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REVIEW ARTICLE

ROLE OF RHIZOSPHERIC BACTERIA IN PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL

Rachna Bhateria and Snehlata*

Department of Environmental Sciences, Maharshi Dayanand University, Rohtak, Haryana-124001

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ABSTRACT

Environmental pollution with toxic heavy metals is spreading throughout the world along with industrial progress. Copper, chromium, cadmium and nickel are few of the known heavy metals. Elevated level of heavy metals not only decrease soil microbial activity and crop production but also threatens human health through the food chain. Rhizoremediation is emerging as a potential cost effective solution for the remediation of heavy metal-contaminated soils in opposition to the conventional chemical and physical remediation technologies that are generally too costly and often harmful to soil characteristics. Rhizosphere plays a significant role in phytoremediation of heavy metals contaminated soil in which microbial population are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes. This paper reviews the important beneficial plant-microbe interaction that promote plant health and development. Role of rhizospheric bacteria that may promote plant growth by producing siderophore, synthesizing phytohormones and enzymes has also been discussed. The role of transgenic plants, genetically engineered microbes and tolerance of plants to heavy metals in stress environment for enhancing phytoremediation is also explained.

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INTRODUCTION

Phytoremediation is described as a natural process carried out by plants and trees in the cleaning up and stabilization of contaminated soils and ground water (Alberto and Gilbert, 2013). Cobalt, copper, manganese, nickel, and zinc in trace amounts are essential for growth of microorganisms, but at high concentrations they have noxious effects on various organisms and on human health (Filali *et al*., 2000). The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Copper, chromium, cadmium and nickel are known to be most commonly used heavy metals and the most widespread contaminants of the environment (Singh *et al.,* 2011). The concept of using plants for remediation of organic pollutants emerged a few decades ago with the recognition that plants are capable of metabolizing toxic compounds such as 1, 1, 1-trichloro-2, 2-*bis*-(4'-chlorophenyl) ethane (DDT) and benzo (α) pyrene. Heavy metals exist both in bioavailable and non-bioavailable forms. Their mobility depends on two factors: (I) the metallic element that precipitates as positively charged ions and (II) the one, which makes up negatively charged component of salt (Ahemad 2012). Microorganisms possess a variety of mechanisms to deal with high concentration of heavy metals and often are specific to one or a few metals (Silver and Phungh (1996), Piddock, 2006). Heavy metals in soil are associated with a number of soil components which determine their behaviour in the soil and influence their bioavailability (Boruvka and Drabek, 2004). Microbes have developed mechanisms to tolerate the metals either by presence of heavy metals through efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Haferburg and Kothe 2010). Soil microorganisms inhabiting the rhizosphere environment interact with plant roots and mediate nutrient availability, e.g. those forming useful symbiotic associations with the roots and contribute to plant nutrition. Implications of plants and their symbionts like mycorrhizal fungi, N-fixing rhizobia, and free living rhizosphere population of bacteria which promote plant growth need to be fully exploited and encouraged by inoculating nutrient poor agricultural soils with appropriate microbes (Khan *et al*., 2002). Phytoremediation, the use of plants to extract, sequester and / or detoxify pollutants through physical, chemical, and biological processes (Cunningham and Ow, 1996; Saxena *et al*., 1999; Wenzel *et al*.,1999), has been reported to be an effective, in situ, non-intrusive, low-cost, aesthetically pleasing, ecologically benign, socially accepted technology to remediate polluted soils (Alkorta and Garbisu, 2001; Garbisu *et al.,* 2002; Weber *et al.,* 2001). The rhizosphere harbors an extremely complex microbial community including saprophytes, epiphytes, endophytes, pathogens and beneficial microorganisms (Avis *et al.,* 2008). Beneficial rhizosphere organisms are classified into two broad groups based on their primary effects, (i) microorganisms with

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****Corresponding author: Snehlata,***
Department of Environmental Sciences, Maharshi Dayanand University, Rohtak, Haryana-124001.

direct effects on plant growth promotion [plant growth promoting microorganisms (PGPM)] and (ii) biological control agents (BCA) that indirectly assist with plant productivity through the control of plant pathogens (Whipps, 2004; Vassilev *et al.,* 2006). Heavy metal contamination of soil and water is a major threat to human and ecosystem health, making the cleanup of metal-contaminated sites a high priority (Ensley, 2000). The persistent nature of heavy metal contaminants in the environment has meant that the most commonly used methods of cleanup of contaminated soil are excavation and removal, incineration, and chemical treatment (Pilon-Smits, 2005). The United States spends $6–8 billion per year on environmental cleanup, and globally the cost is $25–50 billion a year (Tsao, 2003; Pilon-Smits, 2005). This paper reviews the benefits of rhizosphere bacteria on plant nutrition. The interaction between plant and microbes has been discussed along along with the role of rhizosphere bacteria in soil nutrient availability.

