

## **Review: Plant Microbe-Interaction in Heavy Metal Contaminated Soils**

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### **Abstract**

**Heavy metals are the most important inorganic pollutants, which are not degraded and progressively accumulate in the environment. The use of plants for rehabilitation of heavy-metal-contaminated soils is an emerging area of interest, because it provides an ecologically and environmentally sound and safe method for restoration and remediation. Although a number of plant species are capable of hyper-accumulation of heavy metals, however, this approach is not applicable for remediating sites with multiple contaminants. The biogeochemical capacities of microorganisms seem almost limitless and they can adsorb and accumulate metals in their cells and are being used in microbial leaching and also as agents of cleaning the environment.**

**Keywords:** Bioremediation, Heavy metals, Plant growth-promoting-rhizobacteria, Plant microbe-interaction, Rhizosphere

### **Introduction**

Heavy metal pollution affects the production and quality of crops, the quality of atmosphere and water bodies and thus threatens the human and animal health (Narula et al. 2011). The metal species commonly found in the soils as a result of human activities include, copper (Cu), zinc (Zn), nickel (Ni), lead (Pb), cadmium (Cd), cobalt (Co), mercury (Hg), chromium (Cr) and arsenic (As) etc. Some of these acts as micronutrients at small concentrations for living organisms for their normal physiological activities, but their accumulation are toxic to most life forms (Khan et al. 2005). The most common human activities resulting in entry of heavy metal into land are disposal of industrial effluents, disposal of waste such as sewage sludge, atmospheric deposition from industrial activities, mining activities, domestic and industrial wastes, land fill operations and use of agrochemicals. Release of heavy metals from various industrial sources, agrochemicals and sewage sludge present a major threat to the soil environment. Generally, heavy metals are not degraded biologically and persist in the environment indefinitely (Walker et al. 2003).

The toxic heavy metals inversely affect the microbial compositions, including plant growth promoting rhizobacteria (PGPR) in the rhizosphere, and their metabolic activities. In addition, the elevated

concentration of metals in soils and their uptake by plants adversely affect the growth, symbiosis and consequently the yields of crops (Wani et al. 2008) by disintegrating cell organelles, and disrupting the membranes, acting as genotoxic substance disrupting the physiological process, such as photosynthesis (Wani et al. 2007) or by inactivating the respiration, protein synthesis and carbohydrate metabolism (Shakolnik 1984). The remediation of metal-contaminated soils thus becomes important, as these are rendered unsuitable for sustainable agriculture. This review provides a short overview on plant-microbe interactions towards phyto- and bio-remediation.

### **Phytoremediation:**

Phytoremediation approach involves the cultivation of metal accumulating higher plants to remove contaminants from metal polluted soils (Brooks 1998). In this approach, plants capable of accumulating high levels of metals are grown in contaminated soils. At development metal enriched above ground biomass is harvested and soil metal contamination is detached. Successful plant-based decontamination of even moderately contaminated soils would have need of crops able to concentrate metals in excess of 1-2%. Accumulation of such high levels of heavy metals is highly toxic and would certainly kill the common non accumulator plant. However, in hyper-accumulator species, such concentrations are attainable. Nevertheless, the extent of metal removal is ultimately limited by the plants ability to extract and tolerate only a

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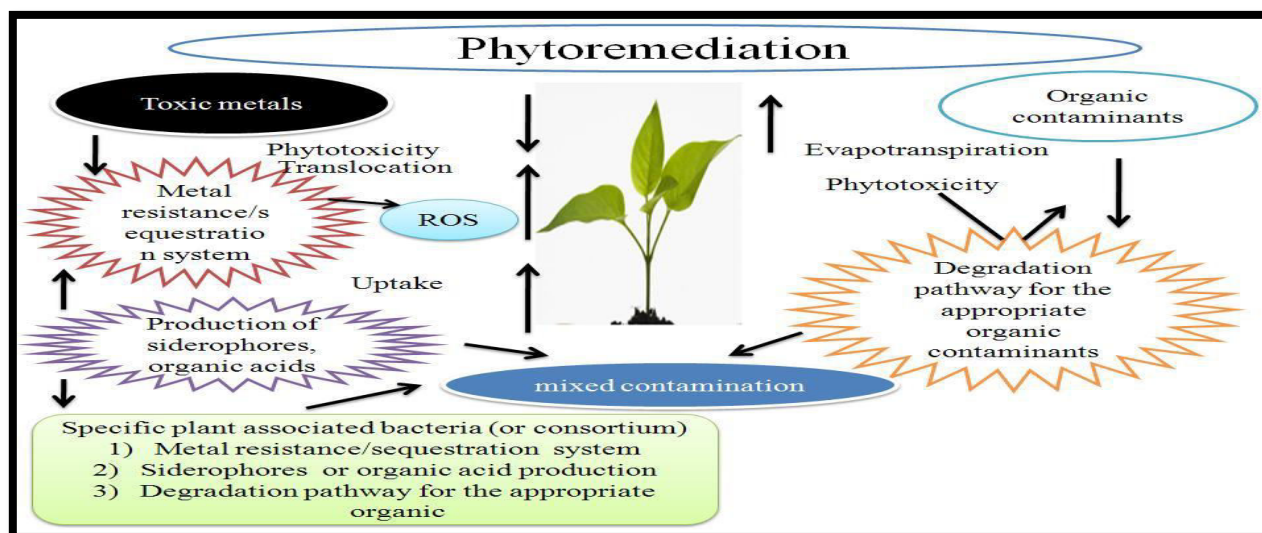
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finite amount of metals. On a dry weight basis, this threshold is around 3% for Zn and Ni, and considerably less for more toxic metals, such as Cd and Pb. The other biological parameter which limits the potential for metal phytoextraction is biomass production. With highly productive species, the potential for biomass production is about 100 tons fresh weight/hectare. The values of these parameters limit the annual removal potential to a maximum of 400 kg metal/ha/yr. It should be mentioned, however, that most metal hyper-accumulators are slow growing and produce little biomass. These characteristics severely limit the use of

