Plant–Microbe Interaction for the Removal of Heavy Metal from Contaminated Site

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Abstract

The diversity of microbes present in the rhizosphere plays a significant role in nutrient cycling and soil sustainability. Plant–microbe-modulated phytoremediation is a viable technology for the cleanup of contaminated environments. Several plants that were identified have various degrees of capacity to eliminate, degrade or detoxify, metabolize, or immobilize a wide range of soil contaminants. Plant-based remediation technologies are not yet commercialized because of its major limitation of slow process and restricted bioavailability of the contaminants, and it is greatly influenced by the climatic factors. The extensive use of plants can overcome most of the limitations by exploring the potential of microbe–plant–metal interaction. The biogeochemical process occurring in the root zone can influence on several rhizobacteria and mycorrhizae directly linked with microbial metabolite synthesis. Thus, a holistic approach of novel remediation technologies and understanding of plant–microbe–contaminant interaction would help for customizing phytoremediation process in relation to site-specific contamination. There is a huge challenge to remediation of contaminated sites by long-term accumulation of heavy metal. Unlike organic contaminants, metals are very much resistant to degradation, and in the long run, continuous accumulation may cause food chain contamination. It is very important to decontaminate the polluted sites in order to reach safe level of metal concentration below the threshold limit of toxicity. Recent studies revealed that phytoextraction, mainly the use of hyperaccumulator plants to extract toxic metals from the contaminated sites, has emerged as a cost-effective, eco-friendly cleanup technology. Novel, efficient microbes and their potential use in the plant rhizosphere could further enhance the phytoremediation for wider range of soil contaminants.

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11.1 Introduction

The era of industrial revolution and rapid urbanization caused various degree of soil contamination. The elevated levels of heavy metal at a long time in the soil are excessively absorbed by plant roots and translocated to aboveground parts, leading to impaired metabolism and reduced plant growth (Bingham et al. 1986). The severe soil contamination with various heavy metals tremendously hampered the soil biological function and soil fertility (McGrath et al. 1995) as well as food chain contamination (Richards et al. 2000). The contamination of the soil environment in the long run is considered as a potential threat to the soil ecosystem services. The soil contaminant bioavailability is highly influenced by various factors such as nature of pollutants, clay content, pH, moisture content, hydrogeology, microbial community dynamics, temperature, and redox potential (Dua et al. 2002). Thus, understanding the plant–microbe–heavy metal has received a great attention for the remediation of contaminated site. Biological means of remediation for the contaminated environment are a promising technique that offers the possibility to degrade or detoxify various contaminants by employing plants and microbes. The approaches of bioremediation are more economically viable, environment-friendly, and an aesthetically pleasing approach which is most widely used for the purpose of remediation of contaminated site. Developing sustainable remediation technologies by employing plant and microbes is a promising solution to reestablish the natural state of soil health (Jansen et al. 1994). However, introduction of numerous waste including toxic heavy metals into the soil leads to considerable loss of the microbial diversity, despite their vital role for the growth and survival of microbes at very low concentrations. The plants employing for cleanup of contaminated environments is quite old concept. More than 300 years ago, plants were used for the treatment of contaminated wastewater. During the nineteenth century, *Thlaspi caerulescens* and *Viola calaminaria* were reported as the first plant species to accumulate higher levels of metals in shoots (Baumann 1885). Several reports were available for the heavy metal accumulation plants like genus *Astragalus* which have a high potential to accumulate selenium up to 0.6 % in dry shoot biomass; some plants were indentified for Ni accumulator (1 %) in shoots (Minguzzi and Vergnano 1948), and *Thlaspi caerulescens* for high Zn accumulation (Rascio 1977). The plants used for phytoextraction of metals from the contaminated soil were developed and reintroduced by Utsunamyia (1980) and Chaney (1983). The first field trial for phytoextraction was conducted for Zn and Cd (Baker et al. 1991). Many plants that are classified as hyperaccumulator depend on type of metal and accumulation behavior from the soil. The diversity of plant rhizosphere microbes and mycorrhiza also play key role for the remediation of contaminated site with heavy metals. The key for successful bioremediation depends on the nature and bioavailability of pollutants. The comprehensive understanding is still required to learn the mechanisms and crucial factors influencing the plant–microbe–toxicant interaction in soils for the success of phytoremediation.

11.2 Rhizosphere Microbe-Assisted Phytoremediation

Phytoremediation involves the use of green plants to extract, sequester, degrade, and/or detoxify pollutants by means of biological processes (Wenzel et al. 1999) and has been reported to be an in situ, nonintrusive, cost-effective, ecologically benign, aesthetically pleasing, socially acceptable technology to remediate contaminated soils (Garbisu et al. 2002). It also helps to prevent landscape deterioration and enhances the diversity of soil microorganisms to maintain healthy ecosystems; hence, it is considered to be a more attractive technique than traditional approaches that are currently in use for heavy metal decontamination.

Phytoremediation process can be classified according to the method and nature of the soil pollutants (Salt et al. 1995). Various aspects of phytoremediation process in relation to organic and inorganic contaminants are depicted in the Fig. 11.1.

