

Chapter 8

Role of Rhizospheric Microbes in Heavy Metal Uptake by Plants

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Abstract Due to industrialization, excessive use of pesticides and fertilizer and improper waste management practices cause heavy metal accumulation in both soil and water. Due to the nondegradable and persistent nature, heavy metals can be accumulated in soils for hundreds of years. They enter the bodies of plants and animals and thereby cause negative health impacts to the environment. Even though the soil heavy metal remediation is a must, it is not an easy task to achieve. Among many physical and chemical methods, phytoremediation plays an important role, due to its efficient and convenient nature. Rhizosphere microbes play an important role in phytoremediation. Since, rhizosphere is the immediate vicinity of the root, the chemical and physical changes in that environment can easily effect heavy metal uptake by the plant. By siderophore production, acidification, releasing plant growth promoters, reducing the plant stress conditions and through redox changes rhizosphere enhances the phytoremediation processes. However, plants can bioconcentrate (phytoextraction) and also bioimmobilize the toxic heavy metals through rhizospheric processes. This chapter summaries the role of rhizospheric organisms for facilitation of heavy metal uptake, the different mechanisms of enhancing the availability of heavy metals in the rhizosphere, the genetic diversity, and the microbial genera that involve in these processes.

Keywords Phytoremediation • Rhizosphere • Agricultural soils • Bioimmobilization • Plant growth promoters

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8.1 Introduction

The term heavy metals is defined as metals with high relative atomic weight and atomic number greater than 20 (Raskin et al. 1994). Some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are required in minute quantities by organisms, and they are defined as trace elements. However, heavy metals such as Pb, Cd, Hg, and As do not have any biological role in living cells, but they cause hazardous effects so that they are labeled as toxic metals. Due to industrialization, excessive use of pesticides and fertilizer and improper waste management practices cause heavy metal accumulation in both soil and water. As they enter to the soil system, they harbor in different fractions; exchangeable ions, insoluble inorganic metal compounds such as carbonates and phosphates, soluble metal compound or free metal ions in the soil solution, metal complex of organic materials, and metals attached to silicate minerals (Marques et al. 2009). Not like many other pollutants, heavy metals do not degrade either chemically or biologically. Therefore, the exchangeable fraction easily enters into the plant and animal bodies and causes bioaccumulation. Bioaccumulation of heavy metals causes a many diseases and disorders in humans, animals, and plants.

Since soil remediation is a global concern, where a variety of physical, chemical, and biological methods are used. Physical remediation techniques include soil washing and soil vapor extraction, whereas chemical remediation involves the use of chemicals to extract pollutants from contaminated media. Soil physical and chemical remediation are quite costly and need skilled labor. In biological remediation, both plants and microbes are used in the process of pollutant removal (Khan et al. 2000). Bioremediation is a cost-beneficial and an environmental friendly method.

Phytoremediation is a branch of bioremediation that uses plants for the removal of pollutants from contaminated soils. It is effective for contaminated sites with pollutants that are distributed within the root zone of the plant (Garbisu and Alkorta 2003). The rhizosphere bacteria which inhabit the root zone of the plant play an important role in phytoremediation process, via various mechanisms.

Rhizosphere is the immediate vicinity of the root. Since most of the physical and chemical activities which take place in the rhizosphere have a direct impact on the root system. It is well understood that plant–microbe interactions determine the efficiency of metal extraction. Metalliferous plants are able to grow on trace element-enriched soils and rocks without any symptoms of toxicity. Comparisons between sterile and nonsterile soil systems showed that heavy metal accumulators achieve their full accumulation capacity only in the presence of their indigenous rhizosphere microflora (de Souza et al. 1999). Whiting et al. (2001) showed that *Thlaspi caerulescens* plants inoculated with rhizosphere bacteria accumulated the highest amounts of Zn. Different mechanisms, EPS production, rhizosphere acidification through organic acids, siderophore production, indole-3-acetic (IAA) or 1-amino-1-cyclopropanoic acid (ACC) deaminase production, or the release of growth-limiting

