alternative to traditional contaminated land remediation methods. To enhance phytoremediation as a viable strategy, fast growing plants with high metal uptake ability and rapid biomass gain are needed. This paper provides a brief review of studies in the area of phytoaccumulation, most of which have been carried out in U.P. Particular attention is given to the role of phytochelators in making the heavy metals bio-available to the plant and their symbionts in enhancing the uptake of bio-available heavy metals.

Keywords: Phytoextraction, phytostabilization, phytochelators, remediation.

Introduction

Soil contains numerous components and metals are one of them. Plants require different metals as micronutrients, these are part of soil. Biosphere has become polluted due to toxic metals and the same continues anabated at an accelerated speed, thanks to industrial revolution. Air, water and soil all are getting polluted in various ways and forms. Speciation in soils is determined by sequential extraction using specific extract ants, which solubilise different phases of metals[1]. The physical and chemical characteristics of soil determine the speciation and mobility of heavy metals[2].

Human activities such as mining and smelting of metals, electroplating, gas exhaust, energy and fuel production, fertilizer, sewage and pesticide application, municipal waste generation, etc.[3] have led to metal pollution become one of the most severe environmental problems today. Excessive accumulation of heavy metals is toxic to most plants. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth[4,5]. Contamination of heavy metals in water and soil poses a major environmental and human health hazard on the other excessive metal concentrations in contaminated soil results in decreased, soil microbial activity and soil fertility leadig to yield losses[6]. Cadmium, as a non-essential, toxic heavy metal to plants, which may well demonstrate the problem, can inhibit root and shoot growth, affect nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops[7]. Thus, when Cd-enriched crop products are consumed by animals and humans, it can cause diseases. On condition that soil Cd pollution is cumulative with levels increasing over time, the soil may eventually become unusable for crop production. Similarly, contamination of soil with Cd can negatively affect biodiversity and the activity of soil microbial communities[8].

Remediation Technologies

Heavy metals cannot be destroyed biologically (no “degradation”, change in the nuclear structure of the element, occurs) but are only transformed from one oxidation state or organic complex to another[9], remediation of heavy metal contamination in soils is more difficult. Until now, methods used for their remediation such as excavation and land fill, thermal treatment, acid leaching and electro reclamation are not suitable for practical applications, because of their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the contaminants of concern, soil properties, site conditions, and so on. Thus, the development of phytoremediation strategies for heavy metals contaminated soils is necessary since it is a less expensive clean up technology[10,11,12].

Phytoremediation is a process that uses plants to remove, transfer, stabilize, and destroy contaminants in soil and sediment. The mechanisms of phytoremediation include enhanced rhizosphere biodegradation, phyto-extraction (also called phyto-accumulation), phyto-degradation, and phyto-stabilization.

Enhanced Rhizosphere Biodegradation

Enhanced rhizosphere biodegradation takes place in the soil immediately surrounding plant roots. Natural substances released by plant roots supply nutrients to microorganisms, which enhances their biological activities. Plant roots also loosen the soil and then die, leaving paths for transport of water and aeration. This process tends to pull water to the surface zone and dry the lower saturated zones. The most commonly used flora in phytoremediation projects are poplar trees, primarily because the trees are fast growing and can survive in a broad range of climates. In addition, poplar trees can draw large amounts of water (relative to other plant species) as it passes through soil or directly from an aquifer. This may draw greater amounts of dissolved pollutants from contaminated media and reduce the amount of water that may pass through soil or an aquifer, thereby reducing the amount of contaminant flushed though or out of the soil or aquifer.

Phyto-Accumulation: Phyto-accumulation is the uptake of contaminants by plant roots and the translocation/accumulation (phytoextraction) of contaminants into plant shoots and leaves.

Phyto-Degradation: Phyto-degradation is the metabolism of contaminants within plant tissues. Plants produce enzymes, such as dehalogenase and oxygenase that help catalyze degradation. Investigations are proceeding to determine if both aromatic and chlorinated aliphatic compounds are amenable to phyto-degradation.

Phyto-Stabilization: Phyto-stabilization is the phenomenon of production of chemical compounds by plant to immobilize contaminants at the interface of roots and soil.

Advantages and Limitations of Phytoremediation

Advantages of Phytoremediation include the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body[13]. Phytoremediation avoids dramatic landscape disruption, and preserves the ecosystem.