hyper-accumulator plants for environment cleanup. Practices have been residential to increase the potential of common non accumulator plants for Pb phytoextraction. Particularly, the uptake-inducing properties of synthetic chelates open the possibility of using high biomass-producing crops for Pb phytoextraction. Under chelate-induced conditions, maize (Huang and Cunningham, 1996) and Indian mustard (Blaylock et al., 1997) have been successfully used to remove Pb from solution culture and contaminated soil, respectively.



**Figure 1.** Mechanism of phytoremediation process

Physical characteristics of soil contamination are also important for the selection of remediating plants. For example, for the remediation of surface-contaminated soils, shallow rooted species would be appropriate to use, whereas deep-rooted plants would be the choice for more deep contamination (Fig. 1). The identification of metal hyperaccumulators, plants capable of accumulating high metal levels, demonstrate that some plants have genetic potential to clean up metal contaminated soils. In general the concentration of metals in hyperaccumulators are about 10-100 fold higher than most other plants growing on metal contaminated soils. It has been possible through bioengineering to develop plants (Raskin 1996)

capable of removing methyl mercury from the contaminated soil. To detoxify this compound, such bioengineered plants express modified bacterial genes *merB* and *merA* which convert methyl mercury to elemental mercury. About 400 plant species have been identified as hyper-accumulator accumulators. The Indian mustard plant (*Brassica juncea*) can extract both heavy metals and radionuclides from soil. Panwar et al. (2002) reported that *B. juncea* has the potential to be hyper-accumulator of Ni. Survey of literature reveals that the rate of metal removal depends upon the plant species, soil contaminating heavy metal(s) and biomass harvested and metal concentration in harvested biomass as summarized in Table 1.

**Table 1.** Plants capable of hyperaccumulating metals

Plant species	Metal accumulated	Accumulated concentration mg/kg dry matter
<i>Thlaspi caerulescens</i> (Brassicaceae)	Zn	10,000
<i>Seberattia acuminata</i> (Sapotaceae)	Ni	10,000
<i>Alyssum lesbiacum</i> (Brassicaceae)	Ni	20,000
<i>Arbidopsis halleri</i> (Brassicaceae)	Cd	1,000
<i>Thlaspi rotundifolium</i>	Pb	8,200
<i>Astralagus</i> sp.(leguminosae)	Se	1,000
<i>Pteris vittata</i> (Fern)	As	22,630

### Rhizosphere

Rhizosphere is the zone of soil surrounding a plant root where in biology and chemistry of the soil is influenced by the roots, rather it is an area of intense activity (biological, chemical and physical) influenced by compounds exuded by roots and by micro-organisms feeding on these compounds (Kumar et al. 2007). Generally, soil contains bacterial numbers in the range of  $10^7$  to  $10^{10}$  cells per gm dry soil. But microbiological activity in the rhizosphere is much greater ( $10^8$ ) than in soil away from plant roots ( $10^5$ ) and also microorganisms provide or make available nutrients for the plants (Walker et al. 2003). Many of these microbes live there as a part of a distinct community surrounding plant roots. Heterotrophic bacteria are able to use organic compounds excreted in root exudates, whereas their metabolites can be used by other microbes, which in the end creates a network of closely associated microorganisms. This phenomenon of highly active micro-organisms in root-associated soil is known as the "rhizosphere effect". Thus microbial population is one of the essential parts of the rhizosphere that affect the rhizosphere soil by its various activities such as water and nutrient uptake, exudation, and all the biological transformations. Among the fast growing and early colonizing bacteria attracted by the plant exudates are members of genera *Bacillus* and *Pseudomonas* besides N fixing bacteria *Azospirillum* and *Rhizobium*. Cultivation based methods show that the *Pseudomonas* spp are generally more abundant in rhizosphere than in the bulk soil. But number of related clones to *Pseudomonas* is higher in rhizosphere of ryegrass and white clover. On the basis of 16s rRNA gene clones, plant roots have further been shown to have a selective effect towards r-proteobacteria leading to majority of *Pseudomonas* spp in rhizosphere.as compared to bulk soil. Free living and associative diazotrophs are more abundant in the rhizosphere than