Plant Growth-Promoting Rhizobacteria (PGPR)

The recognition of plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. Increased growth and yields of potato, sugar beet, radish and sweet potato (Farzana *et al*., 2009). PGPR is a group of bacteria that can actively colonize plant roots and can enhance plant growth (Stefan *et al.,* 2012, Vessey, 2003). PGPR potentiality in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. PGPR in the rhizosphere along with their colonization ability and mechanism of action should facilitate their application as a reliable component in the management of sustainable agricultural system (Bhattacharyya and Jha, 2012, Stefan *et al.,* 2012). PGPR are reported to secrete some extracellular metabolites called siderophores. Siderophores are secreted to solubilize iron from their surrounding environments, forming a complex ferric-siderophore that can move by diffusion and be returned to the cell surface (Andrews *et al*., 2003). Most of the bacterial siderophores are catecholates, and few are hydroxamates and carboxylates, whereas most fungal siderophores are hydroxamates (Schalk *et al*., 2011). A number of bacterial species belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth (Tilak *et al*., 2005; Egamberdiyeva, 2005). The important role is played by plants in selecting and enriching the types of bacteria by the constituents of their root exudates. PGPR in rhizosphere can enable plants to achieve high levels of biomass in contaminated soils despite extreme conditions (Reed and Glick 2004). The ability of siderophore production is one of the key factors that allow plants to cope with toxic metal concentrations (Dimkpa *et al*., 2009; Meda *et al*., 2007). Siderophores are commonly referred to as microbial Fe-chelating low molecular weight compounds. The presence of siderophore-producing PGPR in rhizosphere increases the rate of Fe^{3+} supply to plants and therefore enhance the plant growth and productivity of crop (Singh, 2013). PGPR have great role in reducing plant diseases (Millan, 2007). They can directly facilitate plant growth and may fix atmospheric nitrogen and supply it to plants; synthesize siderophores which can sequester iron from the soil and provide it to plant cells which can take up the bacterial siderophore–iron complex and synthesize phytohormones such as auxins, cytokinins and gibberelins, which can act to enhance various stages of plant growth; solubilize minerals such as phosphorus, making them more readily available for plant growth (Lazcano *et al.,* 2010).

Rhizobacteria can be divided into two major groups according to their relationship with the host plants: (1) symbiotic rhizobacteria and (2) free-living rhizobacteria (Khan, 2005), which could invade the interior of cells and survive inside intracellular PGPR (e.g., nodule bacteria), or remain outside the plant cells, extracellular PGPR (e.g., *Bacillus, Pseudomonas, Azotobacter* etc.) (Khan *et al.,* 2009). PGPR are usually in contact with the root surface, and improve growth of plants by several mechanisms, e.g., enhanced mineral nutrition, phytohormone production, disease suppression (Tarkka *et al.,* 2008). Two groups of PGPR were described: one group is involved in the nutrient cycling and plant growth stimulation (biofertilizers) (Vessey, 2003) and the second group is involved in the biological control of plant pathogens (biopesticides) (Whipps, 2001). Biofertilizers are based on living microorganisms which (when applied to seed, plant surface or soil) colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). Biopesticides promote plant growth by the control of deleterious organisms, e.g., through the production of antibiotics. PGPR can increase the availability of nutrients, e.g., by enzymatic nutrient mobilization from organic matter and production of siderophores (Anderson *et al.,* 1993; Whiting *et al.,* 2001; Jing *et al.,* 2007). Bacteria producing extracellular degrading enzymes are major decomposers of organic matter. They contribute essentially to the soil aggregation and nutrient availability (Johansen and Binnerup, 2002). PGPR can also promote the root growth. This can be caused by the ability of most rhizobacteria to produce phytohormones, e.g. Indole-3-Acetic Acid (IAA), cytokinins, gibberellins, ethylene which promote cell division and cell enlargement, extension of plant tissue and/or other morphological changes of roots (Baum *et al.,* 2006).