There are certain limitations to phytoremediation system. Among them are being time-consuming method, the amount of produced biomass, the root depth, soil chemistry and the level of contamination, the age of plant, the contaminant concentration, the impacts of contaminated vegetation, and climatic condition. Phytoremediation can be a time-consuming process, and it may take at least several growing seasons to clean up a site. The intermediates formed from those organic and inorganic contaminants may be cytotoxic to plants[14]. Phytoremediation is also limited by the growth rate of the plants. More time may be required to phytoremediate a site as compared with other more traditional cleanup technologies. Excavation and disposal or incineration takes weeks to months to accomplish, while phytoextraction or degradation may need several years. Therefore, for sites that pose acute risks for human and other ecological receptors, phytoremediation may not be the remediation technique of choice[15]. Phytoremediation might be best suited for remote areas where human contact is limited or where soil contamination does not require an immediate response[16].

Role of Arbuscular Mycorrhizae

Arbuscular mycorrhizal fungi (AMF) are one of the important endophytic fungi living in the roots of most terrestrial plants. This symbiosis confers benefits directly to the host plant’s growth and development through the acquisition of phosphorous and other mineral nutrients from the soil by the fungus. In addition, they may also enhance the plant's resistance to biotic and abiotic stresses[17]. Potential roles of AMF associations have repeatedly been demonstrated to alleviate metal stress of plants[18]. Besides AMF, there are other beneficial microorganisms that may contribute to the plants, tolerance to HM-contamination. Plant growth-promoting rhizobacteria (PGPR) improves plant growth through several mechanisms, such as increased nutrient uptake, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances, phytohormone production and nitrogen fixation. Although PGPR was first used for promoting the plant growth, much attention has recently been paid on the application of PGPR to remediate contaminated soils in association with plants[19,20,21]. Nowadays, it has been shown that improvement of the interactions between beneficial rhizosphere microorganisms and plants can significantly lower the stress placed on plants by the presence of HMs, increase the availability of metal for plant uptake and subsequently are considered to be an important tool for phytoremediation technology[22,23]. For example, AMF could enhance uptake of nutrient elements as well as water by host plants through their extraradical mycelial networks and protecting the host plants against HM toxicity[24]. This AMF-induced plant nutrient uptake is of more importance in alkaline and/or calcareous soils of arid- semiarid regions in which the bioavailability of P and most of the cationic micronutrients is limited. Calcareous soils have also lower water holding capacity due to the presence of carbonates[25]. Furthermore, Khodaverdiloo and Homaee[26] and Davari et al.[27] reported a significant reduction in plant transpiration with an increase in soil HM concentration. It has been suggested that heavy metals, such as Cd, can affect root hydraulic conductivity by multiple mechanisms operating on the apoplastic and/or the symplastic pathway[28]. Recently, the ability of microorganisms to improve the growth of plants including canola and tomato seedlings treated with toxic concentrations of As, Cd, Ni, Pb, Se and Zn has been demonstrated[29,30,31,32].

It is hypothesized that AM fungi were instrumental in the colonization of land by ancient plants[33]. This hypothesis is supported by observation that AM can now be found worldwide in the angiosperms, gymnosperms as well as ferns, suggesting that the nature of the association is ancestral. Furthermore, the origin of AM fungi coincides with that of vascular plants suggesting the nature of the association and supporting the hypothesis that AMF were instrumental in the colonization of land by ancient plants. There have been few analytical studies of AM in polluted soils. While some workers observed that the external mycelium of AMF was the main site for trace element localization[34,35], others reported selective exclusion of toxic and non-toxic elements by adsorption onto chitinous cell walls[36], or onto extra-cellular glycoprotein, glomalin[37], or intra-cellular precipitation. All these mechanisms have implications in reducing a plant's exposure to potentially toxic elements, i.e. mycorrhizoremediation technology. Gonzalez-Chavez et al. (2002)[38] studied the form and localization of Cu accumulation in the extra-radical mycelium of three AM fungi isolated from the same polluted soil contaminated with Cu and As. The authors reported differential capacity of AMF to sorb and accumulate Cu as determined by TEM and SEM. However, the nature of accumulation and mechanisms involved require further studies in order to better understand the participation of AMF in plant tolerance and its ecological significance in polluted soils.

