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Phytoremediation of Heavy Metal Toxicity and Role of soil in Rhizobacteria

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Abstract- Our surrounding is filled up with a large number of toxicants in different forms. They contaminate our water, land and atmosphere where we live. Heavy metal pollution of soil is a significant environmental problem and has its negative impact on human health and agriculture. Rhizosphere, as an important interface of soil and plant, plays a significant role in phytoremediation of contaminated soil by heavy metals, in which, microbial populations are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes. Phytoremediation of toxic heavy metals could be carried out by using specific metallophytes. Green plants are the lungs of nature with unique ability to purifying impure air by photosynthesis and remove or minimize heavy metals toxicity from soil and water ecosystem by absorption, accumulation and biotransformation process. This article paper reviews some recent advances in effect and significance of rhizobacteria in phytoremediation of heavy metal toxicity in contaminated soils. There is also a need to improve our understanding of the mechanisms involved in the transfer and mobilization of heavy metals by rhizobacteria and to conduct research on the selection of microbial isolates from Rhizosphere of plants growing metal contaminated soils for specific restoration programmes.

***Index Terms*-** Environmental, heavy metal toxicity, Rhizobacteria, Phytoremediation, Rhizosphere, Metalophytes, Metal accumulation.

I. INTRODUCTION

A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leach ate and contribute towards contamination of our ecosystem. A wide range of inorganic and organic compounds cause contamination, these include heavy metals, combustible and putriscible substances, hazardous wastes, explosives and petroleum products. Major component of inorganic contaminates are heavy metals they present a different problem than organic contaminants. Soil microorganisms can degrade organic contaminants, while metals need immobilization or physical removal. Although many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of free radicals. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function [3]. Thus, metals render the land unsuitable for plant growth and destroy the biodiversity. Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as captions, ligand specificity, etc.) and an atomic number >20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Ni. Metals are natural components in soil with a number of heavy metals being required by plants as micronutrients. However, pollution of biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution. As a result of 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. (Kabata-Pendias and Pendias, 1989), metal pollution has become one of the most severe environmental problems today. Human eagerness to perform better and better with respect to production of food, energy and convenience product in order to ameliorate. The ways of living are the causes of chemicals especially heavy metal pollution. This eagerness led to a tremendous growth in production and use of various chemicals like Arsenic, Chromium, Cadmium, Cupper, Mercury etc. are common toxic heavy metal. Some heavy metals in trace amount are essential for normal growth in both animal and plant but it’s become harmful if taken in excess Metal and metalloid occur in variety of form as ions, compound and complexes in the environment over use of these metals and metal based heavy industrialization resulted metal pollution in the biosphere. Mercury, Cadmium, Chromium, Lead and Nickels are chief toxic metal. These metals caused much severe toxic and deleterious effect on human health by producing various kinds of metabolic problems and even chronic diseases. In plant they also reduced productivity due to lose in there chlorophyll and protein contents. Exposure of algae Chlorella, Botryococus, Dunaliella, Nostoc and anabaena to elevated concentration of all these test metal exerted toxic effect on metabolic machinery. Phytoremediation of toxic heavy metals could be carried out by using specific metallophytes. Green plants are the lungs of nature with unique ability to purifying impure air by photosynthesis and remove or minimize heavy metals toxicity from soil and water ecosystem by absorption, accumulation and biotransformation process. Vascular plant absorb toxicant either directly from the atmosphere through the leaves or from soil or water through roots. Studies on aquatic plants have shown that uptake of heavy metal is mostly initiated by a phase of rapid and passive absorption. In Elodea and Eichornia the uptake of water born toxicants by the stem and leaves where more important than the absorption by the root. The metal tolerant plants including algae protect themselves from metal toxicity by producing extra amount of some secondary metabolites & Remediation technology.