Phytoremediation techniques can be studied under different strategies such as: (a) Phytoextraction: It is the process by which plants absorb metal from the contaminated site and transfer it to aboveground parts of the plants. These plants have a high degree of potential to absorb and accumulate or translocate metals or metalloids to the aboveground biomass. (b) Phytostabilization: It involves restriction of the mobility of metals in the soil. The reduced mobility of the contaminants may be achieved by accumulation and absorption onto roots, or precipitation within the rhizosphere. (c) Phytostimulation: It is also called plant-assisted biodegradation. Phytostimulation is the process where root-induced microbial activity is capable of degrading the organic contaminants. (d) Phytovolatilization/rhizovolatilization: In this approach, plants take up contaminants from the soil and transformed it into volatile compounds into the atmosphere through transpiration. These methods are highly used for the metal(loid)s in the soil such as mercury (Hg), selenium (Se), and arsenic (As). (e) Phytodegradation: It is the process of enzymatic degradation of

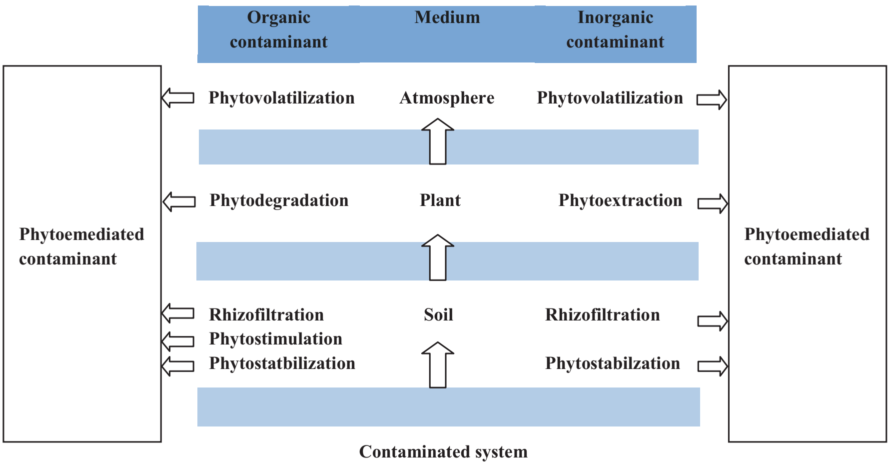

Fig. 11.1 Phytoremediation processes for organic and inorganic contaminants

complex organic molecules to simpler ones by means of enzymatic action or the incorporation of these molecules into plant tissues or into new plant material. (f) Rhizofiltration: It is primarily used to remediate aquatic systems with low levels of contaminant. It can be used for heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), and chromium (Cr) which are generally retained within the roots and do not translocate to the shoots. This method can be explored in both terrestrial and aquatic plants for in situ or ex situ purposes.

11.2.1 Interactions in the Rhizosphere

Efficient phytoremediation techniques rely on the complex interactions among soil, contaminants, microbes, and plants.

11.2.1.1 Plant–Microbe Interactions

The interaction between plant roots and wide range of soil microbes, especially rhizospheric one, is the major determinants of the phytoremediation potential (Glick et al. 1995). Both the micropartner, i.e., plant-associated microbes and the host plant, control the functioning of associative plant–microbe symbioses in the contaminated soil. In plant bacterial symbiosis, plant provides specific carbon source to the bacteria inducing the bacteria to reduce the heavy metal phytotoxicity. Alternatively, in nonspecific association between plants and bacteria, plant metabolic processes stimulate the microbial community through root exudates, which in turn enable the microbes to degrade the contaminants in soil. Moreover, the adaptation capabilities of both the partners of associative symbiosis and the bioremediation potential of the microsymbiont play a vital role in minimizing the heavy metal toxicity.

11.2.1.2 Heavy Metal–Microbe Interactions

Rhizosphere microbes are empowered with different traits that can modify the solubility and bioavailability of the heavy metals in soil (Lasat 2002; McGrath et al. 2001; Whiting et al. 2001). Rhizobacteria may release different chelating substances by which acidification of the environment takes place through production of organic acid and changes the redox potential (Smith and Read 1997). Soil pH reduction mediated through *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *M. arabinogalactanolyticum* has been reported to enhance the Ni uptake in *Alyssum murale* grown in a serpentine soil (Abou-Shanab et al. 2003). An earlier study reported that the metal-polluted sites have negative impact on soil microbial diversity and microbial activities (Giller et al. 1998).

11.2.1.3 Plant–Bacteria–Soil Interactions

The soil condition also dictates the specificity of the plant–bacteria association. Different soil conditions regulate the bioavailability of soil contaminant such as composition of root exudate and levels of nutrient, influencing the bacterial metabolic activity as well as phytoremediation potential. Moreover, the requirements for heavy metals for bacterial metabolism may also govern whether the plant–bacteria interaction would be specific or nonspecific. Along with metal toxicity, there are several other factors that limit plant growth in the contaminated soils including harsh climatic conditions, poor soil structure, low water retention, and nutrient deficiency.

