Rhizoremediation: A Pragmatic Approach for Remediation of Heavy Metal-Contaminated Soil

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Abstract

Soil pollution is the primary source that transmits pollutants like heavy metals from environment to living organisms. From soil, plants adsorb and accumulate heavy metals. Through the food chain, heavy metals enter the animal kingdom including humans and cause health risks. Few physicochemical and phytoremediation approaches have been proved effective in removing heavy metals from contaminated soils. However, soil characteristics and recycling of soil constituents have made their practicability questionable. One pragmatic way to reduce the deleterious effect of heavy metals in soil is rhizoremediation, in which plant–microbe interaction is explored for remediation purposes. In this strategy, the plant growth-promoting rhizobacteria (PGPR) either accumulate or detoxify the heavy metals and thereby prevent the uptake and accumulation of heavy metals in plants. In addition, PGPRs act as biofertilizer that enhance the crop yields in different ecological niches. In this chapter, rhizoremediation strategy is described and portrayed as the pragmatic way for remediation of heavy metals in soil.

9.1 Introduction

Heavy metal, the poorly defined term, is the subset of 40 elements including transition metals, metalloids, lanthanides, and actinides (Appenroth 2010) that have specific density of more than 5 g/cm^3. They possess metallic characteristics such as ductility, conductivity, stability as cations, ligand specificity, etc. Though the term heavy metal is announced as a meaningless term by IUPAC, it is widely

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used in biology (Duffus 2002). Biologically, they are classified as essential and nonessential heavy metals. Some of the heavy metals like cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo) are essential elements whose role in metabolism is known in both prokaryotes and eukaryotes. In contrast, no nutritional function is known for heavy metals like silver (Ag), cadmium (Cd), arsenic (As), lead (Pb), mercury (Hg), and uranium (U) (Appenroth 2010). While in case of chromium (Cr), the role of Cr^{3+} ions in sugar and lipid metabolism is known, but the role of Cr^{6+} ions is unknown (Vincent 2000).

The discharges of heavy metals from natural and anthropogenic activities cause the accumulation of metals into the environment. Natural input includes withering and erosion of parent rocks that transfer large quantities of metals to water bodies and lands (Gadd 2010). As per the World Health Organization (WHO) report (1992), 15,000 metric tons (mt) of Cd is added to the oceans every year. A higher level of heavy metal accumulation is reported in the marine sedimentary rocks, marine phosphates, and phosphorites. Volcanic eruptions (Hong et al. 1996) and forest fires (Shcherbov et al. 2008) also contribute to natural inputs. In addition, the use of heavy metals by humans is known for years (Nriagu 1996). The uses include mining, smelting, fuel combustion, synthetic fertilizers, metal alloys, electroplating, and Ni–Cd batteries, as pigment in plastics and as stabilizer in PVC, in electronic goods, and in solar cells (Gadd 2010). Because of these activities, the contents of Pb, Hg, and Cd in the pedosphere (earth's outermost soil layer) are about 10, 6, and 5 times, respectively, higher than in the lithosphere (earth's crust and outermost mantle layer) (Han et al. 2002). Here, we summarize the toxicity of heavy metals, different approaches employed for soil remediation, and the distinctive properties of rhizoremediation approach, which have made this technology user-, eco-, and economic friendly.

9.2 Soil Pollution

Soil is the major reservoir for most of the metals and nonmetals including the nutrients and is the prime site of biogeochemical cycling of elements. Over the years, continuous cropping and other agricultural activities have resulted in depletion of nutrients in soil (Zhang et al. 2006), requiring application of plant nutrients from external sources. In this context, synthetic agrochemicals especially phosphate fertilizers are excessively applied, which have resulted in heavy metal pollution of soil (Mortvedt 1996; McGrath and Tunney 2010). Besides these, sewage sludge application and industrial activities also add heavy metals to agricultural soil (Kelly et al. 1999; Han et al. 2002). The major factors influencing metals speciation, adsorption, and distribution in soils include pH, soluble organic matter, hydrous metal oxide, clay content and type, organic and inorganic ligands, and competition from other metal ions (Dube et al. 2001).