Table 8.1 Heavy metal adsorption by rhizospheric bacteria

Name of the organism	Heavy metal	Reference
<i>Mesorhizobium amorphae</i>	Zn	Hao et al. (2014)
<i>Paenibacillus jamilae</i>	Pb, Cd, Co, Ni, Zn, and Cu	Pérez et al. (2008)
<i>Pseudomonas aeruginosa</i>	Cu, Pb, Zn	Teitzel and Parsek (2003)
<i>Azotobacter</i>	Cd, Cr	Joshi and Juwarkar (2009)
<i>Rhizobium etli</i>	Mn(II)	Pulsawat et al. (2003)
<i>Bacillus firmus</i>	Pb, Cu, and Zn	Salehizadeh and Shojaosadati (2003)
<i>Enterobacter cloacae</i>	Cr(VI)	Iyer et al. (2004)
<i>Ralstonia</i>	Cd	Chompothawat et al. (2010)
<i>Micrococcus luteus</i>	Cu, Pb	Puyen et al. (2012)

nutrients from the soil, are involved in improving the rate of heavy metal accumulation in plants. This chapter discusses the role of rhizospheric microorganisms in heavy metal uptake, the different mechanisms engaged, the rhizospheric microorganisms involved in plant heavy metal uptake, the research needs, and the future direction.

8.2 Role of Microbial EPS Production in Bioremediation

Among many different mechanisms, extracellular polymeric substances (EPS) play a very important role in biosorption of heavy metals, and EPS are produced by most bacteria (Table 8.1). Rhizosphere bacteria produce more EPS than non-rhizospheric isolates (Kunito et al. 2001). The exopolymer production increased in the presence of Cu, and this was more prominent for the isolates from the rhizosphere. The harmful effect of Cu on the growth rate was small for the Cu-resistant bacteria which produce a high quantity of exopolymers. This was explained as the involvement of exopolymers in the detoxification of Cu (Kunito et al. 2001). Exopolymers that are produced by bacteria are able to bind strongly with trace elements (Bitton and Freihofer 1977) and form organo-metallic complexes, which are difficult to degrade or decompose naturally (Hattori 1996). Moreover, the trace element concentrations enhance the production of exopolymers (Kidambi et al. 1995). The EPS production is reported as a potential mechanism of mercury tolerance in bacteria (Cruz 2014). EPS with different chemical compositions was tested for their ability to sorb mercury, and it was observed that the EPS containing hexosamines was the most effective in removing mercury from the solution whereas EPS consisting neutral sugars removed the least amount of mercury from the solution (Cruz 2014). Studies reported that EPS producing *Azotobacter* spp. was able to bind CrO_4^{2-} and Cd^{2+} (Joshi and Juwarkar 2009). Other than bacteria, biofilms, which are communities of microorganisms, are also able to produce EPS (Flemming et al. 2007). It has been reported that bacterial EPS production is involved in many heavy metal adsorption (Lau et al. 2005). The adsorption of heavy metals is higher in bacterial biofilms

compared to their planktonic counterparts. A number of studies have shown the ability of biofilms in heavy metal adsorption (Jang et al. 2001; Ueshima et al. 2008; Wilson et al. 2001). EPS production is enhanced under low N, P, and S contains and also in the presence of high content of C (Czaczyk and Myszka 2007).

EPS comprise a mixture of polysaccharides, mucopolysaccharides, humic substances, and proteins, which depends on the strain and its culture conditions (Ahemad and Kibret 2013). The proportion of EPS in biofilms can comprise between approximately 50 and 90 % of the total organic matter (Donlan 2002). The polysaccharides in Gram-negative bacteria are neutral or polyanionic. Uronic acids or ketal-linked pyruvates enhance their anionic properties which enhance the adsorption ability of divalent cations to the biofilm (Vu et al. 2009). In some Gram-positive bacteria, the chemical composition of their EPS could be slightly different due to their cationic nature (Sutherland 2001). It was revealed that the EPS produced by Ni-resistant *Cupriavidus pauculus* bacteria isolated from serpentine soil was a homopolymer of rhamnose containing uronic acid, protein, and nucleic acid (Pal and Paul 2013).