Phytoremediation assisted by soil Rhizobacteria

Plant growth-promoting bacteria may facilitate plant growth either indirectly or directly (Glick *et al.,* 1995). The ability of plant growth-promoting bacteria to act as biocontrol agents against phytopathogens and thus indirectly stimulate plant growth may result from any one of a variety of mechanisms including antibiotic production, depletion of iron from the rhizosphere, induced systemic resistance, production of fungal cell wall lysing enzymes and competition for binding sites on the root (Glick *et al.,* 1995; 2007a). Rhizobacteria produce siderophores that have an important role in sequestering metals (Dell *et al.,* 2005) and has more affinity to plants (Kamaludeen and Ramasamy, 2008). *Thlaspi caerulescens* has a remarkable ability to hyperaccumulate Zn from soils containing mostly nonlabile Zn (Kamaludeen and Ramasamy, 2008). *Pseudomonas maltophilia* was shown to reduce the mobile and toxic Cr $^{6+}$ to nontoxic and immobile Cr^{3+}, and also to minimize environmental mobility of other toxic ions (Hg, Pb, Cd) (Blake *et al.,* 1993). *Pseudomonas putida* 06909) resulted in marked decrease in Cd phytotoxicity and increase in Cd accumulation (Huang *et al*., 2005). *Escherichia coli and Moraxella sp*. expressing EC20 (with20 cysteines) on the cell surface or intracellularly have been shown to accumulate up to 25-fold more cadmium (Bae *et al.,* 2000) or mercury (Bae *et al*., 2003).

Heavy metal tolerant Bacteria

Heavy metals are identified as any metallic chemical element with a relatively high density and are toxic or poisonous at low concentrations (Anigma, 2010). Heavy metals are continuously being added to soils through various agricultural and industrial activities such as the use of agrochemicals and the long term deposition of urban sewage sludge on agricultural soils, waste disposal, waste incineration and vehicle exhausts (Venkatesan *et al*., 2011). A large array of heavy metal tolerant bacteria including species of *Pseudomoans, Azotobacter, klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus and Serratia* have reported to enhance plant growth (Kloepper *et al.,*1989 and Glick, 1995). Heavy metal resistant microorganisms can be used as successful bioremediation agents. Some microorganisms under heavy metals stress may develop resistance against the elevated levels of these toxic metals and evolve various strategies to resist against the metal stress. Therefore, the metal resistant microorganisms including bacteria can be exploited as bioremediation, biosorption and bioaccumulation processes. (Ahemad 2012; Khan *et al.,* 2009). Heavy metal transport through bioaccumulation has been reported in many bacterial genera. For eg. *Citrobacter sp*. (lead and cadmium), *Thiobacillus ferrooxidans* (silver), *Bacillus cereus* (cadmium), *Bacillus subtilis* (chromium), *Pseudomonas aeruginosa* (uranium) *Micrococcus luteus* (strontium) *Rhizopus arrhizus* (mercury), *Aspergillus niger* (thorium), *Saccharomyces cerevisiae* (uranium) (Rani and Goel*,* 2009; Ahemad and Malik*.,* 2011; Umrania, 2006; Spain and Alm, 2003). Different species of bacteria and heavy metal which they uptake are summarised in Table 1.

Table 1. Heavy metal tolerant bacteria(Rajbansi, 2008)

S.No.	Bacteria	Heavy metal
1..	*Staphylococcus sp.*	Chromium
2	*Escherichia coli*	Chromium
3	*Klebsiella sp.*	Chromium
4	*Citrobacter sp.*	Cadmium
5	*Acinetobacter sp.*	Cadmium
6	*Flavobacterium sp.*	Cadmium
7	*Pseudomonas sp.*	Copper
8.	*Staphylococcus sp.*	Nickel
9.	*Bacillus sp.*	Nickel

Plant and Microbes interaction

Plant–microbe interactions may occur at phyllosphere, endosphere and rhizosphere. Phyllosphere is related with the aerial parts of the plants and endosphere with internal transport system. Rhizosphere, the term, can be defined as any volume of soil specially influenced by the plant roots or in association with the roots and plant-produced material. Plant-root interactions in rhizosphere may include root–root, root-insect and root-microbe interactions, resulting in the production of more root exudates that ultimately favours maximum microbial populations (rhizosphere engineering) in this ecologically significant region. Changes in rhizobacterial community structure have been reported with the application of Polymerase Chain Reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE) resulting in significant alterations in plant–microbes interactions (Herschkovitz *et al*., 2005). Currently >450 metal hyperaccumulators occur in over 34 different families, 25% of hyperaccumulating species are in the family *Brassicaceae* (Rascio and Navari-Izzo, 2011), the best known of which are in the genera *Alyssum and Noccaea* (formerly, *Thlaspi*) (Verbruggen *et al.,* 2009). Roots of plants interact with a large number of different microorganisms, with these interactions being major determinants of the extent of phytoremediation (Glick, 1995). Soil microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests and plant growth (Elsgaard *et al.,* 2001; Filip, 2002; Giller *et al.,* 1998). The plant-microbe interaction in rhizosphere can be beneficial, neutral or variable for plant growth (Bhatia and Malik*,* 2011). Microbial attachment to and proliferation on roots is generally referred to as root colonization. Root colonization is an important factor in plant pathogenesis of soil-borne microorganisms as well as in beneficial interactions used for microbiological control,biofertilisation, phytostimulation, and phytoremediation (Thomas *et al.,* 2008). PGPR may colonize the rhizosphere, the surface of the root, or even superficial intercellular spaces (McCully, 2001). It is influenced by many factors: biotic, such as genetic traits of the host plant and the colonizing organism or abiotic such as growth substrate, soil humidity, soil and rhizosphere pH and temperature (Stefan *et al*., 2012). The specificity of the plant – bacteria interaction is dependent upon soil condition, which can alter contaminant bioavailability, root exudates composition and nutrient level (Jing *et al*., 2007).