Even though heavy metals in soil seem to be immobile, they interact with biotic components especially the plant roots. Thereafter, they are transported to all parts of the plants including the fruits, vegetables, and seeds. Consequently, heavy metals enter the food chain of animals including humans (Fig. 9.1). It is reported that terrestrial foods account for 98% of the ingested toxic heavy metals, while 1% each of aquatic foods and drinking water (Van Assche 1998). The Joint Food and Agricultural Organization (FAO)/WHO Expert Committee on Food Additives (JECFA) determined the provisional tolerable weekly intake (PTWI) for the toxic heavy metals which is listed in Table 9.1. Among the toxic heavy metals, Hg and Cd have PTWI values less than 10 μg/kg body weight showing their high toxicity, while Cu and Zn being the essential metal ions have PTWI values of 3,500 and 7,000 μg/kg body weight, respectively. Various studies reported the presence of heavy metals higher than the PTWI values in vegetables, canned foods, and other eatables in all parts of the world. The technical report of Imperial College of London prepared in collaboration with Indian universities has revealed the heavy metal contamination of vegetables in Delhi, India (Marshall et al. 2003). According to the Indian Council of Medical Research (2003) report, nearly 50% of the tested mother's milk samples had Cd eight times more than stringent limits. Since heavy metals are not quickly eliminated from the human system, they bioaccumulate to

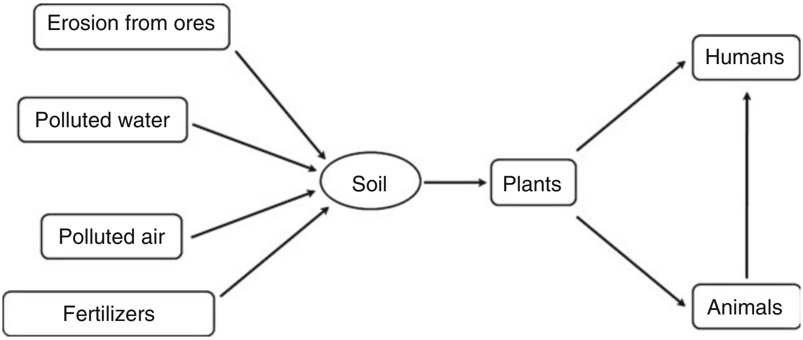

Fig. 9.1 Food chain exhibiting the transport of heavy metals

Table 9.1 Provisional tolerable weekly intake (PTWI) for heavy metals

Heavy metals	PTWI (μg/kg body weight)
Mercury	4
Cadmium	7
Arsenic	21
Chromium	23.3
Lead	25
Nickel	35
Silver	50
Copper	3,500
Zinc	7,000

Reports of Joint Food and Agricultural Organization (FAO)/WHO expert committee on food additives (JECFA) toxic levels. The biological half-life of toxic heavy metals ranges between 20 and 30 years (Sugita 1978). The WHO (1987) estimated that the daily intake of 200 μg Cd for longer period can be connected with a 10% prevalence of adverse health effects suggesting threat to food security.

9.3 Heavy Metal Toxicity

Most of the heavy metals cause changes in both the environment and living organisms. Besides the nonessential heavy metals, even the essential heavy metals become toxic, when their level exceeds the physiological value. The prime reason for their toxicity is due to their ability to bind strongly to oxygen, nitrogen, and sulfur atoms because of free enthalpy of the metal–ligand product (Weast 1984). As a result, heavy metals inactivate the enzymes by binding to –SH group, leading to changes in metabolism (Fuhrer 1982). Many enzymes and proteins need essential divalent cations like Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}, which are displaced by the toxic divalent ions. For instance, in case of calmodulin, the protein that is important in cell signaling, the Ca^{2+} ions are displaced by Cd^{2+} ion, leading to loss of activity (Rivetta et al. 1997). The binding ability of heavy metals with nucleic acids allows them to act as mutagens, which lead to misreading of the genetic profile (Wong 1988). In addition, they cause lipid peroxidation and oxidative stress, leading to membrane damage (Howlett and Avery 1997).