11.2.2 Rhizoremediation: Microorganism-Assisted Phytoremedation

Rhizoremediation is a subprocess of phytoremediation where plants along with their rhizospheric microorganisms are being used to enhance the efficiency of contaminant extraction (Jing et al. 2007). It is a beneficial association where the microorganisms enhance the bioavailability of the metals and the plants help in the extraction and removal of such compounds from soil (Chaudhry et al. 2005). It has positive role for both sides, where the plants supply nutrients to microorganisms, which, in turn, grow and proliferate, increasing the potential of degradation by the plant. However, there is a lack of studies about this synergism between plants and microorganisms facilitating phytoremediation (Kavamura and Esposito 2008). Some beneficial associations among plant and rhizospheric microbes that participated in the rhizoremediation are as follows:

11.2.2.1 Plant Growth-Promoting Rhizobacteria and Rhizoremediation

Plant growth-promoting rhizobacteria are generally known to promote the growth of the plants in the following manner:

1. Fix nitrogen from the atmosphere and deliver it to the plants.
2. Produce siderophores that can make complex with iron present in the soil and make available for assimilation to plant cells. Plants can easily take up the bacterial siderophore–iron complex and also through production of plant hormones like auxins, cytokinins, gibberellins, etc. which may stimulate the growth of the plant.
3. Solubilize mineral nutrients such as phosphorus through production of various organic acids, making them more easily available for plant growth.
4. Act as biocontrol agent.

Several experiments were conducted to examine the ability of a wide range of plants for heavy metal extraction and then to translocate those metals from roots to leaves and shoots. However, the potential of heavy metal removal is limited by slow plant growth and low biomass production by hyperaccumulator plants (Raskin and Ensley 2000). In this context, the use of plant growth-promoting rhizobacteria as adjuncts has been found to stimulate significant growth of plants even in the presence of higher concentration of heavy metals in soil (Zhuang et al. 2007; Glick 2010).

11.2.2.2 Endophytic Microorganisms and Rhizoremediation

Endophytic microorganisms can be defined as microbial colonizations in the internal tissues (root cortex or xylem) of plants without causing any symptoms of infection or negative impacts on their host (Schulz and Boyle 2006). Among the most predominant genera of culturable endophytes are *Pseudomonadaceae*, *Burkholderiaceae*, and *Enterobacteriaceae.* Endophytes play a very important role in phytoremediation especially in rhizoremediation. Idris et al. (2004) studied the endophytes and rhizobacteria with *Thlaspi goesingense*, a hyperaccumulator of Ni using both cultivation and cultivation-independent techniques. Results revealed that endophytes are generally culture independent and are more tolerant to higher concentration of Ni as compared to rhizobacteria. Though endophytes hold great promise for heavy metal remediation, the mechanisms by which endophytes enhanced metal accumulation are yet to be well understood. Furthermore, the application of culture-independent endophytes is quite a challenging task (Weyens et al. 2009).

11.2.2.3 Mycorrhizoremediation

Mycorrhizoremediation is an advanced phytoremediation strategy involving contribution from tripartite association among plant, mycorrhiza, and rhizobacteria. Mycorrhizae can be efficiently explored in the soil microsites that are not accessible for plant roots. They can further change the heavy metal bioavailability through competition with roots and other microorganisms for water and metal uptake, protection of roots from direct contact with the heavy metal via development of the ectomycorrhizal sheath, and restricted metal transport by increasing soil hydrophobicity (Lazcano et al. 2010). Ectomycorrhizal associations are reported to enable the host plant to withstand higher heavy metal toxicity. The structure of the fungal sheath, density, and surface area of the mycelium are key factors to determine the efficiency of an ectomycorrhizal association to resist/tolerate metal toxicity and to protect the host plant from pollutant contact (Hartley et al. 1997). Studies also reported increased uptake of metal(loid)s in the presence of arbuscular mycorrhizal fungi; however, there are some contradictory reports indicating negligible effect or decreased accumulation in plant tissues (Lazcano et al. 2010). The controversial results are difficult to interpret and could be attributed to the differential response under greenhouse experiment and field study.

11.2.3 Phytoextraction

Phytoextraction is a subprocess of phytoremediation where the pollutant-accumulating plants are being utilized for removal of heavy metals from contaminated soils by concentrating them in the aboveground biomass (Salt et al. 1998). The selection of plants for heavy metal phytoextraction should possess features like (a) potential tolerance to high levels of heavy metal concentration, (b) fast-growing plants for effective accumulation of heavy metal, (c) ready translocation of heavy metal in the aboveground biomass of plants, and (d) ease of harvest (Vangronsveld et al. 2009).

However, the success of phytoextraction depends upon factors such as bioavailability of heavy metal and the potential of the plant to intercept, take up, and accumulate the metals in shoots (Ernst 2000).

11.2.3.1 Role of Plant-Associated Rhizobacteria in Phytoextraction

To enhance the efficiency and rate of phytoextraction, the role of plant-associated rhizobacteria is highly beneficial. Microorganisms can increase plant uptake of heavy metal in the following way: (1) may increase the root surface area and root hair architecture, (2) enhance the metal bioavailability, and/or (3) increase the metal translocation from the rhizosphere to the plant shoot (Weyens et al. 2009). Further, improving the plant biomass production can influence the efficiency of trace element phytoextraction.

The plant-associated rhizobacteria metabolic performance may help develop new improved phytoremediation strategies. However, the dynamic and variable metabolic capacities of plant-associated rhizobacteria are still poorly highlighted. Plants stimulate the growth of rhizosphere microorganisms due to secretion of different organic molecules by their roots, which in turn improved the bacterial densities in the rhizosphere (Anderson and Coats 1995).