Quorum sensing (QS) is one of the regulatory pathways for EPS production (Masák et al. 2014). QS in EPS production is a very complex process. In *Pseudomonas aeruginosa*, QS is essential for adhesion EPS production and biofilm formation. They have two QS systems, namely LasI/LasR and RhlI/RhlR. Mutant *P. aeruginosa* cells, which do not produce any QS signals were found to be more densely populated with a very thin EPS matrix compared to the wild type (Gupta and Schuster 2012). *Rhizobium meliloti* is a soil bacterium, which fixes nitrogen in symbiotic association with the leguminous plant *Medicago sativa* (Alfalfa). It produces succinoglycan as its major exopolysaccharide which is a polymer of repeating octasaccharide subunits (Leigh and Walker 1994). Similar to other EPSs succinoglycan is also originated from cytoplasmic sugars.

exoR and exoS are involved in the regulation of EPS I synthesis in the free-living state of *Rhizobium meliloti* (Reuber et al. 1991). In addition, they have discovered that *R. meliloti* has a latent capacity to synthesize a second exopolysaccharide (EPS II) that can substitute for the role(s) of EPS I in nodulation of alfalfa (Glazebrook et al. 1990). Products generated by exoR and exoS play a negative roles in EPS synthesis.

Pseudomonas sp. is a model organism which has been used to study the EPS production (Wei and Ma 2013). It has been reported that three exopolysaccharides (Psl, Pel, and alginate) are produced by *P. aeruginosa*. The Psl cluster consists of 15 co-transcribed genes (*pslA* to *pslO*, PA2231-2245) which encodes for proteins to synthesize Psl. Psl was found to contain a repeating pentasaccharide consisting of D-mannose, D-glucose, and L-rhamnose (Byrd et al. 2009). Pel polysaccharide is a glucose-rich and cellulase-sensitive extracellular substance which is synthesized by the products of the *pel* gene cluster (*pelA-F*, PA3058-PA3064) (Cruz 2014). Alginate is the exopolysaccharide that is mainly produced by *P. aeruginosa* during pathogenicity.

EPS are a complex mixture of biomolecules, consist of proteins, humic-like substances, polysaccharides, uronic acid, nucleic acid, lipids, and glycoproteins, surrounding the bacterial cells (Sheng et al. 2010). The major functional groups of

EPS can be identified by Fourier transform infrared (FTIR) spectroscopy. These substances contain ionizable functional groups such as carboxyl, phosphoric, amine, and hydroxyl groups, which enable EPS to sequester heavy metals (Liu and Fang 2002; Seneviratne et al. 2015). Most of these functional groups are negatively charged at neutral pH and thereby able to form organometallic complexes with multivalent cations through electrostatic attraction, ion exchange, complexation with functional groups of negatively charged, adsorption and precipitation are the mechanisms involved in metal biosorption (Gutnick and Bach 2000; Zhang et al. 2006). These substances thus detoxify metals by complex formation or by forming an effective barrier surrounding the cell (Rajkumar et al. 2010). The FTIR spectral region between 4000 and 400 cm^{-1} holds the major characteristic bands of the various bonds in EPS functional groups.