Sources of heavy metals for plants are the soil, irrigation water, and air emissions. Plants take up heavy metals primarily from the soil and accumulate in the plant tissues (Fig. 9.2a). Following accumulation, heavy metals cause changes in the metabolic pathways like photosynthesis (Clijsters and Van Assche 1985; Somasundaram et al. 1994; Pandey and Tripathi 2011), protein and nitrogen metabolism (Hemalatha et al. 1997; Llorens et al. 2001; Manios et al. 2002; Priti et al. 2009), uptake of nutrients

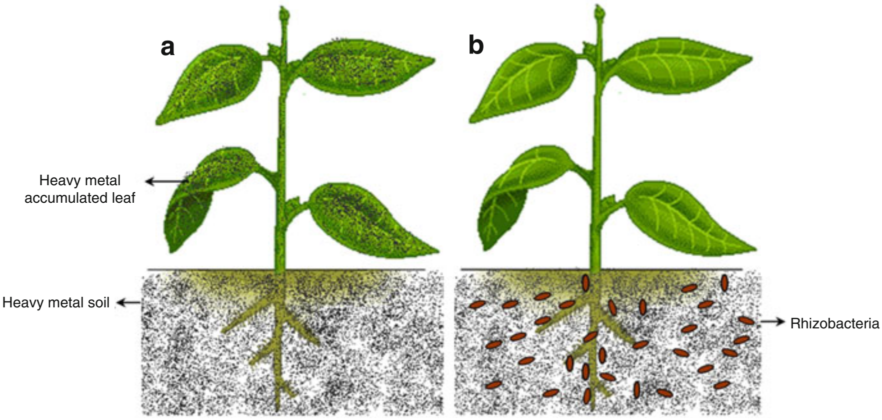

Fig. 9.2 Schematic representation of rhizoremediation

(Veselov et al. 2003), and sugar and water metabolism (Pandey and Tripathi 2011; Stobrawa and Lorenc-Plucińska 2007; Babula et al. 2008). Heavy metals also lead to hormonal imbalances and especially elevate ethylene synthesis (Arteca and Arteca 2007) and decrease cytokinin level due to oxidation (Hare et al. 1997). These effects lead to poor crop productivity. For humans, the heavy metals enter through polluted food, water, air, and occupational exposure. Heavy metals are carcinogens that alter the gene expression, leading to cell proliferation by the induction of proto-oncogenes or by interference with genes involved in cell growth (Beyersmann 2002). International Agency for Research on Cancer (IARC) grouped Cd, Cr, and As in group 1 (proven human carcinogens) and Ni, Co, Hg, and Pb in group 2B (possibly carcinogenic to humans). The teratogenic effects of heavy metals like Cd, Pb, Hg, and U are also reported (Emmanouil-Nikolussi 2007). The other health risks include renal tubular damage, bone demineralization, cardiac failure, nervous, respiratory disorders, and loss of fertility (Duruibe et al. 2007). Even low levels of Cd, Pb, and Hg exposure are reported to diminish intellectual capacity and brain development of children (Drum 2009).

9.4 Soil Remediation Approaches

Many different physical, chemical, and biological methods are proposed for the remediation of metal-contaminated soil.

9.4.1 Physicochemical Methods

The conventional *ex situ* methods like land filling, incineration, leaching, and chemical methods are adopted to remediate metal-contaminated soils, but they are not effective (Lambert et al. 2000). Other *in situ* approaches like vitrification and electrokinetics that involve the application of electrical voltages are efficient, but labor safety and cost factors are of major concern (Mulligan et al. 2001). All these techniques just transfer the contaminants from soils to some other material, which needs to be transported and recycled. Hence, the low efficiency, high cost, safety problems, recycling, transfer, and need to analyze the nature of the contaminants and type of soil are the major setbacks for these approaches. In addition, the ex situ modes and transfer of absorbed material in other techniques pose possibilities of spread of pollutants during transport. Furthermore, the nonbiological methods disrupt the soil characteristics and ecology that make the land unsuitable for agriculture and other purposes.