11.2.4 Bacterial Heavy Metal Resistance

The plant-associated rhizospheric bacteria have several benefits conferred to their hosts; the major qualification for protecting plants from heavy metals stress is resistance of the bacteria to heavy metals. Along with dynamic metabolic capacity of the bacteria, metal resistance operon is also important to empower the bacteria against heavy metal toxicity. Among the heterotrophic bacteria, members of the β-proteobacteria have the maximum levels of heavy metal resistance. *Alcaligenes eutrophus* is a potential member of this group. *A. eutrophus* CH34 species is the extensively reported that harbors two endogenous megaplasmids encoding genes for multiple heavy metal resistance. Plasmid pMOL28 is 180 kb and codes for resistance to various heavy metals such as cobalt, nickel, chromate, mercury, and thallium. Resistance genes are organized with the *chr*, *mer*, *and cnr* operons, coding for resistances to chromate, mercury, and both cobalt and nickel, respectively, (Mergeay et al. 1985; Taghavi et al. 1997). The plasmid from strain CH34 is pMOL30 (240 kb) responsible for resistance against some heavy metals. This plasmid also consists of organized operon out of which the *mer*, *cop*, and *pbr* operons encode resistance to heavy metal mercury, copper, and lead, respectively. The *czc* operon encodes for heavy metal cadmium, zinc, and cobalt resistance.

11.3 Plant–Microbe Association for Heavy Metal Transformation in Soil–Plant System

Rhizospheric microbes play an important role in improving phytoremediation process by changing the metal bioavailability through altering redox reactions, soil pH, or release of some chelators like siderophores, organic acids, biosurfactants, etc. (Zarei et al. 2010; Miransari 2011; Rajkumar et al. 2012) (Fig. 11.2).

Metabolites or reactions produced by plant-associated microbes have been reviewed and summarized in Table 11.1

11.3.1 Siderophores

Most plant-associated microorganisms can produce iron chelator siderophores at low levels of iron concentration in soil; however, siderophore can also form stable complex with other heavy metals such as Al, Cd, Cu, Ga, In, Pb, and Zn (Glick and Bashan 1997; Schalk et al. 2011) and cause solubilization of unavailable form of heavy metal to available form, thus improving efficacy of phytoextraction (Braud et al. 2009b; Rajkumar et al. 2010). Pyoverdine and pyochelin produced by *Pseudomonas aeruginosa* are responsible for enhancing the bioavailability of Cr and Pb in the rhizosphere of maize (Braud et al. 2009b). Similarly, siderophores produced by *Streptomyces tendae* F4 significantly enhanced uptake of Cd by sunflower plant (Dimkpa et al. 2009). Nevertheless, there are also contradictory reports

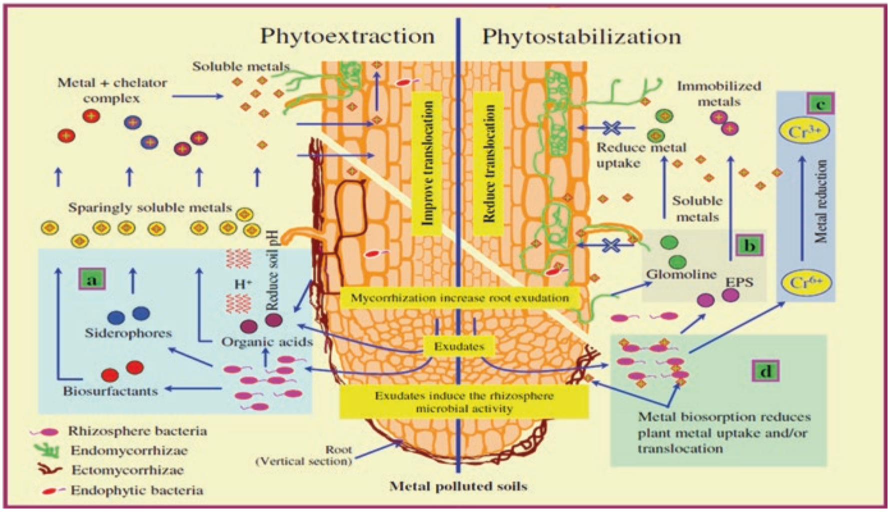

Fig. 11.2 Schematic representation of role of rhizospheric microbes for phytoremediation (**a**) by producing metal-mobilizing chelators, (**b**) by excreting metal-immobilizing metabolites, (**c**) by reducing metal reduction, and (**d**) by metal biosorption. EPS, extracellular polymeric substances (Source: Rajkumar et al. 2012)

Table 11.1 Potential of microbial metabolites/actions to mobilize/immobilize metals by plants