Role of Phytochelators

The unique superfamily of thiol-containing metal binding proteins called metallothioneins (MT) are known to modulate internal levels of metal concentrations between deficient and toxic levels by binding toxic metals through closely spaced cystein thiol groups. These polypeptides have been given the name phytochelators. Various researchers in the past two decades have provided evidence to show that plants, algae and certain fungi also produce MT, which differs from the classical MT first discovered by Margoshes and Vallee[39]. Glutathione (GSH) is the most abundant cellular thiol-rich heavy metal-binding peptide (PC) in plants, animals and fungi[40]. The role of PCs in metal detoxification has largely been studied using Cd and plant cell suspension cultures. Cd-tolerant cells bound most of the cellular Cd as Cd-binding complexes; little binding of Cd occurred in non-tolerant cells, which grew poorly and subsequently died[41]. Formation of Cd-binding complexes allowed the Cd-tolerant cells to survive excess Cd due to lower contents of the free metal in the cells, allowing undisturbed metabolism. Sequestration of heavy metals by PCs confers protection for heavy metal sensitive enzymes. Keltjens and Vanbeusiche[42] tested the use of PCs as biomarkers and concluded that PCs seem to be a useful early warning system for heavy metals stress in plants. Plants, depending on their species and genotype, differ in their efficiency in acquisition and utilization of nutrients[43]. Some plants release phytosidophores (PS) under Zn or Fe deficiencies[44,45,46] which mobilizes Mn, Zn and Cu in the rhizosphere, uptake of which is also enhanced[47,48]. Some plants are able to tolerate an excess of heavy metals by involving processes like sequestration in the cell vacuole with organic acids and complexation with metal detoxifying peptides induced on their exposure to heavy metals[49].

Conclusion

Phytoremediation has emerged as a biobased low cost alternative technology employed for cleaning up contaminated soils. The technology is being tested and future of the technique is still being shaped up as it is in research and development phase. The technology even today faces certain technical barriers which need to be addressed. These areoptimization of the process, greater understanding the absorption capacities of plants, translocation and metabolisation of heavy metals, identification of genes responsible for uptake and/or degradation of contaminant, decreasing the length of time needed for phytoremediation to work, disposing biomass so produced and protecting wild life form feeding on plants used for remediation. Since contaminant uptake and tolerance depend on both the variety of plant and factors affecting soil which includes soil microbes, information on microbial interactions such as nitrogen fixing bacteria and the ubiquitous mycorrhizal fungi is another quest in the success of technology. Contribution of mycorrhizal, actinorrhizal and rhizobial symbionts to soil productivity and enhanced heavy metal uptake have not yet been seriously considered as a part of study which are other areas which could influence the technique. In addition to optimizing metal bioavailability, it is recommended to introduce actinorrhizal, mycorrhizal and rhizobial plants as soil improvers to rehabilitate polluted sites by optimizing the uptake of bioavailable metals due to modification of the root/rhizosphere systems. The arbuscular mycorrhizal fungi (AMF) are universal and ubiquitous rhizosphere microflora forming symbiosis with plant roots and acting as biofertilizers, bioprotactants, and biodegraders. In addition to AMF, soils also contain various antagonistic and beneficial bacteria such as root pathogens, plant growth promoting rhizobacteria including free-living and symbiotic N-fixers, and mycorrhiza helping bacteria. Potential role bacteria in phytoremediation of heavy metal (HM) contaminated soils and water is being identified and becoming evident although there is need to completely understand the ecological complexities of the plant-microbe-soil interactions as well as their better exploitation as consortia in remediation strategies employed for contaminated soils. Multitrophic root microbial associations deserve multi-disciplinary investigations using molecular, biochemical, and physiological techniques. Ecosystem restoration of heavy metal contaminated soil practices need to incorporate microbial bio- technology research and development.

References

1. Shuman L.M., Fractionation method for soil micro-elements, *Soil Science,* **140(1)**, 11 **(1985)**

2. Kabata-Pendias A. and Pendias H., Trace Elements in Soils and Plants, *2nd Ed. CRC Press, Boca Raton, FL* **(1992)**

3. Kabata-Pendias A. and Pendias H., Trace Elements in the Soil and Plants, *CRC Press, Boca Raton, FL* **(1989)**

4. Bingham F.T., Pereyea F.J. and Jarrell W.M., Metal toxicity to agricultural crops, *Metal Ions Biol. Syst*., **20,** 119 **(1986)**

5. Foy C.D., Chaney R.L. and White M.C., The physiology of metal toxicity in plants, *Annu. Rev. Plant Physiol***. 29(1)**, 511 **(1978)** [doi:10.1146/annurev.pp.29.060178.00 2455]

6. McGrath S.P., Chaudri A.M and Giller K.E., Long-term effects of metals in sewage sluge on soils, microorganisms and plants. *J. Ind. Microbiol.,* **14(2)**, 94 **(1995)** [doi:10.1007/BF01569890]

7. Sanità di Toppi L. and Gabrielli R., Response to cadmium in higher plants. *Environ. Exp. Bot.,* **41(2)**, 105 **(1999)** [doi:10.1016/S0098-8472(98)00058-6]