9.4.2 Bioremediation

9.4.2.1 Phytoremediation

Remediation of soil contaminated with hazardous substances utilizing the innate capabilities of plants is generally termed phytoremediation, an in situ eco-friendly and perpetual approach which does not require any specialized equipments. In addition, application of plants for abatement/rehabilitation of heavy metal-stressed soil is indeed a promising but emerging area of interest because it is an ecologically sound and environmentally safe method for restoration of degraded lands. In this context, about 0.2% angiosperms (Baker and Brooks 1989) are reported to tolerate and accumulate excessively high concentrations of metals and are often termed hyperaccumulators. Plants like *Alyssum* species, *Brassica juncea, Arabidopsis halleri, Noccaea* sp. (formerly *Thlaspi* sp.), *Viola calaminaria*, and *Astragalus racemosus* are hyperaccumulators. The molecular mechanism underlying hyperaccumulation is attributed to the involvement of metal-specific transporters, chelators such as phytochelatins (PC), metallothioneins (MT), and organic acids (OA) like citrate and antioxidants like glutathione (Kramer 2010). Phytoremediation as a technique broadly involves (1) phytoextraction: uptake and accumulation of metals in plants; (2) rhizofiltration: roots absorb, concentrate, or precipitate the metals; (3) phytostabilization: plant reduces the heavy metal mobility by precipitation; (4) phytodegradation: the pollutants are taken up by plants and degraded by the plant enzymes; and (5) phytovolatilization: uptake and release of metals into air as volatile compounds (Raskin and Ensley 2002). These characteristics are commonly associated with only hyperaccumulators, and hence, the normal plants are genetically engineered by introducing the genes involved in metal chelation, transport, and stress responses (Susan and D'Souza 2005). For example, engineering of human MT and mouse MT genes in different plants like *Arabidopsis*, tobacco, and rapeseed plants has shown enhanced Cd uptake and accumulation (Misra and Gedamu 1989). Thus, phytoremediation is a promising option, but longer time, climatic conditions, recycling of accumulated plants, and soil characteristics are some of the major constraints. In addition, the use of transgenic plants poses many unanswered ecological questions. Therefore, the better strategy that answers the problems with the above strategies is achieved by rhizoremediation that combines the advantages of plant–microbe symbiosis.

9.4.2.2 Rhizoremediation

Rhizosphere is defined as the soil zone of biological activity around the plant roots, which is the sink of nutrients. In this region, an intense interaction between plant roots and microbes takes place. Microbes inhabiting this area are generally termed rhizobacteria and regulate the biogeochemical cycles, degrade organic materials, and preserve the soil chemistry (Haferburg and Kothe 2007). The proven traits for effective root colonization include the synthesis of the O-antigen of lipopolysaccharide and cellulose, thiamine and biotin production, amino acid synthesis, an isoflavonoid inducible efflux pump, and a nine-polar flagellar arrangement (Lugtenberg et al. 2001). Using in vivo expression technology (IVET), about 20 genes were demonstrated to be induced in root-colonizing pseudomonads

(Silby and Levy 2004). Around the roots, they form microcolonies, often called biofilms which are covered by mucoid layer (Lugtenberg and Kamilova 2009).

One way to reduce the deleterious effects of heavy metals taken up from the environment by some plants involves the use of plant growth-promoting rhizobacteria (Khan et al. 2009) and mycorrhizae (Heggo and Angle 1990; Saraswat and Rai 2011), and this strategy is termed rhizoremediation. More precisely, the rhizoremediation is defined as the biological treatment of organic or inorganic contaminants in soils by bacterial or fungal activity in the rhizosphere (Kuiper et al. 2004). All through rhizoremediation, low- (e.g., phenolics, organic acid) and high-molecular-weight (e.g., proteins) exudates released from growing plants stimulate the viability and functionality of the plant growth-promoting rhizobacteria (PGPR), which consequently results in a more efficient transformation/degradation of environmental pollutants. The microbial activity also prevents the uptake and accumulation of heavy metals in different organs of plants (Fig. 9.2b). In general, the heavy metals are, however, toxic to the microbes, which in turn affect the fertility of soil. As a survival strategy, some microbes have evolved resistance/avoidance mechanisms that cause change in metal speciation (White et al. 1997). The strategies include biosorption of heavy metals by cell walls, polysaccharides, and pigments (Gadd 2009) and removal by the efflux pumps and by the synthesis of metal-binding peptides and proteins like MTs (Silver 1996). Thus, the microbial biomass acts as sink for the toxic heavy metals (Gadd 2010). In addition, some microbes detoxify the heavy metals like Cd, Hg, and Pb by enzymatic action (Aiking et al. 1985). Various studies reported the successful rhizoremediation of heavy metals in soil using heavy metal-resistant rhizobacteria and different plant species (Table 9.2). In heavy metal soil, the metal-resistant rhizobacteria also enhance the mycorrhizal and nodulation efficiency in plants (Vivas et al. 2006). Besides native bacteria, transgenic approaches both at the microbial and plant levels are reported for higher efficiency of rhizoremediation. For example, the expression of metal-binding peptide (EC20) in rhizobacteria *Pseudomonas putida* 06909 improved both cell growth and cadmium binding in the presence of the cadmium in sunflower seedlings (Wu et al. 2006). It was shown that the introduction of genetically modified microorganisms designed for rhizoremediation induces changes in native bacteria in the rhizosphere but not in the surrounding soil (Carcer et al. 2007).