in bulk soils, indicating their dependence on organic compounds exuded by roots in the rhizosphere. Various nitrogen fixing micro-organisms have been found to be present in the rhizosphere of agricultural plants, but the contribution of fixed nitrogen to plant nutrition is controversial (Lima et al. 2006). Diazotrophs found in the soil or associated with roots include *Azotobacter chroococcum*, *Azospirillum brasilense* and *Gluconacetobacter diazotrophicus* (formerly *Acetobacter diazotrophicus*) and the positive responses of plants to inoculation with these bacteria are attributed to nitrogen ( $N_2$ ) fixation besides, several other factors like phytohormone and/or ammonium production, etc. (Okon 1985). Plant-associated rhizobacteria and mycorrhizae may significantly increase the bioavailability of various heavy metal ions for their uptake by plants. Also, they are known to catalyze redox transformations leading to changes in heavy-metal bioavailability (Yang et al. 2005). Plant association with diazotrophs or any colonizer indicate a high degree of adaptation between the host plant and the most abundant diazotrophs. The micro habitat provided by the host plant seems to generate a selection pressure in favour of the micro-organisms, which in turn best benefit the host. Nutrients and metals are typically present in the soil solution at low concentrations and tend to form sparingly soluble minerals (except nitrogen, sulfur, and boron), or may be adsorbed to a solid phase through ion exchange, hydrogen bonding, or complexation (White 2003). The extent to which they are transferred from the soil to the biota (i.e., microbes or plants) is dependent on the biogeochemical interactions (N, P, S) and/or processes among the soil, plant roots, and microorganisms in the rhizosphere (Abbot and Murphy 2003). At this interface, the presence of root exudates may influence chemical reaction kinetics within the soil environment and subsequently affect biological activities. The exudates act as messengers that stimulate biological and

physical interactions between root and soil organisms. In addition to the adsorption and conduction, roots also produce hormones and other substances that help to regulate the plants development and structure, help in modifying the biochemical and physical properties of the rhizosphere (Abbot and Murphy 2003) and contribute to root growth and plant survival.

### PGPR (Plant growth promoting rhizobacteria)

Plant growth promoting rhizobacteria (PGPR) are capable of promoting plant growth by colonizing and establishing around the plant root (Narula et al. 2006). PGPRs have been found to play a potential role in developing sustainable systems in crop production (Shoebitz et al. 2009). Soil bacteria have been used as biofertilizer for ameliorating the soil fertility and enhancing crop production for decades. The main functions of these bacteria are (1) to supply nutrients to

crops; (2) to stimulate plant growth, e.g., through the production of plant hormones; (3) to control or inhibit the activity of plant pathogens; (4) to improve soil structure; and (5) to act as bio-accumulator in microbial leaching of inorganics (Ehrlich 1990). More recently, bacteria have also been used in soil for the mineralization of organic pollutants, i.e. bioremediation of polluted soils (Zaidi et al. 2008). Generally, PGPR function in three different ways (Glick 1995, 2001): a) synthesizing particular compounds for the plants (Dobbelaere et al. 2003), b) facilitating the uptake of certain nutrients from the soil (Çakmakçı et al. 2006) and c) lessening or preventing the plants from diseases. Besides their role in protecting the plants from metal toxicity, the plant growth promoting rhizobacteria are also known for their role in enhancing the soil fertility and promoting crop productivity by providing essential nutrients (Zaidi and Khan 2006) and plant growth regulators (Kumar et al. 2007).