EPS contain different complexing sites; “hard” (e.g., carboxylic and phenolic) and “soft” (e.g., nitrogen and sulfur-containing) (Zhu et al. 2012). The main electron donor atoms in the EPS are nitrogen in amino-sugars and oxygen in hydroxyl and carboxyl groups. These atoms can easily bind with soft metal cations of strong covalent characteristics (Pb^{2+} , Cu^{2+} , and Zn^{2+}) (Fang et al. 2011). Whereas, other metal cations such as Ni^{2+} and Cd^{2+} form weaker covalent bonds with EPS ligands (Joshi and Juwarkar 2009). The environmental pH also effect on the metal adsorption process by EPS. Metal ion affinity by EPS varies under different conditions, $\text{Pb} > \text{Cu} > \text{Cd}$ at pH 6 and $\text{Cu} > \text{Pb} > \text{Cd}$ at pH 7–8 (Comte et al. 2008).

8.3 Rhizosphere Acidification and Heavy Metal Mobilization

Recent research studies imply that microorganisms could be the key players in heavy metal mobilization. Soil microflora increases the solubility and speciation of heavy metal ions by secretion of various organic ligands and by decomposition of organic matter. Both bacteria and plant roots are able to produce organic acids, such as malate, citrate, gluconate, 2-oxoglutarate, succinate, and oxalate. Thereby it creates a low pH environment in the rhizosphere and enhances the heavy metal mobilization and uptake. As the microbes consume root exudates, they are involved in the production of a wide range of organic acids especially in situations where nutrients may be limiting (Rózycki and Strzelczyk 1986). This could be the reason for higher heavy metal uptake in contaminated sites since most of those sites contain nutrient low degraded soils. It has been reported that LMWOA influence heavy metal speciation and the bioavailability of heavy metals to plants and microorganisms (Renella et al. 2004).

Organic acids can bind metal ions in soil solution by complexation reaction. However, the stability of the ligand-metal complexes is dependent on several factors: number of carboxylic groups and their position, the binding form of the heavy metals, pH of soil solution (Jones 1998). Organic acids released by plant-associated microbes play an important role in the complexation of toxic and essential ions and increase their mobility for plant uptake. It was observed that Zn solubilizing

Gluconacetobacter diazotrophicus produce gluconic acid derivative, 5-ketogluconic acid, which aids in the solubilization of Zn compounds. Similarly, mobilization of Pb and Zn were observed with inoculation of common metal-resistant *Bacillus* strains (Wani et al. 2007). The metal-resistant endophytic bacteria, *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 have also been reported to enhance Pb accumulation in *Brassica napus* through excretion of organic acid. The organic acid producing fungi, *Aspergillus niger* was able to mobilize large amounts of Pb and P from pyromorphite indicated the presence of organic acid in dissolution of minerals (Sheng et al. 2008).

The role of organic acids, acetic and malic acids was exhibited in a study stimulating Cd uptake by maize roots and reported that the organic acid with low stability constant was able to enhance large amount of Cd accumulation in maize (Han et al. 2006). On the other hand, some studies have reported either no effect or negative effects of organic acids in heavy metal mobilization (Braud et al. 2006). The inoculation of organic acid producing bacteria *Bacillus subtilis* in metal contaminated agriculture soils showed no significant effect on the mobilization of Cr and Pb (Braud et al. 2006).

Organic acids function as natural chelating agents which are capable of solubilizing heavy metals from soil (Wasay et al. 1998). *Pseudomonas fluorescens* is a very important rhizobacterium in rhizosphere (Rodríguez and Fraga 1999; Sivasakthi et al. 2013). It produces a variety of organic acids for various functions in the rhizosphere. The gluconic acid production in fluorescent pseudomonads is catalyzed by membrane-bound glucose dehydrogenase (Gcd). In many Gram-negative bacteria, the synthesis of gluconic acid is dependent on pyrroloquinoline quinone (PQQ) as an enzymatic cofactor of the Gcd (de Werra et al. 2009). Biosynthesis of citric acid, which is also an effective organic acid involves condensation of oxaloacetate (OAA) and acetyl-CoA catalyzed by the enzyme citrate synthase.