Metabolites or reactions	Microorganisms	Microbial potential	References
Siderophores			
Azoto chelin and azotobactin	*Azotobacter vinelandii*	Helps in Mo and V acquisition	Wichard et al. (2009)
Pyochelin	*Pseudomonas aeruginosa*	Chelates many metals like Cd^{2+}, Cr^{2+}, Al^{3+}, Mn^{2+}, Zn^{2+}	Braud et al. (2009a)
Desferrioxamine and coelichelin	*Streptomyces tendae*	Enhanced uptake of Cd and Fe by plants	Dimkpa et al. (2009)
Organic acids			
Oxalic acid, tartaric acid, formic acid, acetic acid, malic acid	*A. niger*, *Burkholderia cepacia*, *Beauveria caledonica*, *Oidiodendron maius*, *Pseudomonas fluorescens*, *Penicillium bilaiae*	Solubilized Zn, Ni, Fe, Pb, and Cd	Arwidsson et al. (2010), Li et al. (2010), and Hoberg et al. (2005)
Gluconic acid, 5-ketogluconic acid	*Gluconacetobacter diazotrophicus*, *Pseudomonas aeruginosa*	Solubilized ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$	Saravanan et al. (2007), and Fasim et al. (2002)
Biosurfactants			
Rhamnolipids, dirhamnolipid	*Pseudomonas aeruginosa*	Mobilized Cu, Cd, and Pb	Venkatesh and Vedaraman (2012), and Juwarkar et al. (2007)
Polymeric substances			
Polymeric substances (extracellular)	*Azotobacter* spp.	Immobilized Cd and Cr	Joshi and Juwarkar (2009)
Glomalin	*Glomus mosseae*	Immobilized Cu, Pb, and Cd	Gonzalez-Chavez et al. (2004)
Redox reaction			
Oxidation and reduction	*Streptomyces lividans* sp., *Rhodococcus* sp., *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*	Increased the mobility of As, Cu, Cd, Hg, and Zn	Yang et al. (2012), Beolchini et al. (2009)

(Sinha and Mukherjee 2008; Tank and Saraf 2009; Kuffner et al. 2010) which generated the need to study the interaction of plant–siderophore-producing microorganisms–metals in the contaminated soils. Siderophore production by microbes is controlled by various factors, viz., iron availability, pH, nutrient status of soils, type, concentration of heavy metals, etc. Therefore, higher heavy metal concentration acts as stimuli to produce more siderophore by microbes. Findings of Braud et al. (2009a) revealed the fact that addition of heavy metals, Al, Cu, Ga, Mn, and Ni, in iron-limited succinate medium induced pyoverdine synthesis by P. *aeruginosa*.

Moreover, the presence of heavy metals such as Cu, Ni, and Cr stimulated pyoverdine synthesis even in the case of iron (Braud et al. 2010).

11.3.2 Organic Acids

Low molecular weight organic acids, synthesized by plant–microbe interaction, play an instrumental role in enhancing the bioavailability of the trace elements and metals in the soil mainly through formation of metal complex. Organic acids work as a ligand which form stable complex with the heavy metals. However, the stability of the complex is regulated by several factors, viz., number and the position of carboxyl groups in organic acids, form of heavy metals, and most importantly pH of the soil solution (Ryan et al. 2001). Different studies have reported that 5-ketogluconic acids and 2-gluconic acids are prime responsible for solubilizing and mobilizing of insoluble ZnO, $Zn_3(PO_4)_2$, and $ZnCO_3$. The bacterial strain involved in gluconic acid productions and Zn solubilization are reported to be *Gluconobacter diazotrophicus* and *Pseudomonas aeruginosa* (Fasim et al. 2002; Saravanan et al. 2007). Similarly, formic acid, succinic acid, oxalic acid, acetic acid, and tartaric acid produced by rhizospheric bacteria have been reported to solubilize Cd and Zn in the rhizosphere of *Sedum alfredii*, a hyperaccumulating plant (Li et al. 2010). Furthermore, organic acids secreted by plant-associated microbes expedite the absorption of Cu (Chen et al. 2005), Pb (Sheng et al. 2008), and Cd and Zn (Li et al. 2010) by plant root. Mycorrhizal fungi, especially ericoid mycorrhizal fungi (*Oidiodendron maius*) and other soil fungi (*Beauveria caledonica*), can also increase solubility of Zn from insoluble sources by releasing citric and malic acids. These organic acids either by chelation or by acidolysis process can increase the solubility and availability of Zn from insoluble ZnO, $Zn_3(PO_4)_2$, and pyromorphite (Martino et al. 2003; Fomina et al. 2005).

Although the role of organic acids seem promising, however, the factors governing the fate and the performance of the organic acids need to be considered for better understanding of their mechanisms. Moreover, the other root-mediated process such as contribution of root exudates and other metabolites in metal mobilization (Wenzel 2009) also need to be taken into account before describing the role of organic acids produced by plant–microbe interaction in heavy metal transformation and solubilization. In this respect, precise quantification of organic acids in rhizosphere and the genetic sequencing of responsible microbes could shed light in understanding organic acid dynamics between soil, plant, and microbe continuum.

11.3.3 Biosurfactants

Biosurfactants are amphiphilic molecules comprising of a nonpolar (hydrophobic) tail and a polar/ionic (hydrophilic) head. Biosurfactant produced by microbes can increase metal solubility and bioavailability through complex formation with heavy metals at the soil interface leading to desorption of metals from soil matrix. The potential of biosurfactant dirhamnolipid produced by *P. aeruginosa* in solubilizing and mobilizing Cd, Pb, and Cu has already been documented in earlier studies (Juwarkar et al. 2007; Venkatesh and Vedaraman 2012). In addition, biosurfactants produced by plant–microorganism association also show high promise for improving the metal (Cd) uptake by rape, maize, Sudan grass, and tomato plants, a desirable trait for plants to be used for phytoextraction. The biosurfactant released from *Bacillus* sp. J119 was capable of enhancing Cd uptake from soil artificially contaminated with different levels of Cd (0 and 50 mg kg^{-1} Sheng et al. (2008)). Hence, the knowledge regarding interactive effect of biosurfactant-producing microbes on plants will enrich our perception about the role of biosurfactant-producing microbes in heavy metal phytoremediation.