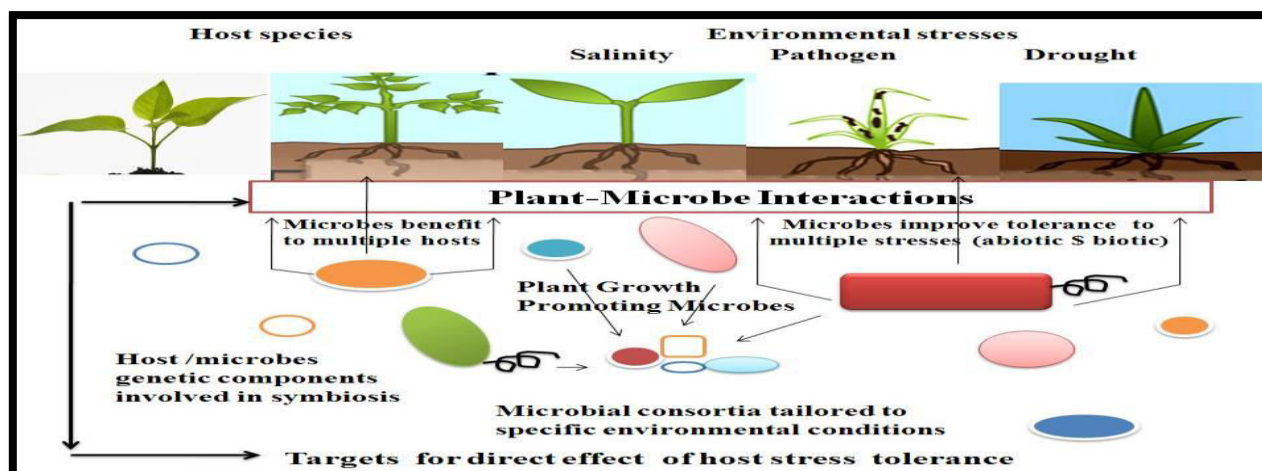


Figure 2. Mechanism of plant-microbe interaction in environmental stresses

### Plant-microbe Interactions in the Rhizosphere

An understanding of the mechanisms, which is important for the initiation and establishment of the association between host and bacterium, can be reached from the analyses of influences exerted by each interaction partner on the other. In addition, there is a need to know how diazotrophs may benefit the plant. Investigations on the production of phytohormones and the action of siderophores produced by *Azotobacter* strains might help to understand this aspect of interactions (Narula et al. 2006). Plant-microbe interactions are important for both the partners i.e., macro as higher plants and micro partners as the plant-associated bacteria (Somers et al. 2004). Microbial partners can induce antagonistic (in

case of phyto pathogens) or symbiotic interactions. Different types of interactions involving plants roots in the rhizosphere have been reviewed by Bais et al. (2006). These include root-root, root-insect, and root-microbe interactions. The rhizosphere represents a highly dynamic front to study interaction between roots and pathogenic as well as beneficial soil microbes, invertebrates, and root systems of competitors (Bais et al. 2006). In recent years several plant scientists have recognized the importance of root exudates in mediating these biological interactions. However, because plant roots are always hidden below ground, many of the interesting phenomena, their attractions, love and hate relationship in which they are involved have remained largely unnoticed. Especially the role of chemical signals (Peters et al. 1986) in mediating

below ground interactions is only beginning to be understood. Chemical signalling between plant roots and other soil organisms, including the roots of neighbouring plants, is often based on chemicals exuded from the roots. The same chemical signals may elicit dissimilar responses from different recipients. Most importantly, chemical components of root exudates may be the one to deter one organism while attracting another, or two very different organisms may be attracted with differing consequences to the plant (Fig. 2).

Therefore, the mechanisms used by roots to communicate and interpret these signals which they receive from other roots and soil microbes in the rhizosphere are largely unknown. The rhizosphere has some positive or negative and neutral associations. Much has to be done still to determine and elucidate whether the chemical signature of a plant root exudates will be perceived as a negative or a positive signal. However, evidences over the years suggest that root exudates is the determining factor to identify interactions in the rhizosphere and, ultimately, plant and soil community dynamics (Narula et al. 2009).

### Conclusion

Microorganisms advantageous and biotechnologically beneficial when occurring in the rhizosphere of metal-tolerant plants (special hyperaccumulator plants), thereby facilitating phytoremediation processes. Interaction of metal ions with biological matter is essential as well as important for various biological processes for all organisms and in related fields (biogeochemistry, bioremediation and phytoremediation, biomining, biotechnology of metal extraction, sorption and recovery, etc.). It can hardly be over emphasized that lack of understanding of molecular mechanisms underlying the effects of soil microorganisms and plant-root exudates on the state of metal compounds in the rhizosphere is a serious impediment in use of phyto-bioremediation technology for cleaning soils contaminated with heavy metals. It can be overcome by widely using a variety of modern powerful physicochemical techniques in environmental and life sciences. On the other hand, knowing the mechanisms and routes of metal transformations may open ways for a variety of practical applications.

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