Root exudates (carbohydrates, amino acids, organic acids) and mucilage-derived nutrients attract deleterious rhizobacteria as well as beneficial and neutral bacteria allowing them to colonize and multiply in rhizosphere (Walker *et al.,* 2003). Root colonisation is a complex procedure involving several steps (Badri *et al*., 2009) and establishment in plant tissue includes several complex mechanisms (Reinhold-Hurek and Hurek, 2011). Microorganisms move towards rhizosphere in response to root exudates, which are rich in amino acids, organic acids, sugars, vitamins, purines/ pyrimidines and other metabolic products (Bais *et al.,* 2006; Compant *et al.,* 2010). The plants and their metal uptake can be influenced by many other factors, since they form an own microcosm in their rhizosphere. Indeed, many organisms living around the plants influence their growth and mineral uptake. Microorganisms are known to react with metals present in their environment therefore many are used to treat wastewaters containing high amounts of metals (AMD) by precipitating them as sulphides, so concentrating or immobilising them, and increasing the pH. These biogeochemical processes are catalysed mainly by sulphate reducing bacteria like *desulfovibrio* or *desulfotomaculum* (Cohen, 2006). Plant roots release a wide range of substances, which, especially the easily decomposed low molecular weight ones, are involved in attracting beneficial microorganisms and forming mutualistic associations in the rhizosphere (Marschner, 2012). PGPR have to be highly competitive to successfully colonize the root zone (Compant *et al*., 2010). *Alyssum murale* is a Ni resistant, siderophore and acid producing bacteria more in rhizposphere than in bulk soil(Abou- shanab *et al*.,2003). Some rhizospheric bacteria can produce siderophores and there is evidence that a number of plant species can absorb bacterial Fe^{3+-}siderophore complexes (Bar-Ness *et al.,* 1991). The significance of bacterial siderophore in the iron nutrition of plants is controversial (Vessey, 2003). Most of PGPR have the ability to produce peptide antibiotics. These are oligopeptides that inhibit synthesis of pathogens cell walls, influence membrane structures of cells, inhibit the formation of initiation complex on small subunit of ribosomes (Maksimov *et al.,* 2011).Figure 1 shows plant- microbes interaction.

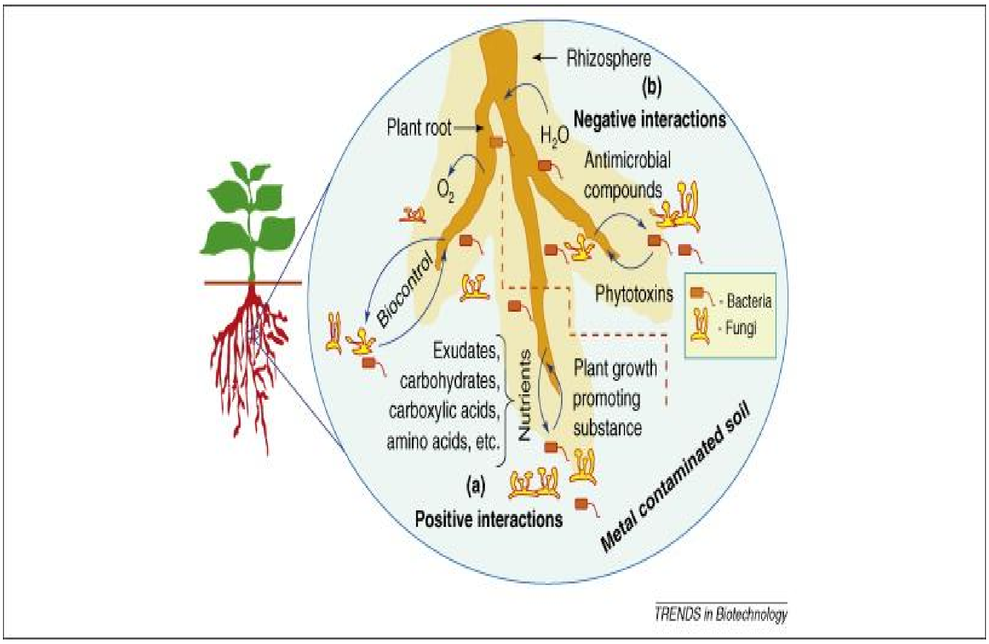

Figure 1. Plant–microbe interactions (Rajkumar *et al*., 2009)