9.5 Plant Growth-Promoting Activities of PGPR

Besides the heavy metal accumulation or detoxification, the plant-growth-promoting activities of rhizobacteria improve the efficiency of rhizoremediation. The PGPR promote the plant growth by hormonal regulation, enhanced mineral uptake, sequestering iron by siderophore production, antagonism by antibiotics production, and root growth stimulators production (Fig. 9.3) (Khan et al. 2009; Lugtenberg and Kamilova 2009; Martínez-Viveros et al. 2010; Ahemad and Khan 2011). Studies with metal-sensitive PGPR have also been found effective in preventing the accumulation

Table 9.2 Some examples of rhizoremediation of heavy metals using plant growth-promoting rhizobacteria

Rhizomicrobe	Microbial characteristics[a]	Heavy metal	Plant	Effect on plants[a]	Reference
Bacillus sp. PSB10	Chromate reducer; produced siderophore, IAA, HCN, ammonia, and solubilized insoluble P	Cr	*Cicer arietinum*	Increased growth, nodulation, chlorophyll, leghemoglobin, seed yield, and grain protein and decreased Cr accumulation	Wani and Khan (2010)
Serratia sp. SY5	Produced IAA and siderophore	Cd, Cu	*Zea mays*	Increased root biomass	Koo and Cho (2009)
Mesorhizobium sp. RC3	Chromate reducer; produced IAA and fixed N	Cr	*Cicer arietinum*	Increased nodulation, dry-matter content, seed yield, and grain protein; higher N content in roots and shoots; and decreased Cr accumulation	Wani et al. (2009)
Pseudomonas aeruginosa MKRh3	Cd^{2+} resistant; produced ACC deaminase, siderophores, IAA, and P	Cd	*Vigna mungo*	Enhanced plant growth with decreased Cd accumulation	Ganesan (2008)
P. aeruginosa KUCd1	Rifampicin-resistant mutant, Cd^{2+} tolerant, produced siderophore	Cd	Pumpkin, *B. juncea*	Enhanced growth with high chlorophyll and iron content and decreased Cd accumulation	Sinha and Mukherjee (2008)
Methylobacterium oryzae CBMB20 and *Burkholderia* sp. CBMB40	Methylotrophic, Ni^{2+}, and Cd^{2+} resistant	Ni, Cd	*L. esculentum*	Enhanced growth, decreased ethylene emission, and low Ni, Cd uptake	Madhaiyan et al. (2007)
Bradyrhizobium japonicum Cb1809	Produced nitrogenase and phytohormones	As	*Glycine max*	Enhanced dry weight and N content. Decreased As uptake	Reichman (2007)
Brevibacillus B-I	Zn^{2+}-resistant and produced IAA	Zn	*Trifolium repens*	Stimulated mycorrhization and nodulation. Increased biomass, P, and N uptake	Vivas et al. (2006)
P. fluorescens PRS_9 Hg^r and GRS_9 Hg^r	Hg^{2+}-resistant mutant; produced IAA, siderophore, and solubilized P	Hg	*Glycine max*	Enhanced plant growth	Gupta et al. (2005)
O. intermedium CrT-2, CrT-3, and CrT-4	Reduced Cr (VI) to Cr (III)	Cr	*Helianthus annuus*	Enhanced germination and plant growth. Increased auxin content and decreased Cr content	Faisal and Hasnain (2005)
Kluyvera ascorbata SUD165	Resistant to Ni^{2+}, Cd^{2+}, Zn^{2+}, CrO^{4-}; displayed ACC deaminase activity	Ni, Pb, and Zn	*L. esculentum, B. juncea, and B. campestris*	High yield. Enhanced chlorophyll and protein content in leaves. Decreased Ni, Pb, and Zn uptake	Burd et al. (1998)