II. MATERIALS AND METHODS

As mentioned above, rhziobacteria secretion may play a major role among mechanisms of phytoremediation assisted by rhziobacteria. Indirect mechanisms include preventing phytopathogens from inhibiting plant growth and development while direct mechanisms include: nitrogen fixation; synthesis of siderophores which can solubilize and sequester iron from the soil; production of phytohormones such as auxins and cytokinins, which can enhance plant growth; and solubilization of minerals such as phosphorus (Kloepper et al., 1989; Glick, 1995; Glick et al., 1999; Patten and Glick, 1996). Rhizobacteria produce metal-chelating agents called siderophores, which have an important role in the acquisition of several heavy metals (Leong, 1986). Microbial siderophores are used as iron chelating agents that can regulate the availability of iron in the plant rhizosphere (Bar-Ness et al., 1992; Loper and Henkels, 1999). It has been assumed that competition for iron in the rhizosphere is controlled by the affinity of the siderophore for iron and ultimately decides the rhizosphere population structure. The important factors, which participate, are concentration of various types of siderophore, kinetics of exchange, and availability of Fe-complexes to microbes as well as plants (Loper and Henkels, 1999). Interestingly, the binding affinity of phytosiderophores for iron is less than the affinity of microbial siderophores, but plants require a lower iron concentration for normal growth than do microbes (Meyer, 2000). Metal contaminated soil can be remediated by chemical, physical and biological techniques. These can be grouped into two categories.

- **Ex-situ method-**

It requires removal of contaminated soil for treatment on or of site, and returning the treated soil to the resorted site. The conventional ex-situ methods applied for remediating the polluted soils relies on excavation, detoxification and/or destruction of contaminant physically or chemically, as a result the contaminant undergo stabilisation, solidification, immobilisation, incineration or destruction.

- **In-situ method-**

It is remediation without excavation of contaminated site. Reed et al. defined in-situ remediation technologies as destruction or transformation of the contaminant, immobilization to reduce bioavailability and separation of the contaminant from the bulk soil. In-situ techniques are favored over the ex-situ techniques due to their low cost and reduced impact on the ecosystem. Conventionally, the ex-situ technique is to excavate soil contaminated with heavy metal and their burial in landfill site. But the offsite burial is not an appropriate option because it merely shifts the contamination problem elsewhere and also because of hazards associated with the transport of contaminated soil. Diluting the heavy metal content to safe level by importing the clean soil and mixing with the contaminated soil can be an alternative of on-site management. On-site containment and barriers provide an alternative; it involves covering the soil with inert material. Immobilization of inorganic contaminant can be used as a remedial method for heavy metal contaminated soils. This can be achieved by completing the contaminants, or through increasing the soil pH by liming. Increased pH decreases the solubility of heavy metals like Cd, Cu, Ni and Zn in soil. Although the risk of potential exposure to plants is reduced, their concentration remains unchanged. Most of these conventional remediation technologies are costly to implement and cause further disturbance to the already damaged environment. Plant based bioremediation technologies have been collectively termed as phytoremediation; this refers to the use of green plants and their associated micro biota for the in-situ treatment of contaminated soil and ground water. The idea of using metal accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years. The generic term 'Phytoremediation' consists of the Greek prefix photo (plant), attached to the Latin root remedium (to correct or remove an evil). This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) and the air. The physico-chemical techniques for soil remediation render the land useless for plant growth as they remove all biological activities, including useful microbes such as nitrogen fixing bacteria, mycorrhiza, fungi, as well as fauna in the process of decontamination. The conventional methods of remediation may cost from 10 to 1000 per cubic meter. Phytoextraction costs are estimated to be as low as 0.05 per cubic meter. Phytoremediation consists of five main processes, shown in Table 1.

Table-1. Phytoremediation includes the following processes and mechanisms of contaminant.

NO.	**Process**	**Mechanism**	**Contaminant**
1	. Rhizofiltration	Rhizosphere accumulation	Organics/Inorganics
2	Phytostabilisation	Complexation	Inorganics
3	Phytoextraction	Phytoextraction Hyper-accumulation	Inorganics
4	Phytovolatilization	Volatilisation by leaves	Organics/Inorganics
5	Phytotransformation	Degradation in plant	Organics

Rhizobacteria have been shown to possess several traits that can alter heavy metals bioavailability (Lasat, 2002; McGrath et al., 2001; Whiting et al., 2001) through the release of chelating substances, acidification of the microenvironment, and by influencing changes in redox potential (Smith and Read, 1997). For example, Abou-Shanab et al.(2003a) reported that the addition of Sphingomonas macrogoltabidus, Micro bacterium liquefactions', and Micro bacterium arabinogalactanolyticum to Alyssum murals grown in serpentine soil significantly increased the plant uptake of Ni when compared with the un-inoculated controls as a result of soil pH reduction. However, heavy metals are known to be toxic to plants and most organisms when present in soils in excessive concentrations. Giller et al. (1998) reported that there was a detrimental effect to soil microbial diversity and microbial activities (indexes of microbial metabolism and of soil fertility) in metal-polluted environment. The specificity of the plant-bacteria interaction is dependent upon soil conditions, which can alter contaminant bioavailability, root exudates composition, and nutrient levels. In addition, the metabolic requirements for heavy metals remediation may also dictate the form of the plant-bacteria interaction i.e., specific or nonspecific. Along with metal toxicity, there are often additional factors that limit plant growth in contaminated soils including arid conditions, lack of soil structure, low water supply and nutrient deficiency.