Interactions are of two types (a) Positive interactions: During plant growth, roots either actively or passively release a range of organic compounds. Among them are exudates, mainly carbohydrates, carboxylic acids and amino acids, which are passively released along concentration gradients that serve as nutrients for microbes in the rhizosphere. Microbes colonize the rhizosphere of many plants and often confer beneficial effects, such as promoting plant growth and reducing susceptibility to diseases caused by plant pathogens such as fungi, bacteria, viruses and nematodes. Microbial plant growth-promoting mechanisms include the fixation of atmospheric nitrogen, utilization of 1-aminocyclopropane-1-carboxylic acid as a sole N source, production of siderophores or production of plant growth regulators(Rajkumar *et al*., 2009; Weyens *et al*., 2009). The mechanisms that are responsible for controlling disease susceptibility include competition of rhizosphere microbes for nutrients, niche exclusion, induced systemic resistance and production of antifungal metabolites(Miethke and Marahiel,2007; Compant *et al*., 2005). (b) Negative interactions: Rhizosphere microbes can also have detrimental effects on plant health and survival by increasing the risk for infection with plant pathogens or parasites. Root exudates containing toxic substances, such as antimicrobials, phytotoxins, nematicidal, and insecticidal compounds might also inhibit the growth of beneficial as well as pathogen organisms in the rhizosphere (Bais *et al*., 2006).

Transgenic plants

Transgenic plants that secrets detoxifying enzymes can be useful for rhizoremediation of wide range of hydrophobic chemicals (Wang *et al.,* 2004). Transgenic plants have been produced for phytoremediation of both heavy metals and organic pollutants (Eapen *et al.,* 2007). Transgenic plants for phytoremediation were first developed for remediating heavy metal contaminated soil sites. For example, *Nicotiana tabaccum* expressing a yeast metallothionein gene for higher tolerance to cadmium, or *Arabidopsis thaliana* overexpressing a mercuric ion reductase gene for higher tolerance to mercury (Misra and Gedama, 1989; Rugh *et al.,* 1996; Abhilash *et al.,* 2009). Transgenic plants increase remediation efficiency by expressing a particular PGPR protein (Denton, 2007). *Arabidopsis thaliana* was engineered to express a modified organomercurial lyase and those transgenic plants grew vigorously on a wide range of concentrations of highly toxic organomercurials, probably by forming ionic mercury which should accumulate in the disposable plant tissues (Bhatia and Malik, 2011). Plants such as *Populus angustifolia, Nicotiana tabacum* or *Silene cucubalis* have been genetically engineered to overexpress glutamylcysteine syntlietase, and thereby provide enhanced heavy metal accumulation as compared with a corresponding wild type plant (Fulekar *et al.,* 2009). *Brassica juncea* was genetically engineered to investigate rate-limiting factors for glutathione and phytochelatin production. To achieve this Escherichia coli gshl gene was introduced (Fulekar *et al.,* 2009).