11.3.4 Polymeric Substances and Glycoprotein

Extracellular polymeric substance (EPS), mucopolysaccharides, and proteins produced by plant-associated microbes can form complex with heavy metals and reduce their mobility in soil. Joshi and Juwarkar (2009) reported that EPS produced by *Azotobacter* spp. could immobilize Cd and Cr through complex formation (15.2 mg g^{-1} of Cd and 21.9 mg g^{-1} of Cr) and reduce the uptake of Cd (−0.5) and Cr (−0.4) by *Triticum aestivum*. Arbuscular mycorrhizal fungi are also reported to produce glomalin which form complex with Cu, Pb, and Cd and extract approximately 4.3 mg Cu, 1.1 mg Pb, and 0.1 mg Cd per gram of glomalin from metal-polluted soils (Gonzalez-Chavez et al. 2004). Therefore, AMF with higher amount of glomalin secretion capacity could play an instrumental role in phytoextraction and phytostabilization effort.

11.3.5 Redox Transformation of Metal in Rhizosphere

Plant-associated microbes can change the mobility of heavy metals through redox transformation reactions. Oxidation of metals by rhizospheric microbes is particularly interesting from a phytoextraction point of view. For instance, Cu mobilization in contaminated soils and its uptake in plant tissue were enhanced in the presence of sulfur-oxidizing bacteria in the rhizosphere (Shi et al. 2011). This enhanced uptake of copper in the presence of sulfur-oxidizing bacteria was due to lowering of the soil pH as a result of conversion of reduced sulfur to sulfates. Potential of Fe-/S--oxidizing bacteria to enhance metal bioavailability in the soils through acidification reaction was also reported by Chen and Lin (2001).

Microbial reduction of heavy metals also sometime immobilizes the heavy metals in the rhizosphere. For example, decreased uptake of Cr by of 37 % in shoot and 56 % in root of green chili grown in Cr(VI)-contaminated soils upon inoculation with *Cellulosimicrobium cellulans* was reported by Chatterjee et al. (2009). This effect was brought about by microbial reduction of mobile and toxic Cr(VI) to non-toxic and immobile Cr(III) in the soil. Abou-Shanab et al. (2007) reported lower Cr translocation from root to shoots of water hyacinth as indicative of the Cr-reducing potential of rhizosphere microbes. Similarly, Di Gregorio et al. (2005) demonstrated the Se-reducing potential of *Stenotrophomonas maltophilia* isolated from the rhizosphere of *Astragalus bisulcatus*. This bacterium significantly reduced soluble and harmful Se(IV) to insoluble and unavailable Se(0), thereby reducing the uptake of Se by plant. These examples demonstrate mechanisms, by which metal-reducing microbes lock the metals within the rhizosphere soil and reflect the suitability of these microbes for phytostabilization applications.

Besides, the synergistic interaction of metal-oxidizing and metal-reducing microbes on heavy metal mobilization in contaminated soils has also been studied. Inoculation of Fe-reducing bacteria and the Fe-/S-oxidizing bacteria together significantly increased the mobility of Cu, Cd, Hg, and Zn by 90 %. This effect was attributed to the coupled and synergistic metabolism of oxidizing and reducing microbes Beolchini et al. (2009). Though these results open new perspectives for the bioremediation technology for metal mobilization, further investigations are needed to utilize such bacteria in phytoextraction process.

11.3.6 Biosorption

Through biosorption mechanism, the plant-associated microbes may also contribute in plant–metal uptake. Biosorption can be defined as the microbial adsorption of soluble/insoluble organic/inorganic contaminant by a metabolism-independent, passive or by a metabolism-dependent, active process (Ma et al. 2011). The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Higher affinity of the sorbent for the sorbate species (metals) is responsible for binding of metals on sorbent by different interactions. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution (Das et al. 2008). The efficiency of biosorption depends upon factors like initial metal concentration, pH, temperature, and biomass weight in solution. Several researchers have pointed out the restricted entry, reduced bioavailability, and lower metal uptake by plant due to biosorption. For instance, Madhaiyan et al. (2007) reported inoculation of metal-binding fungi *Magnaporthe oryzae* and bacteria *Burkholderia* sp. reduced Ni and Cd accumulation in roots and shoots of tomato. These effects of inoculation of *Trifolium repens* with *Brevibacillus* sp. B-I decreased the concentration of Zn in shoot tissues compared to respective uninoculated control due to the increased Zn biosorption by *Brevibacillus* sp. B-I Vivas et al. (2006).

The mycorrhizal fungi have also been reported to act as a filtration barrier against the translocation of heavy metals from plant roots to shoots. Experiments revealed that the inoculation of pine seedlings with *Scleroderma citrinum*, *Amanita muscaria*, and *Lactarius rufus* reduced translocation of Zn, Cd, or Pb from roots to