- **Role of Rhizobacteria on Phyoremediation-**

Rhizosphere microorganisms, which are closely associated with roots, have been termed plant growth promoting rhizobacteria (PGPR) (Glick, 1995). Plant growth-promoting rhizobacteria include a diverse group of free-living soil bacteria that can improve host plant growth and development in heavy metal contaminated soils by mitigating toxic effects of heavy metals on the plants (Belimov et al., 2004). Table-2 Bioavailability of toxic heavy metals Soil rhizobateria can also directly influence metal solubility by changing heavy metal speciation in the rhizosphere. Study of the roles of mycorrhiza in metal speciation in the rhizosphere and the impact on increasing host plant tolerance against excessive heavy metals in soil showed that speciations of Cu, Zn and Pb changed significantly in the rhizosphere of AM (arbuscular mycorrhiza) infected and non-infected maize in comparison to bulk soil; The greatest change was exchangeable Cu that increased by 26% and 43% in non-infected and AM-infected rhizosphere, respectively, than in bulk soil. With the exception of organic bound Cu in AM, other speciations were stable in the rhizosphere of AM and non-AM treatments. It is understandable that Cu was activated by inducing rhizobacteria (Huang et al., 2005). The organic bound Zn and Pb increased significantly in the rhizosphere in comparison to those in the bulked soil. In contrast, carbonate and Fe-Mn oxides of Zn and Pb did not exhibit significant changes. The results might indicate that mycorrhiza could protect its host plants from the phytotoxicity of excessive copper, zinc and lead by changing the speciation from bioavailable to the non-bioavailable form. The fact that copper and zinc accumulation in the roots and shoots of mycorrhiza infected plants were significantly lower than those in the non-infected plants might also suggest that mycorrhiza efficiently restricted excessive copper and zinc absorptions into the host plants (Huang et al., 2005).

- **Rhizofiltration-**

It is defined as the use of plants, both terrestrial and aquatic; to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots. Rhizofiltration can partially treat industrial discharge, agricultural runoff, or acid mine drainage. It can be used for lead, cadmium, copper, nickel, zinc and chromium, which are primarily retained within the roots. The advantages of rhizofiltration include it ability to be used as in-situ or ex-situ applications and species other than hyper accumulators can also be used. Plants like sunflower, Indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove lead from effluent, with sunflower having the greatest ability. Indian mustard has proven to be effective in removing a wide concentration range of lead (4 – 500 mg/l). The technology has been tested in the field with uranium (U) contaminated water at concentrations of 21-874 μg/l; the treated U concentration reported by Dushenkov was < 20 μg/l before discharge into the environment.

- **Future of Phytoremediation-**

One of the key aspects to the acceptance of phytoextraction pertains to the measurement of its performance, ultimate utilization of by-products and its overall economic viability. To date, commercial phytoextraction has been constrained by the expectation that site remediation should be achieved in a time comparable to other clean-up technologies. So far, most of experiments have taken place in the lab scale, where plants grown in hydroponic setting are fed heavy metal diets. While these results are promising, scientists are ready to admit that solution culture is quite different from that of soil. In real soil, many metals are tied up in insoluble forms, and they are less available and that is the biggest problem, said Kochian. The future of phytoremediation is still in research and development phase, and there are many technical barriers which need to be addressed. Both agronomic management practices and plant genetic abilities need to be optimised to develop commercially useful practices. Many hyper accumulator plants remain to be discovered, and there is a need to know more about their physiology. Optimisation of the process, proper understanding of plant heavy metal uptake and proper disposal of biomass produced is still needed. One of the key aspects to the acceptance of phytoextraction pertains to the measurement of its performance, ultimate utilization of by-products and its overall economic viability. To date, commercial phytoextraction has been constrained by the expectation that site remediation should be achieved in a time comparable to other clean-up technologies. So far, most of the phytoremediation experiments have taken place in the lab scale, where plants grown in hydroponic setting are fed heavy metal diets. While these results are promising, scientists are ready to admit that solution culture is quite different from that of soil. In real soil, many metals are tied up in insoluble forms, and they are less available and that is the biggest problem, said Kochian. The future of phytoremediation is still in research and development phase, and there are many technical barriers which need to be addressed. Both agronomic management practices and plant genetic abilities need to be optimised to develop commercially useful practices. Many hyper accumulator plants remain to be discovered, and there is a need to know more about their physiology. Optimisation of the process, proper understanding of plant heavy metal uptake and proper disposal of biomass produced is still needed.