8.4 Siderosphere Production by Bacteria

Siderophores are low-molecular-weight (>10 kDa) iron chelating compounds that are mainly produced under low Fe conditions by bacteria, fungi, and plants to facilitate uptake of iron (Chu et al. 2010; Hider and Kong 2010). Siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Whereas they can also form stable complexes with other heavy metals (Glick and Bashan 1997; Rajkumar et al. 2010). Hence, enhance their bio-availability in the rhizosphere. Binding of the siderophore to a metal increases the soluble metal concentration. Screening with 16 different metals (Ag^+ , Al^{3+} , Cd^{2+} , Co^{2+} , Cr^{2+} , Cu^{2+} , Eu^{3+} , Ga^{3+} , Hg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Sn^{2+} , Tb^{3+} , Tl^+ , and Zn^{2+}) it is revealed that pyoverdine siderophores produced by *Pseudomonas aeruginosa* are able to chelate all these metals (Braud et al. 2009a).

Table 8.2 Siderophore production by rhizospheric bacteria

Bacterial sp.	Metal	Plant	Reference
<i>Streptomyces tendae</i>	Cd	<i>Helianthus annuus</i>	Dimkpa et al. (2009a)
<i>Pseudomonas eruginosa</i>	Pb, Cr	<i>Zea mays</i>	Braud et al. (2009b)
<i>Bacillus</i> sp. SLS18	Mn, Cd	<i>Sorghum bicolor</i>	Luo et al. (2012)
<i>Psychrobacter Bacillus weihenstephanensis Bacillus cereus</i>	Ni	<i>Alyssum serpyllifolium and Phleum phleoides</i>	Ma et al. (2009)
<i>Pseudomonas putida</i>	Pb, Cd	<i>Vigna radiata</i>	Tripathi et al. (2005)

About 500 different siderophores have been identified. Even though they differ in overall structure, the functional groups that coordinate Fe are not diverse. They are broadly classified into three main groups based on the chemical nature of the moieties donating the oxygen ligands for Fe(III) coordination, classified as hydroxycarboxylate, catecholate, or hydroxamate type siderophores (Raymond and Dertz 2004). The biosynthetic pathways of siderophores are tightly connected to aerobic metabolism involving molecular oxygen and acids (citrate, succinate, and acetate) originating from the final oxidation of the citric acid cycle.

The production of siderophores in the rhizosphere involves in growth-promoting effect of bacteria on plants (Braud et al. 2009b). The role of SPB in metal uptake of hyperaccumulator plants are extensively studied (Table 8.2). Siderophores produced by soil microbes play an important role in complexing toxic metals and radionuclides and in increasing their mobility in soils. Metal-resistant SPB can increase the efficiency of phytoextraction directly by enhancing the metal accumulation in plant tissues (Dimkpa et al. 2009b; Rajkumar et al. 2010). Siderophores produced by rhizosphere bacteria solubilize unavailable forms of heavy metal-bearing minerals by complexation (Braud et al. 2009b). Plants can then uptake metals from metal–siderophore complexes possibly by root-mediated processes, such as chelate degradation and release of metals, the direct uptake of siderophore–metal complexes or by a ligand exchange reaction. The production of siderophores has also been demonstrated in some mycorrhizal fungi also. It has been reported that the ectomycorrhizal fungi (EMF), *Scleroderma verrucosum*, *Suillus luteus*, and *Rhizopogon luteolus* produce catecholates and hydroxamates siderophores under iron-deficient conditions (Goodell et al. 1997; Machuca et al. 2007). It is suggested that siderophore-producing microbes are possible to improve heavy metal uptake in plants. However, the mechanisms essential for the plant metal uptake through microbial siderophore-mediated processes are still under research.