Heavy Metal-Bacteria interactions

Plant growth-promoting rhizobacteria include a vast 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). PGPR like *Azotobacter chroococcum HKN-5, Bacillus megaterium HKP-1, Bacillus mucilaginosus HKK-1, Bacillus subtilis SJ-101, Brevundimonas sp. KR013, Pseudomonas fluorescens CR3, Rhizobium leguminosarum bv. trifolii NZP561, Kluyvera ascorbata SUD165 are used* (Kamaludeen and Ramasamy, 2008). Rhizobacteria possess several traits that can alter heavy metals bioavailability (Lasat, 2002; 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.,*2003) reported that the addition of *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *Microbacterium arabinogalactanolyticum* to *Alyssum murale* 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. Only about 1% of the bacteria occurring in the environment can be cultured on laboratory media (Amann *et al.,* 1995). Rhizosphere isolates collected from *Alyssum murale* were mainly Proteobacteria (Mengoni *et al.,* 2001), those from *Thlaspi goesingense* mostly alphaproteobacteria and Actinobacteria (high-GC gram positive bacteria) (Idris *et al*., 2004). The most important mutualisms exist between plants and mycorrhizae or rhizobacteria (Badri *et al*., 2009), which can also exist simultaneously and influence each other. *Bacillus subtilis* is able to reduce Au^{3+} ions to produce octahedral gold nanoparticles(Mandal *et al.,* 2006). Microorganisms can interact directly with the trace metals to reduce their toxicity and/or influence their bioavailability: strong acids such as H_2SO_4 (i.e. *Thiobacillus*) lead to metal dissolution (Abhilash *et al.,* 2009; Natarajan, 2008; Pathak *et al.,* 2009); organic acids chelate metals to form metal-organic molecules; ammonia or organic bases precipitate metal hydroxide. Rhizosphere microbes play an important role for the soluble metal pool in soils by altering the solubility, availability and transport of trace elements and nutrients by reducing soil pH, secretion of chelators and siderophores or redox changes but can also reduce the extent of contaminant uptake or translocation to aerial parts of plants by decreasing the bioavailability of metals. It has been shown that different microbial inocula can enhance metal uptake and plant growth at the same time: mycorrhizal fungi and followed by yeast treatment were shown to be highly effective in enhancement of uptake of Zn, Cu, and Cd by corn and sunflower plants (Usman and Mohamed, 2009).

Rhizospheric microorganism

The microorganism community structure of the rhizosphere population is important in the context of plant growth. The highest portion of microorganisms which inhabit the rhizosphere are fungi and bacteria. When considering the rhizosphere effect on their abundance, the fungal abundance is 10–20 times higher and the bacterial abundance 2–20 times higher in the rhizosphere than in the bulk soil (Morgan *et al*., 2005). This is largely attributed to the finding that microbial populations often establish some sort of positive cooperation with the host plant system. For example, soil pollution with heavy metals could lead to the appearance of heavy-metal resistant rhizobateria in the soil of industrial regions (Aleem *et al*., 2003). High level of heavy metal decrease rhizobacteria metabolic activity, biomass and diversity (Gremion *et al.,* 2004; Sandaa *et al*., 1999). It was revealed that a high proportion of metal resistant bacteria persist in the rhizosphere of the hyperaccumulators *Thalaspi caerulescens* (Delorme *et al.,* 2001) and *Alyssum bertolonii* (Mengoni *et al*., 2001) or *Alyssum murale* (Abou-Shanab *et al.,* 2003) grown in soil contaminated with Zn and Ni or Ni, respectively. The presence of rhizobacteria increased concentrations of Zn (Whiting *et al.,* 2001), Ni (Abou-Shanab *et al.,* 2003) and Se (de Souza *et al.,* 1999) in *T.caerulescens*, *A. murale* and *B. juncea*, respectively. The rhizobacteria is used or manipulated with three main objectives for remediation of metal contaminated soils a) hyperaccumulation of metals in plants b) reducing the uptake of metals and c) *in-situ* stabilization of the metals as organo complexes. Most of the associative and endophytic bacteria fix atmospheric nitrogen and supply it to the associated host plants. A variety of nitrogen fixing bacteria like *Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Derxia, Enterobacter, Gluconoacetobacter, Herbaspirillum, Klebsiella, Pseudomonas, Serratia and Zoogloea* have been isolated from the rhizosphere of various crops, which contribute fixed nitrogen to the associated plants (Jha *et al*., 2013). *P. putida PCL1444* effectively utilizes root exudates, degrades naphthalene around the root, protects seeds from being killed by naphthalene, and allows the plant to grow normally. In-situ inoculation of *P. putida W619-TCE* reduced evapotranspiration of trichloroethylene by 90% under field condition (De Bashan *et al*., 2012).