- **Effect Heavy Metals toxicity in soil-**

Heavy metals are elements having atomic weight between 63.54 and 200.59, and a specific gravity greater than. Trace amount of some heavy metals are required by living organisms, however any excess amount of these metals can be detrimental to the organisms. Nonessential Heavy metals include arsenic, antimony, cadmium, chromium, mercury, lead, etc; these metals are of particular concern to surface water and soil pollution. Stimulation of transport protein. Bacterial survival and proliferation in the environment as well as within various hosts are critically dependent on the uptake and sequestration of transition metals such as manganese, zinc, and iron. For example, cells may stringently regulate intracellular zinc levels, since high concentrations of zinc are toxic to cellular functions and have evolved several types of proteins involved in binding and transport of zinc (Claverys, 2001). Bacteria may also stimulate the sulfate transport protein, located in the root plasma membrane, which also transports selenate (Leggett and Epstein, 1956). Inorganic Hg uptake in higher plants has not been well investigated, but has been linked to the passive uptake of lipophilic chloride complexes in phytoplankton (Mason et al., 1996). Heavy metal toxicity consists of five main processes, shown in Table 2.

Table-2. Heavy metal toxicities include the following sources and Effect of contaminant.

S.N.	Metal	Sources	Effects
1	Arsenic	Industrial dusts, Polluted water.	Perforation of nasal septum respiratory cancer, peripheral neuropathy, dermatoses, skin cancer,
2	Cadmium	Industrial dusts & fumes & Polluted water & food.	Glucosuria, Osteomalacia, aminoaciduria and emphysemia.
3	Chromium	Polluted water & food, Industrial dusts.	Ulcer and respiratory cancer.
4	Lead	Industrial dusts & fumes & Polluted food.	Anemia, Peripheral, neuropathy.
5	Mercury	Industrial fumes, vapour, polluted water & food.	Chronic rhinitis and sinusitis, respiratory cancer, dermatitis.

III. CONCLUSION

Phytoremediation is a fast developing field, since last ten years lot of field application were initiated all over the world, it includes Phytoremediation of Organic, Inorganic and Radionuclide's. This sustainable and inexpensive process is fast emerging as a viable alternative to conventional remediation methods, and will be most suitable for a developing country like India. Most of the studies have been done in developed countries and knowledge of suitable plants is particularly limited in India. When evaluating the effect of rhizobacteria on phytoremediation in contaminated soil, regardless of the precise effects used by the bacterium to protect plants, the results from literature suggest that certain bacteria may eventually find a use in the development of phytoremediation strategies. In this regard, heavy metals may be removed from polluted soil either by increasing the metal-accumulating ability of plants or by increasing the amount of plant biomass. In heavily contaminated soil where the metal content exceeds the limit of plant tolerance, it may be possible to treat plants with plant growth-promoting rhizobacteria, increasing plant biomass and thereby stabilizing, revegetating, and remediating metal-polluted soils. However, there are many areas of poor understanding or lack of information where more research is needed. They include:-

1. Little has been done to investigate the microorganism induced changes in the rhizosphere of hyper accumulator plants in relation to metal accumulation. Similarly, it is difficult to clarify specific features of microbial-plant and microorganism-soil interactions in the rhizosphere.
2. Further research is also needed to quantify the effect of rhizospheric processes induced by rhizobacteria on the phytoavailability of heavy metals.
3. Minimal work has been done to examine heavy metal speciation changes in the rhizosphere and to determine whether such changes could have altered the accumulation and distribution of heavy metals.
4. Rhizobacteria encounter soil solution before it enters the root and the sequestration of heavy metals by rhizobacteria from soil solution may play an important part in plant metals uptake. The role played by bacteria from soil solution in plant Cd uptake is still poorly understood.
5. Finally, we need to further understand the mechanisms involved in mobilization and transfer of metals in order to develop future strategies and optimize the phytoextraction process. Such knowledge may enable us to understand the role and mechanism of soil rhizobacteria on phytoremediation.

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