8. McGrath S.P., Effects of Heavy Metals from Sewage Sludge on Soil Microbes in Agricultural Ecosystems, In: Ross*, S.M. (Ed.), Toxic Metals in Soil-Plant Systems*. Wiley, New York, 247-273 **(1994)**

9. Garbisu C. and Alkorta I., Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment, *Bioresour, Technol*. 77(3), 229 **(2001)** [doi:10.1016/S0960-8524(00)00108-5]

10. Chaney R.L., BrownLi S.L., Y.M., Angle J.S., Stuczynski T.I., Daniel W.L., Henry C.L., Siebelec G., Malik M. and J.A. Ryan M., et al.,. Progress in Risk Assessment for Soil Metals, and In-situ Remediation and Phytoextraction of Metals from Hazardous Contaminated Soils, US-EPA *Phytoremediation: State of Science, 2000 May 1*, Boston, MA **(2000)**

11. Cheng S., Grosse W., Karrenbrock F. and Thoennessen M., Efficiency of constructed wetlands in decontamination of water polluted by heavy metals, *Ecol. Eng.,* **18(3),** 317 **(2002)** [doi:10.1016/S0925-8574(01)00091-X]

12. Lasat H.A., Phytoextraction of toxic metals: a review of biological mechanisms, *J. Environ. Qual.,* **31(1)**, 109 **(2002)**

13. Hinchman R.R., Negri M.C. and Gatliff E.G., “Phytoremediation: using green plants to clean up contaminated soil, groundwater, and wastewater,” Argonne National Laboratory Hinchman, Applied Natural Sciences, Inc **(1995)** http://www. treemediation. Com /Technical/Phytoremediation_1998.pdf

14. Mwegoha W.J.S., The use of phytoremediation technology for abatement soil and groundwater pollution in Tanzania: opportunities and challenges, *J. of Sust. Dev. in Africa,* **10(1)**, 140 **(2008)**

15. U.S. Environmental Protection Agency, Introduction to Phytoremediation, National Risk Management Research Laboratory, EPA/600/R-99/107, **(2000)**, http://www.clu-in.org/download/remed/introphyto.pdf.

16. Salido A.L., Hasty K.L., Lim J.M. and Butcher D.J. Phytoremediation of arsenic and lead in contaminated soil using Chinese Brake ferns (Pteris vittata) and Indian mustard (Brassica juncea*), Int. J. of Phytorem.*, **5(2)**, 89 **(2003)**

17. Harrier L.A. and Sawczak J. Detection of the 3phosphoglycerate kinase protein of Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe, *Mycorrhiza,* **10,** 81 **(2000)**

18. Hildebrandt U., Regvar M. and Bothe H., Arbuscular mycorrhiza and heavy metal tolerance, *Phytochem,* **68**, 139 **(2007)**

19. Huang X.D., El-Alawi Y., Penrose D.M., Glick B.R. and Greenberg B.M., Responses of Three Grass Species to Creosote During Phytoremediation, *Environ. Pollut*, **130**, 453 **(2004)**

20. Huang X.D., El-Alawi Y., Gurska J., Glick B.R. and Greenberg B.M., A Multi-Process Phytoremediation System for Decontamination of Persistent Total Petroleum Hydrocarbons from Soils, *Microchem. J*., **81**, 139 **(2005)**

21. Narasimhan K., Basheer C., Bajic V.B. and Swarup S., Enhancement of plant-microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls, *Plant Physiol*, **132,** 146 **(2003)**

22. Glick B.R., Phytoremediation: synergisticuse of plants and bacteria to clean up the environment, *Biotechnol. Adv*., **21**, 383 **(2003)**

23. Glick B.R., Using soil bacteria to facilitate phytoremediation, *Biotechnol. Adv*., **28**, 367 **(2010)**

24. Leyval C., Turnau K. and Haselwandter K., Effect of heavy metal pollution on mycorrhizal colonization and function: Physiological, ecological and applied aspects, *Mycorrhiza,* 7, 139 **(1997)**

25. Khodaverdiloo H. and Homaee M., Modeling phytoremediation of Cd and Pb from contaminated soils using plant transpiration reduction functions, *Iranian J. Irrig. Drain*., **2(1),** 7 **(2008)**

26. Khodaverdiloo H. and Homaee M., Modeling of Cadmium and Lead Phytoextraction from Contaminated Soils, *Soil Sci*., **41 (2)**, 149 **(2008)**

27. Davari M., Homaee M. and Khodaverdiloo H., Modeling Phytoremediation of Ni and Cd from Contaminated Soils Using Macroscopic Transpiration Reduction Functions, *J. Sci. Technol. Agric. Natural. Resour. Water Soil Sci*., **14(52)**, 75 **(2010)**