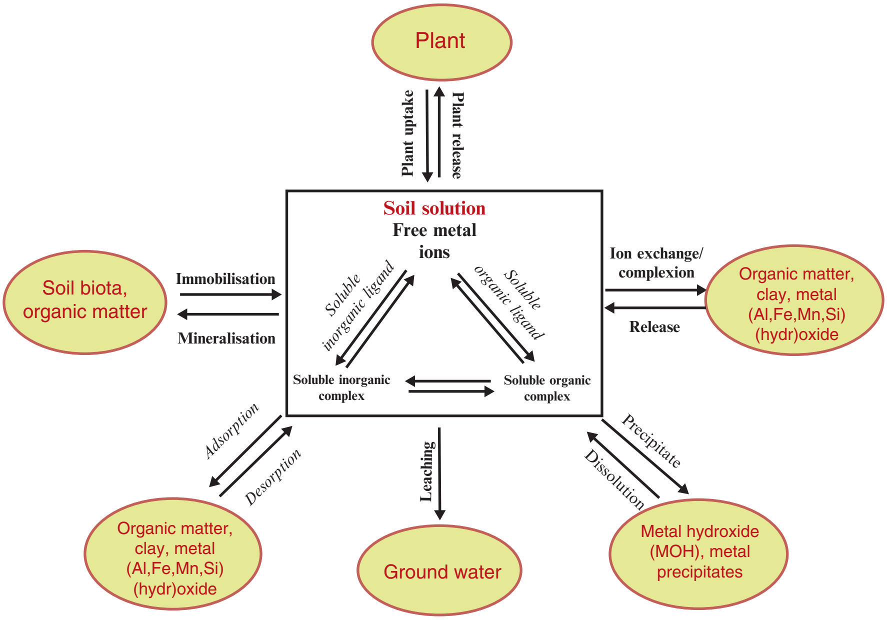

Fig. 11.3 Possible reactions involved in physical, chemical, and biological transformation of metal(loid)s in soil (Source: Seshadri et al. 2015)

shoots by increased metal biosorption in outer and inner components of the mycelium (Krupa and Kozdrój 2007). Large surface area of mycorrhizal fungi endows mycorrhizal fungi with a strong capacity for adsorbing heavy metals from soil. The fungal cell wall components (e.g., chitin, extracellular slime, etc.) and intracellular compounds (e.g., metallothioneins, P-rich amorphic material) may also immobilize/arrest the metals in the interior of plant roots (Meharg 2003). An exhaustive compilation of microbes for biosorption of heavy metals was made by Volesky and Holan (1995). Although inoculation of plants with metal-binding microbes could be a suitable approach for plant protection against heavy metals and phytostabilization of metal-polluted soils, many authors believe that the reduction in accumulation and translocation of metal in plants is not due to biosorption/bioaccumulation alone (Babu and Reddy 2011).

Plant-associated microorganisms differ in their ability to alter heavy metal bioavailability and its uptake by plants through metal-mobilizing/metal-immobilizing metabolites/processes. Colonization and survival of these microbes also greatly influence the quantity of metal accumulation in plants growing in metal-contaminated soils which in turn is governed by soil physicochemical–biological properties such as metal toxicity, indigenous microbial communities, adverse pH, nutrient deficiency, etc.

The general mechanisms involved in the transformation of metal(loid) ions in the soil lead to retention (mediated by sorption, precipitation, and complexation reactions) or loss (plant uptake, leaching, and volatilization) of heavy metal(loid)s (Fig. 11.3). Although most metal(loid)s do not undergo volatilization-related losses, some metal(loid)s such as As, Hg, and Se tend to form gaseous compounds (Bolan et al. 2013). A greater understanding of the microbiological (activity) and chemical (exudates) changes occurring in the rhizosphere would identify the mechanisms involved in the transformation of heavy metals in the contaminated soil.

11.4 Role of Mycorrhiza and PGPR for Heavy Metal Removal from Metal-Contaminated Site

Rhizosphere microbes have played a key role for nutrient cycling and soil sustainability. Arbuscular mycorrhizal fungi (AMF) are a group of endophytic fungi infecting the roots of majority of the terrestrial plants. This symbiosis association between mycorrhiza and host plants has very important role on the plant's growth and development through the acquisition of phosphorous and other essential mineral nutrients from the soil. Plant growth-promoting rhizobacteria (PGPR) is a group of bacteria that colonize plant roots and promote growth and yield (Wu et al. 2005). However, PGPR are known to increase root system uptake properties of colonized plants, thus facilitating better supply of plant nutrient such as N, P, and Fe. The potential application of mycorrhizal plants for land decontamination has several benefits such as increased plant biomass, plant phosphorus nutrition, and tolerance to heavy metal stress. Mycorrhizal species influences metal toxicity to plants through decreasing translocation of heavy metals and its concentration.