[a] Includes only reported parameters, but other characteristics might be absent or not tested

and toxicity of heavy metals in plants (Table 9.2). The different PGPR activities are discussed below in correlation with heavy metal toxicity in plants.

9.5.1 Heavy Metal Stress Tolerance

Primarily, heavy metal stress may cause hormonal imbalance in plants, leading to reduced root growth. Ethylene synthesis, for example, is increased upon treatment with Cd, Cu, and Zn. In the case of Cd and Cu, this increase is due to an upregulation of ACC synthase transcription and enhanced activity (Waldemar 2007). The microbial enzyme 1-aminocyclopropane carboxylic acid (ACC) deaminase catabolizes the immediate ethylene precursor ACC (Fig. 9.3), which is released in the root exudates. Thus, rhizobacteria acts as a sink for ACC by stimulating plants to exude more ACC and thereby reducing ethylene stress in plants (Penrose and Glick 2001). On treatment with Cd, the abscisic acid (ABA) hormone content rapidly increased in rice (*Oryza sativa*) seedlings (Hsu and Kao 2003). Secretion of cytokinin by the microbes decreases the ABA content and its effects (Cowan et al. 1999). In addition, PGPR also produce antioxidants like catalases and pyrroloquinoline quinine (PQQ) that helps in degrading the reactive oxygen species (ROS) which is synthesized during stress conditions (Fig. 9.3) (Yang et al. 2009).