Table 8.3 Biosurfactant production by bacteria

Class of the surfactant	Microorganism	References
Rhamnolipids	<i>Pseudomonas aeruginosa</i> , <i>Acinetobacter</i> , <i>Enterobacter</i>	Toribio et al. (2010), Hořková et al. (2013)
Trehalolipids	<i>Mycobacterium tuberculosis</i> , <i>Rhodococcus erythropolis</i> , <i>Arthrobacter</i> sp.	Kuyukina and Ivshina (2010), Shao (2011), Desai and Banat (1997)
Sophorolipids	<i>Torulopsis bombicola</i>	Inoue and Ito (1982)
Corynomycolic acid	<i>Corynebacterium lepus</i>	Cooper et al. (1979, 1981a)
Fatty acids, phospholipids, and neutral lipids	<i>Rhodococcus erythropolis</i> , <i>Acinetobacter</i> sp.	Rahman and Gakpe (2008)
Surfactin	<i>Bacillus subtilis</i>	Cooper et al. (1981b)
Lichensyn	<i>Bacillus licheniformis</i>	Yakimov et al. (1996)
Polymeric biosurfactants (emulsan, alasan, lipomanan liposan)	<i>Acinetobacter calcoaceticus</i> , <i>Candida lipolytica</i>	Rubinovitz et al. (1982), Rufino et al. (2007)

8.5 Biosurfactants

Biosurfactants are microbial metabolites that facilitate the metal mobilization and improve phytoremediation is microbially produced. These are microbial compounds that demonstrate high surface activity and emulsifying activity. They are amphiphilic molecules with a nonpolar (hydrophobic) tail and a polar/ionic (hydrophilic) head. A hydrophilic group consists of mono-, oligo-, or polysaccharides, peptides or proteins, and a hydrophobic moiety usually contains saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols (Lang 2002). Biosurfactants are categorized as glycolipids, lipopeptides, phospholipids, fatty acids, and neutral lipids. They are either anionic or neutral (Mulligan et al. 2001).

The microorganisms produce low- and high-molecular-weight biosurfactants. The low-molecular-weight types are generally glycolipids or lipopeptides. The glycolipids include trehalose tetraesters, dicorynomycolates, fructose lipids, sophorolipids, and rhamnolipids. Lipopeptides include surfactin, viscosin, and polymixin (Table 8.3).

The role of surfactants possesses different mechanisms in metal removal process. According to the le Chatelier's Principle, metals in a nonionic form can complex with biosurfactants and removed from the surface. Cationic surfactants also have the ability to reduce the association of metals by competition (Herman et al. 1995). The biosurfactants produced by microbes form complexes with heavy metals at the soil interface, desorbs metals from soil matrix, thus increasing metal solubility and bioavailability in the soil solution. Soil type, soil pH, cation exchange capacity (CEC), and particle size also influence biosurfactant action. They act as a soil washing agent due to their ability to solubilize metals within their micellae.

Due to their anionic nature, low toxicity, biodegradability, and excellent surface-active properties of biosurfactants are used in heavy metal removal in soil. The ability of biosurfactants to remove heavy metals from an oil-contaminated soil was demonstrated by batch washes with surfactin, a rhamnolipid, and a sophorolipid, respectively, by *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Tomlopsis bornhicola* (Mulligan et al. 1999), whereas they observed surfactin or rhamnolipid could remove the organically bound copper and that the sophorolipid with acid could remove the carbonate and oxide-bound zinc.

8.6 Metal Reduction and Oxidation

Oxidation or reduction reactions are also involved to alter the bioavailability of heavy metals in the plant microbial system. Metal oxidation by rhizosphere microbes is also an interesting and an important process for phytoextraction process. Sulfur-oxidizing bacteria was found to be better than acid treatment for heavy metal solubilization in sulfidic municipal sludges (Blais et al. 1992). A study compared the leaching potential of indigenous sulfur-oxidizing bacteria with acid treatment and they concluded that indigenous sulfur-oxidizing bacteria can be used for heavy metal mobilization (Seidel et al. 1998).