11.4.1 Role of Mycorrhiza for the Remediation of Contaminated Site

Remediators choose the applicable and suitable microbial species that are used as inoculants to plant growth promotion and bioremediation process. The arbuscular mycorrhizal (AM) fungi have several critical roles for improving the plant's resistance to various biotic and abiotic stresses (Harrier 2001). AM fungi also have great advantage to alleviate heavy metal toxicity of plants (Hildebrandt et al. 1999). AM fungi has significant role for improving the uptake of nutrient and water by host plants through their mycelial networks and protecting the host plants from heavy metal toxicity. Besides AM fungi, there are several other beneficial microorganisms in the rhizosphere that may also help for heavy metal tolerance to the plants. According to Khan et al. (2000), mycorrhizal species enhance the bioavailability of toxic metals by altering the microenvironment of the rhizosphere through decontamination. This AM fungi may improve the plant nutrient uptake in alkaline and calcareous soils of arid and semiarid regions in which the bioavailability of P and several cationic micronutrients is limited. The presence of carbonates in calcareous soils is also limiting water holding capacity. Furthermore, plant transpiration is significantly reduced with an increase in soil heavy metal concentration (Davari et al. 2010). It has been reported that heavy metals like Cd can affect the hydraulic conductivity of root by multiple mechanisms occurring on the apoplastic and/or the symplastic pathway (Shah et al. 2010). The ability of beneficial microorganisms to promote the growth of canola and tomato seedlings treated with toxic concentrations of various metal(loid)s such as As, Cd, Ni, Pb, Se, and Zn has been demonstrated. There have been few analytical studies available on AM fungi in the contaminated soils. While some workers highlighted that the external mycelium of the arbuscular mycorrhizae was the primary site for various heavy metal localization (Kaldorf et al. 1999; Turnau 1998), other reports emphasized the selective exclusion of toxic and nontoxic metals by adsorption onto chitinous cell wall structure (Zhou 1999), or onto extracellular glycoprotein called glomalin (Wright and Upadhyaya 1998), or intracellular crystallization. These mechanisms have great significance in reducing a plant's exposure to potentially toxic metals, which is called mycorrhizoremediation. Localization of Cu accumulation in the extraradical mycelium (ERM) of different AM fungi differed in their capacity for sorption of Cu which was directly related to the cation exchange capacity of ERM of AM fungi (Gonzalez-Chavez et al. 2002). Difference exists in accumulation and tolerance for different heavy metals among the species of AM fungi. Hence, mechanism involved in tolerance and accumulation of heavy metals require future research in order to explore the contribution of AM fungi in plant tolerance and its ecological significance in polluted soils.

11.4.2 Role of PGPR for the Remediation of Contaminated Site

Plant growth-promoting rhizobacteria (PGPR) colonize in the rhizosphere and improve plant growth through various mechanisms, such as plant nutrient uptake, suppressing harmful phytopathogens by producing antibiotics and siderophores or other bioactive compounds, phytohormone production, and fixation or solubilization of plant nutrient and making it available to the plants. Better colonization of rhizospheric microorganism increases stress endurance of a plant and improves the metal bioavailability. Many isolated strains of PGPR used to enhance crop yield and improve agriculture sustainability (Begonia et al. 2005). PGPR are known to increase root system uptake properties of colonized crops by facilitating ion nitrate adsorption, phosphate solubilization, and iron chelation (Islam et al. 2009). Maize seed inoculation with rhizobacteria such as *Pseudomonas cepacia*, *P. fluorescens*, and *Streptomyces aurantiacus* in combination with nitrogen increased 25 % more crop yield than the non-rhizobacterium-colonized control.

When Indian mustard (*Brassica juncea*) and canola (*Brassica campestris*) seeds grow in the presence of PGPR strain, the plants produce siderophores, and this plays an important role in the remediation of Ni-, Pb-, and Zn-contaminated site (Burd et al. 1998). According to Belimov et al. (2001), growth of *Brassica napus* plant is improved by inoculating recalcitrant PGPR through ACC-deaminase activity, and growth of barley plants is improved by biological nitrogen fixation and auxin production with PGPR inoculation in Cd-contaminated soil (Belimov and Dietz 2000). The rhizosphere is a type of microenvironment where microorganisms form a special type of communities with plant growth-promoting capabilities present to remove the toxic contaminants (Ma et al. 2009). Findings of Idris et al. 2004 confirmed that metal mobility and bioavailability to the plants are enhanced by rhizospheric bacteria by releasing chelating agents, acidification, phosphate solubilization, and redox changes.

Thus, interactions between plants and useful rhizosphere microbes can improve biomass production and accumulation of heavy metals. Growth of crop plant is promoted by PGPR which help in decreasing the plant stress related with phytoremediation methods (Reed and Glick 2005). Selection of highly potential microbial combination is a big challenge for developing phytoremediation strategies.

11.5 Conclusion

As an economic and green approach for decontamination of polluted soil and water, phytoremediation is an optimistic technology. Association of microbes has shown improved efficiency of phytoremediation in many cases. The capability of soil function is mostly regulated by the soil biological component. Plant–microbe interaction plays a critical role to remediate extensive contaminated sites and recover to health state of soil. Though the mechanism involved in reducing the load of contaminating metal through plant assisted by microbes is complex and involves several processes occurring simultaneously in a habitat, thorough understanding of processes will further improve the efficiency of phytoremediation by manipulating the interaction depending upon nature of pollutant, condition of microhabitat, concentration of contaminant, type of associated microbial community, etc. Further, identification of specific biomarker associated with the promising microbes for efficient microbe-assisted phytoremediation will further improve the remediation efficiency. Although promising response of inoculation of beneficial microbes particularly plant growth-promoting bacteria and/or mycorrhizae has been reported under laboratory conditions, the result under field condition showed limited effectiveness because of complexity of soil environment and competing microbes. Characterizing the physicochemical and biological features of target contaminated soils may be important for making successful microbe-assisted phytoremediation technology. The colonization and survival of inoculums in metal-contaminated soil is necessary to exhibit beneficial traits for improving the plant growth and overall phytoremediation process in metal-contaminated soils. Advancing the knowledge on identification of favorable soil condition, efficient microbes with multiple metal resistance/tolerance potential, survival, and compatibility with other microbes may be important to utilize the potential of inoculants for phytoremediation purpose. Identification of efficient microbes for bioaccumulation of heavy metal and understanding biochemical and molecular mechanisms of interaction of plant–microbe toxicant play a major role in the processes involved in phytoremediation.

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