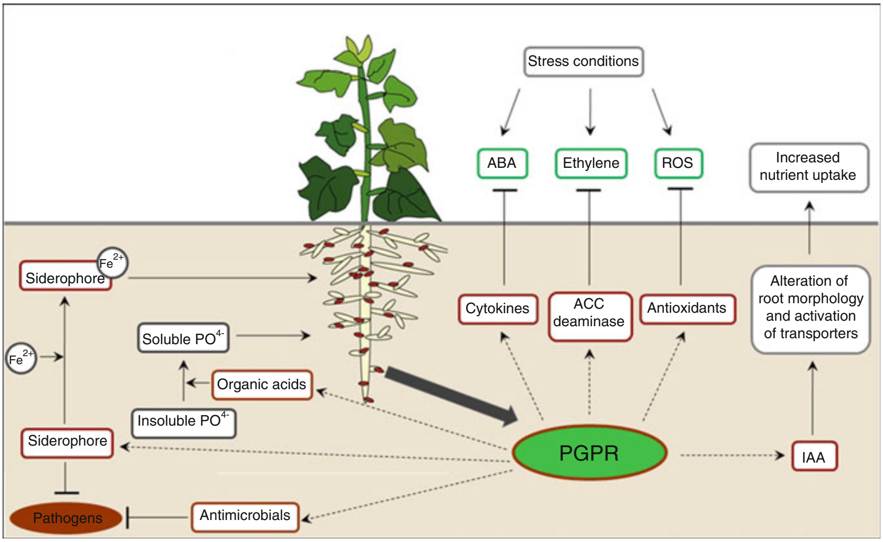

Fig. 9.3 Plant growth-promoting activities of rhizobacteria

9.5.2 Mineral Uptake

Various studies indicated the reduced uptake of minerals like iron, phosphate, nitrate, and other nutrients by plants grown in soils contaminated with heavy metals (Rubio et al. 1994; Huang et al. 2007). The deficiency of such elements in plants results in different types of symptoms on plants. For example, leaf chlorosis is one of the key morphological effects of heavy metals in plants due to iron starvation. Siderophores, the low-molecular iron-chelating substances produced by rhizobacteria, help in sequestration of iron by both microbes and plants (Neilands 1995). Siderophore-overproducing mutant of *Kluyvera ascorbata* SUD165, for example, enhanced the plant growth, chlorophyll contents in foliage, and protein content and decreased the heavy metal accumulation in tomato plants grown in metal-contaminated soil (Burd et al. 2000). Siderophore production is reported to be induced by heavy metals like Cd, Pb, Al, and Zn in *Pseudomonas* and *Rhizobium* spp. (Roy and Chakrabartty 2000; Sinha and Mukherjee 2008; Ganesan 2008), but the molecular mechanism underlying the synthesis of siderophore is not well explained.

Heavy metal ions also disrupt some of the important plant enzymes like nitrate and nitrite reductases, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase (Llorens et al. 2001), leading to reduced uptake of ammonium and nitrate and low-protein content. This effect was circumvented by PGPR like Rhizobia and diazotrophs capable of producing nitrogenase. Due to these activities, legume–Rhizobia symbiosis has shown higher efficiency in rehabilitation of heavy metal-poisoned soils (Pajuelo et al. 2008; Wani et al. 2009). Similarly, phosphate-solubilizing microbes and arbuscular mycorrhizal fungi (AMF) enhance the P uptake in plants (Khan et al. 2007; Zaidi and Khan 2007; Zaidi et al. 2009; Lugtenberg and Kamilova 2009). Besides these, the production of hormones like indole acetic acid (IAA), cytokinin, and other metabolites (Fig. 9.3) promotes the root growth, modifies the root architecture, and induces the membrane transporters, which leads to the enhanced nutrient uptake by plants (Waldemar 2007).

9.6 Eco-economics

In spite of the billions of funding and development of newer technologies and programs aimed at restoring heavy metal-polluted soils, the severity of heavy metal problems is increasing alarmingly every year around the world. This is partly due to the lack of awareness but largely due to economic constraints mostly in developing countries. However, when applied, the comparative estimates and additional factors involved in remediation of metals, for example, cadmium per ton of soil (Glass 1999) employing various approaches, are presented in Table 9.3. The cost given in this table suggests that the rhizoremediation approach when employed properly with sound understanding is inexpensive. The estimates shown here include only the remediation cost, while the other expenses like cost of transport, recycling, and monitoring may further increase the overall cost of remediation. Further, since

Table 9.3 Estimates of cost for cadmium remediation per ton of the soil by different approaches

Remediation approaches	Estimates (US $/ton)	Additional factors and expenses
Land filling	100–500	Transport/excavation
Vitrification	75–425	Long-term monitoring
Chemical treatment	100–500	Recycling
Electrokinetics	20–200	Monitoring
Phytoremediation	5–40	Recycling and monitoring
Rhizoremediation	5–20	Monitoring

Adapted from Glass (1999)

rhizoremediation approach involves the use of cheap renewable resources like PGPR having multiple properties, this technology could be more profitable than other remedial technology. The biocontrol activities like antagonism and competition for nutrients and niches (CNN) (Lugtenberg and Kamilova 2009) add further strength to the economic friendliness of rhizoremediation approach by cutting off the costs for pesticides and thereby circumventing phytopathogens naturally. Thus, rhizoremediation approach is made environmentally as well as economically more pragmatic.

Conclusion

Rhizoremediation approach is aesthetically pleasing and low cost, uses solar energy, requires minimal maintenance, presents no need for further recycling, and preserves the soil fertility and ecology. As a result, this strategy is gaining wider acceptance. Besides remediation and earning, it ensures the food security for humans and prevents them from a lot of ailments. However, large-scale field trials and its assessment are required to guarantee the practicability of rhizoremediation. However, how this technology could be useful in the rehabilitation of metal contaminated but nonagricultural soils with poor nutrients or nutrient deficient soils is indeed a challenge before scientists. Considering different facets of remediation methods, it is evident that all these methods in general provide only a temporary solution for the abatement of polluted lands and not complete destruction of metals from the contaminated sites. Hence, the practice of organic farming along with the remedial technology should be promoted in order to prevent metal pollution in agricultural soil.

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