Iron- and sulfur-oxidizing bacteria, *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, respectively, were enriched from contaminated soil and were able to leach >50 % of the metals present (As, Cd, Co, Cu, Ni, V, Zn, B, and Be) (Gomez and Bosecker 1999). It was observed that sulfur-oxidizing rhizosphere bacteria are able to enhance Cu mobilization in contaminated soils and its uptake in plant tissue (Shi et al. 2011). The authors showed that the sulfur-oxidizing bacteria are able to reduce the rhizospheric soil pH by the conversion of reducing sulfur to sulfates, thus enhances the Cu availability for plant uptake. Applications of sulphur-oxidizing bacteria in combination with mycorrhizal infection resulted in a significant additive effect on root's Cd uptake and root bioaccumulation (Khorrami Vafa et al. 2012). While (Yang et al. 2012) reported that addition of the As-reducing bacteria promoted the growth of *P. vittata*, it has increased As accumulation (44 %), activated soil insoluble As, and reduced As leaching compared to the untreated control.

The synergistic interaction of metal-oxidizing and -reducing microbes on heavy metal mobilization in contaminated soils has also been studied. The co-inoculation of Fe-reducing bacteria and the Fe/S-oxidizing bacteria significantly increased the mobility of Cu, Cd, Hg, and Zn by 90 % (Beolchini et al. 2009).

8.7 Stress Reduction

The phytoremediation process is also dependent on the plant's ability to tolerate heavy metal toxicity and also to yield a certain biomass. Heavy metals (HMs) are toxic for plant growth development and reproduction. It is one of the major abiotic

stresses that cause detrimental effects to the plants growth. The redox-active HMs are directly involved in the redox reactions in cells and result in the reactive oxygen species. Redox-inactive HMs also results in oxidative stress through indirect mechanisms such as interaction with the antioxidant defense system, disruption of the enzymatic reactions, or induction of lipid peroxidation. The result of HM toxicity is the excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, and DNA damages in plants. The oxidative stress causes discolouration of leaves, deformation of leaves, growth retardation, leaf curling, and disorders in physiological and biochemical reactions. Thereby, it reduces the accumulation of sufficient biomass of the pollutant.

Production of ethylene is one major signal molecule that induces the stress effects that enhances senescence. The endogenous production of ethylene is enhanced significantly, and it causes harmful effects on root growth and thus the growth of whole plant. Certain plant growth-promoting rhizobacteria (PGPR) contain the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which regulates ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into alpha-ketobutyrate and ammonia (Saleem et al. 2007). Inoculation of plants with rhizobacteria containing ACC deaminase activity or transgenic plants expressing ACC deaminase genes produce longer roots and greater root density.

Most of the ACC are synthesized by plant roots, which are subjected to hydrolysis by ACC deaminase bacteria to produce ammonia and a-ketobutyrate. Microbes are able to uptake and then hydrolyze the ACC, and thereby it results subsequent reduction of ACC amount outside the plant. To maintain the equilibrium between the internal and external ACC levels, plants secrete ACC from inner tissues into the rhizosphere. Rhizobacteria containing ACC deaminase activity stimulates ACC exudation from plant roots. And consumption of ACC by microorganisms, which acts as a source of carbon and nitrogen is a driving force for ACC secretion by the plant. The growth of microorganisms containing ACC deaminase is more prominent in the rhizosphere compared to the bulk soil. And consumption of ACC by microorganisms, acts as a driving force for ACC secretion by the plant. As a result, it decreases the level of ACC within the plant, which leads to a reduction in the endogenous ethylene biosynthesis (Glick et al. 1998). The stress reduction in plants cause elimination in root inhibition and enhance root growth which cause an enhanced uptake of inorganic contaminants. It has been reported that due to the inoculation of ACC deaminase-producing heavy metal-resistant bacterium, *Kluyvera ascorbata* was able to protect canola (*Brassica napus*) and tomato (*Lycopersicon esculentum*) seeds from the toxicity of high concentrations of nickel chloride (NiCl_2) grown under gnotobiotic conditions (Burd et al. 1998). They ascribed this effect to the ability of the bacterium to lower the level of stress ethylene induced by Ni.