Nitrogen fixers were found in Archea (*Methanosarcina)* and many bacterial genera, mainly proteobacteria as *Sphingomonas* (*S. azotifigens*), *Burkholderia*, *Pseudomonas*, *Azotobacter, Devosia*, *Bradyrhizobium*, *Rhodobacter*, *Agrobacterium*, *Rhizobium*, *Frankia*, *Rhodococcus, Alcaligenes, Ralstonia,* some firmicutes (*Paenibacillus*), cyanobacteria as *Nostoc* sp. *Rhizobium, Rhodococcus, Paenibacillus and Pseudomonas* (Franche *et al.,* 2009; Wang *et al.,* 2008). Bacteria which can solubilise phosphorus includes *Azotobacter chroococcum, Bacillus spp., Enterobacter agglomerans, Pseudomonas chlororaphis, Pseudomonas putida, and Rhizobium and Bradyrhizobium spp.* (Weyens *et al.,* 2009). Rhizosphere microbes play an important role for the water-soluble metals pool in soil by altering the solubility, availability and transport of trace elements and nutrients. This happens through modifying soil pH, secretion of chelators and siderophores or redox changes (Usman and Mohamed, 2009). The main aspect of metal uptake is driven by the production of siderophores, which can make Iron(III)-hydroxide available for reduction to Fe^{II}; this is crucial especially in alkaline (calcareous) soils with decreased Fe availability (Kidd *et al.,* 2009; Weyens *et al.,* 2009). Siderophores take up Fe^{III}, which is then reduced intra-cellularly. Siderophores are generally only for Fe, but also Al and other metals as Cd, Ni or micronutrients such as Mn, Co, Zn can be transported in some cases (Dimkpa *et al*., 2009; Kidd *et al.,* 2009). The most common microorganisms causing acidification and used for leaching are *Acidithiobacillus ferrooxidans* (Fe and S oxidiser), *Leptospirillum ferrooxidans* (Fe oxidiser), *Acidithiobacillus thiooxidans* (S–oxidiser, cannot oxidise pyrite), *Thiobacillus thioparus* (sulphide-oxidiser, neutrophilic), sulphate reducing bacteria (metal precipitation as sulphide). Microorganisms catalyse chemical reactions, like *Streptomyces thermocarboxydus* that transforms Cr^{VI} into $Cr^{III,}$ which decreases its toxicity (Shen and Yang, 2008).

Genetically engineered microbes for enhanced phytormediation

A genetically modified organism (GMO) is an organism whose genetic material has been altered using genetic engineering techniques. Organisms that have been genetically modified include micro-organisms such as bacteria and yeast, insects, plants, fish, and mammals. GMOs are the source of genetically modified foods, and are also widely used in scientific research and to produce goods other than food. The bacteria capable of degrading certain kind of organic pollutant, such as polychlorinated biphenyls (PCBs) have been isolated from a range of sites (Brazil *et al.,* 1995). Most of these bacteria cannot survive in the near-starvation conditions found in the rhizosphere (Normander *et al*., 1999). For heavy metals, *Arabidopsis thaliana* gene for phytochelatin synthase (PCS; PCSAt)into *Mesorhizobium huakuii* subsp. rengei strain B3 and then established the symbiosis between *M. huakuii* subsp. rengei strain B3 and *Astragalus sinicus*(Sriprang *et al*., 2003). The gene was expressed to produce phytochelatins and accumulate $Cd^{2+,}$ under the control of bacteroid-specific promoter, the nifH gene. The first organophosphorus degrading (opd) gene was found in *P. diminuta* and was shown to be present on a plasmid (Serder *et al.,* 1982; Bhatia and Malik, 2011).

Plant internal physiological mechanism

Plants employs internal physiological mechanisms of metal detoxification to avoid toxicity (Kramer, 2010). Detoxification involves chelation of the metal cation by ligands or organic acids, or vacuolar or cell wall sequestration away from metabolic sites in the cytoplasm, usually within localized areas in the shoot (Baker *et al*., 2000; Lasat and Kochian 2000; Salt *et al.,* 2000; Broadhurst *et al.,* 2004; Chaney *et al.,* 2007; Memon and Schroder, 2009).

A.*Chelation, ion uptake and metal loading*

The three main steps in inorganic ion transport in the symplastic pathway are active transport of metal across root membranes; entry of metal into symplast during translocation from root to shoot; and chelation and sequestration of metals into specific compartments in the leaves(Maestri *et al.,*2010). The uptake of metal requires transport across the root cell membrane into the symplast. This process involve specific membrane transporter proteins (Williams *et al.,* 2000; Maser *et al*., 2001). The role of genes in transporting metals across electrochemical gradients and in translocation. The overexpression of certain genes in hyperaccumulators leads to greater amounts of chelators and transporters within the plant (Verbruggen *et al.,* 2009). Different organic acids and ligands have been found to be associated with various metals in distinct parts of different plants; for example, in *T. caerulescens,*most Zn in roots was associated with histidine, while in shoots it was associated with organic acids (Verbruggen *et al.* 2009). In *Arabidopsis halleri,* Zn was mostly stored in the vacuoles of mesophyll, while in *T. caerulescens* it was in the vacuoles of the epidermal cells (Verbruggen *et al*., 2009). *Histidine* also plays a key role as a chelator in the tolerance as well as the high rate of translocation of Ni in *A. lesbiacum* (Kramer, 2010).