28. Shah F.R., Ahmad N., Masood K.R., Peralta-Videa J.R. and Ahmad F.D., Heavy Metal Toxicity in Plants. In: *Plant Adaptation and Phytoremediation. (M. Ashraf · M. Ozturk · M.S.A. Ahmad, Eds.) Springer Dordrecht Heidelberg* London New York. 71 **(2010)**

29. Burd G.I., Dixon D.G. and Glick B.R., Plant growth-promotingbacteria that decrease heavy metal toxicity in plants, *Can. J. Microbiol*., **46**, 237 **(2000)**

30. Burd G.I., Dixonand D.G. and Glick B.R., A plant growth-promoting bacterium that decreases nickel toxicity in seedlings, *Appl. J. Environ Microbiol,* **64**, 3663 **(1998)**

31. Belimov A.A., Hontzeas N., Safronova V.I., Demchinskaya S.V., Piluzza G, Bullitta S. and Glick B.R., Cadmium-tolerant plant growth- promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.), *Soil Biol. Biochem,* **37**, 241**(2005)**

32. Stearns J.C., Shah S., Greenberg B.M., Dixon D.G. and Glick B.R., Tolerance of transgenic canola expressing 1-aminocyclopropane-1-carboxylic acid deaminase to growth inhibition by nickel, *Plant Physiol. Biochem*, **43**, 701 **(2005)**

33. Simon L.K., Bousquet J., Levesque R.C. and Lalonde M., Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants, *Nature*, **363(6424),** 67 **(1993)** [doi:10.1038/363067a0]

34. Kaldorf M., Kuhn M., Schroder W.H., Hildebrandt U. and Bothe H., Selective element deposits in maize colonized by a heavy metal tolerance conferring arbus-cular mycorrhizal fungus, *J. Plant Physiol*, **154**, 195 **(1999)**

35. Turnau K., Heavy metal content and localization in mycorrhizal *Euphorbia cyparissias* from zinc wastes in Southern Polland, *Act. Soc. Bot. Pol*., **67**, 105 **(1998)**

36. Zhou J.L., Zn biosorption by *Rhizopus arrhizus* and other fungi. *App. Microbiol. Biotechnol,* **51(5),** 686 **(1999)** [doi:10.1007/s002530051453]

37. Wright S.F. and Upadhyaya A., A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil,* **198(1)**, 97 **(1998)** [doi:10.1023/A:1004347701584]

38. Gonzalez-Chavez C., D'Haen J., Vangronsveld J.J. and Dodd J.C., Copper sorption and accumulation by the extraradical mycelium of different Glomus spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil, *Plant and Soil*, **240(2),** 287 **(2002)** [doi:10.1023/A:10157946 22592]

39. Margoshes M. and Vallee B. L., *J. Am. Chem. Soc*., **79**, 4813 **(1957)**

40. Singh R.P., Tripathi R.D., Sinha S.K., Meheshwari R. and Srivastava H.S., Responses of higher plants to lead contaminated environment, *Chemosphere,* **34**, 2467 **(1997)**

41. Klapheck S., Fliegner W. and Zimmer I., Hydroxymethyl-phytochelatins [(c-glutamylcysteine)(N)-serine] are metal induced peptides in the Poaceae, *Plant Physiol,* **104,** 1325 **(1994)**

42. Keltjnes W.G. and Vanbeusichem M.L., Phytochelatins as biomarkers for heavy metal toxicity in maize – single metal effects of copper and cadmium, *J. Plant Nutri,* **21**, 635 **(1998)**

43. Baird C., Environmental Chemistry. W.H. Freeman. New York **(1997)**

44. Marschner H., Mineral Nutrition of Higher Plants (2nd Edn), Academic Press, London **(1995)**

45. Walter A., Romheld V., Marschner H. and Mori S., Is the release of phytosiderophores in zinc-deficient water plants a response to impaired iron utilization, *Physiol. Pl*., **92**, 493 **(1994)**

46. Marschner H. and Romheld V., Strategies of plants for acquisition of iron, *Plant Soil,* **165**, 262 **(1995)**

47. Zhang F.S., Romheld V. and Marschner H., Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron-deficient wheat, *Plant Nutri,* **37**, 671 **(1991)**

48. Graham M.J., Nickell C.D., and Hoeft R.G., Effect of manganese deficiency on seed yield of soybean cultivars, *J. Plant Nutri,* **17**, 1333 **(1994)**

49. Rauser W.E., Phytochelatins, *Ann. Rev. Biochem*, **59**, 61 **(1990)**