8.8 Plant Growth Promotion

It has been known that 80 % of microorganisms that isolated from the rhizosphere of various crops possess the ability to synthesize and release plant growth regulators (Patten and Glick 1996). IAA is the major plant growth regulator synthesized by the microorganisms. The endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria (Arshad et al. 2007; Glick 2012). IAA stimulates seed and tuber germination, increases the root development, and initiates lateral and adventitious root formation and helps to develop resistance to stressful conditions in plants. IAA produced by rhizobacteria interferes with the above ground physiological processes of plants by changing the plant auxin pool. Whereas bacterial IAA increases root surface area and length, and thus increase the soil nutrient uptake (Glick 2012).

It has been well documented that there are at least five different pathways for the synthesis of IAA (Spaepen and Vanderleyden 2011; Patten and Glick 1996). (1) IAA formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in a majority of bacteria, namely, *Erwinia herbicola*, *Pseudomonas*, *Bradyrhizobium*, *Rhizobium*, *Azospirillum*, *Klebsiella*, and *Enterobacter*, (2) The conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway, which is found in pseudomonads and azospirilla, (3) IAA biosynthesis via indole-3-acetamide formation, which is reported for phytopathogenic bacteria *Agrobacterium tumefaciens*, *Pseudomonas syringae*, and *E. herbicola*, (4) IAA biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in the cyanobacterium (*Synechocystis* sp.), and (5) the tryptophan-independent pathway which is more common in plants and cyanobacteria (Ahemad and Kibret 2014).

8.9 Future Perspectives

Even though phytoremediation is a very important aspect in pollution remediation, the role of rhizospheric organisms is very poorly understood. In several studies, it has been revealed that inoculation of heavy metal-resistant microbes is able to enhance the metal uptake. However, further studies should be conducted to determine the mechanisms behind their role.

- The role of siderophores in plant heavy metal uptake has been discussed in many research studies, but there are no exact mechanisms that have developed to understand the role of siderophores in plant metal uptake and more research towards siderophore interactions with heavy metals will add knowledge into science.
- Only a few researches have focused on the production of low-molecular-weight organic acids and their role in rhizosphere acidification. Further studies may enhance the understanding how the low-molecular-weight organic acids increase

rhizosphere acidity to dissolve more metals into the soil solution. In this case, the monoprotic, diprotic, and triprotic organic acids may need more attention to observe their role in reducing pH of the soil solution.

- Use of biosurfactants in removing organic pollutants have been investigated well in recent years; however, it has not been investigated against heavy metals. Therefore, more attention towards this may increase the potential of biosurfactants in the field of environmental sciences.
- Many laboratory and pilot scale studies already showed that rhizoremediation can contribute to the restoration of polluted sites. However, a selection of suitable rhizoremediation system consisting of a plant inoculated with a bacterium or a consortium with degradation capacity has not been investigated thoroughly.
- Interaction of multiple metals with multiple plants and microorganisms is also an aspect which needs further attention as in the actual field various different types of microorganisms or heavy metals may hinder the rhizoremediation. Heavy metals in diverse valance forms, anionic and cationic forms behave in a different way. Depending on various conditions such as microorganisms present, their ability to secrete EPS, biosurfactants, pH of the rhizosphere, etc., the rhizoremediation capacity may vary. This needs further studies.
- The microbial processes can enhance phytoextraction either by increasing the solubility of the metals in the soil through the production of siderophore, organic acid or biosurfactant or promote plant growth via the IAA or ACC deaminase or the release of growth-limiting nutrients from the soil. However, only a modest attention has been given to elucidate the processes that have the greatest impact on phytoextraction efficiency. More research on these areas will generate new information and knowledge.
- Studies using molecular and biotechnological advances towards biosurfactants, siderophores, plant growth promoters, etc. may yield useful novel information where engineered systems can be constructed for phytoremediation where artificially altered microbial functions supports plant growth promotion and phytoextraction. Such advanced systems will be an interesting tool to further improve and develop bioremediation.

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