B.Translocation

For root shoot translocation of heavy metals, metal transporters export metal ions out of the root symplast into the xylem apoplast(Marschner,1995).The major difference between hyperaccumulators and metal excluders seems to be that the hyperaccumulators translocate a significantly greater percentage of accumulated heavy metal ions to their shoots for sequestration in the leaves, whereas metal excluders, while still being able to contain large amounts of heavy metals (Lasat and Kochian, 2000; Broadhurst *et al.*, 2004; Memon and Schroder, 2009; Richau *et al.,* 2009; Salt *et al*., 2000; Verbruggen *et al.,* 2009).

C.Root anatomy and physiology

Mench *et al.* (2009) found that *Thlaspi caerulescens* develops a periendodermal layer, a zone in the roots with thickened inner tangential cell walls which form a continuous layer, externally attached to the endodermis. Richau *et al.* (2009) also compared Ni translocation patterns of *T. caerulescens* and *T. arvense*. They found that the high rate of Ni translocation in *T. caerulescens* compared to *T. arvense* seems to be dependent on two factors: greatly enhanced concentration of root histidine in *T. caerulescens* and a strongly decreased ability to accumulate histidine-bound Ni in root cell vacuoles in *T. caerulescens.*

D. Metal Localization and Sequestration

One class of metal chelating molecules that may play role in sequestration- they are upgraded under codition of high metal availability – are the metallothioneins (MTs). Broadhurst *et al.* (2004) found that the majority of hyperaccumulated Ni in five *Alyssum* hyperaccumulator species grown in Ni-enriched soils is stored in either the leaf epidermal cell vacuoles or in the basal portions of the numerous stellate trichomes. Ghasemi *et al.* (2009) measured Ni accumulation in the trichomes of the serpentine-endemic Ni-hyperaccumulator *Alyssum inflatum.* Galeas *et al.* (2006) looked at seasonal differences in the uptake of Se and S (chemically similar elements) in two hyperaccumulators, including *Stanleya pinnata,* over two growing seasons.

Metal resistance mechanisms

Some metals such as, zinc, copper, nickel and chromium are essential or beneficial micronutrients for plants, animals and microorganisms (Olson *et al*., 2001) while others (e.g., cadmium, mercury and lead) have no known biological and/or physiological functions. When the bacterial cells are exposed to the high concentrations of heavy metals, the metals react within cells with various metabolites and form toxic compounds. Mechanisms for uptake of these metal species are present in the bacterial cell through which heavy metals enter the cell. Generally, there are two types of uptake mechanisms for heavy metals: one of them is quick and unspecific which is driven by a chemiosmotic gradient across the cell membrane and consequently, does not require ATP. In contrast, the second process of metal uptake is comparatively, slower and more substrate-specific and is dependent upon the energy released from ATP hydrolysis (Ahemad, 2012; Spain and Alm, 2003). Heavy metal, copper is utilized by bacterial cells in small quantities in biosynthesis of metabolic enzymes like, cytochrome c oxidase. Bacteria in different ecosystems including soil and water, are exposed to very high concentration of this metal as high levels of copper exists in soil ecosystem due to its wide application in mining, industry processes, and agricultural practices (Singh *et al*., 2007). For survival under metal-stressed environment, bacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions (Nies, 1999). These mechanisms include (1) exclusion-the metal ions are kept away from the target sites (2) extrusion-the metals are pushed out of the cell through chromosomal/plasmid mediated events (3) accommodation-metals form complex with the metal binding proteins (e.g. metallothienins, a low molecular weight proteins) (Umrania, 2006; Kao *et al*., 2006) or other cell components (4) bio-transformation-toxic metal is reduced to less toxic forms and (5) methylation and demethylation. One of the best known metal hyperaccumulators is *Thlaspi caerulescens*, which is a member of the *Brassicaceae* family and a Cd/Zn hyperaccumulator (Kochian *et al*., 2002).

Conclusion

Rhizoremediation process is a promising approach to restore contaminated sites. However, challenging issues remain to be overcome. Efforts are required to understand how plants will respond to the presence of high levels of toxic metabolites. 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. Microbial activity in the rhizosphere contributes significantly to the sustainability of agriculture and forestry as well as to the remediation of disturbed soils. Selective promotion of desirable rhizosphere processes requires a fundamental understanding of the complex microbial interactions in the rhizosphere. Heavy metal-resistant and plant growth-promoting bacteria can protect plants from the toxic effects of metals, or even enhance metal uptake by hyperaccumulator plants. Finally, it is important to keep in mind that a variety of remediation approaches may be required to accomplish all reclamation goals at a contaminated site. The type of approach or approaches chosen will most likely be site-specific and depend on the desired speed of reclamation as well as the number of dollars dedicated to the reclamation effort.

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