



Microbe-Assisted Phytoremediation in Reinstating Heavy Metal-Contaminated Sites: Concepts, Mechanisms, Challenges, and Future Perspectives

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Abstract

Pollution has become a serious matter of environmental and political concerns in the world. Our natural environment has been contaminated by various organic and inorganic contaminants, which are being used in many industrial processes and released along with industrial effluents. Among them, heavy metals are highly toxic pollutants, which cause serious environmental pollution and severe health hazards in living beings, and there is a public outcry to ensure the safest and healthiest environment for living beings. Phytoremediation, a type of bioremediation, has been emerged as an eco-sustainable technology that uses plants and their associated microbes to clean up heavy metal-contaminated soils, water, and wastewaters as compared to various physicochemical remediation technologies currently being applied for environmental restoration. However, in current scenario, phytoremediation assisted by plant-associated microorganisms, i.e., microbe-assisted phytoremediation (use of microbes, i.e., plant growth-promoting rhizobacteria, endophytes, and arbuscular mycorrhizal fungi, in assisted phytoremediation), is highly preferred for the remediation of heavy metal-contaminated sites as they have potential to alleviate the heavy metal toxicity in plants through their own metal resistance system and facilitate and

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improve the growth of host plants under heavy metal stress. In this line, this chapter aims to provide an overview on microbe-assisted phytoremediation, illustrate various mechanisms elicited for plant growth promotion and heavy metal phytoremediation (accumulation/detoxification), and discuss drawbacks and future challenges.

Keywords

Heavy metals · Environmental pollution · Toxicity · Microbe-assisted phytoremediation · Contaminated sites

6.1 Introduction

Environmental pollution is of serious ecological concern worldwide with a continually rising public outcry to ensure the safest and healthiest environment. A variety of organic and inorganic pollutants have been reported to cause environmental pollution and severe health hazards in living beings (Maszenan et al. 2011; Saxena and Bharagava 2017). Among them, heavy metals (HMs) are highly notorious pollutants due to their high abundance and nonbiodegradable and persistent nature in the environment. Hence, they cause soil/water pollution and toxic, genotoxic, teratogenic, and mutagenic effects in living beings (Dixit et al. 2015; Sarwar et al. 2017). They also cause endocrine disruption and neurological disorders even at low concentration (Yadav 2010; Maszenan et al. 2011; Dixit et al. 2015; Sarwar et al. 2017). Any naturally occurring metal/metalloid having an atomic number greater than 20 and elemental density greater than 5 g/cm³ is termed as HM. They include copper (Cu), cadmium (Cd), chromium (Cr), cobalt (Co), zinc (Zn), iron (Fe), nickel (Ni), mercury (Hg), lead (Pb), arsenic (As), silver (Ag), and platinum group elements (Ali et al. 2013; Ali and Khan 2018). Among them, Cd, As, Hg, and Pb do not have any biological function in the body and thus are nonessential elements. They can cause severe health hazards and are listed as priority pollutants by many environmental protection agencies worldwide (Jaishankar et al. 2014; Dixit et al. 2015; Sarwar et al. 2017). Therefore, the removal of HMs from the contaminated matrix is an urgent need to safeguard the environment and human health.

Currently, applied physicochemical approaches are environmentally destructive in nature and are also costly to apply. However, bioremediation is considered as the most eco-friendly approach and employs microbes and plants or their enzymes to degrade/detoxify the organic and inorganic pollutants from contaminated environments. Phytoremediation has been identified as an emerging, low-cost, and eco-sustainable solution for HM pollution prevention and control. It is the most suitable alternative to conventional physicochemical remediation technologies, which are highly expensive and technically more suited to small areas, create secondary pollution and deteriorate soil fertility, and, thus, adversely affect agroecosystem (Ali et al. 2013; Chandra et al. 2015; Mahar et al. 2016; Muthusaravanan et al. 2018).

Phytoremediation is the engineered use of green plants with associated soil beneficial microbes to remove toxic pollutants via degradation and detoxification

mechanisms from contaminated soil and water/wastewaters (Bharagava et al. 2017; Mukhopadhyay and Maiti 2010; Ali et al. 2013). It is an eco-friendly, nonintrusive, and aesthetically pleasing remediation technology that removes metal pollutants from the contaminated sites (Lee 2013; Chandra et al. 2015; Chirakkara et al. 2016). It can be commercialized, and income can be generated, if metals removed from contaminated sites could be used to extract usable form of economically viable metals (i.e., phytomining) (Chandra et al. 2015; Mahar et al. 2016). In addition, energy can be generated through the burning of plant biomass, and land restoration could be achieved for sustainable agricultural development or general habitation (Stephenson and Black 2014; Mahar et al. 2016). The rationale, mechanisms, and economic feasibility of phytoremediation have been discussed elsewhere (Ali et al. 2013; Wan et al. 2016; Sarwar et al. 2017). However, a longtime frame required for phytoremediation and physiological damage to remediating plants under toxic metal stress is a major issue. Hence, plant–microbe interactions (PMIs) could be exploited to enhance the plant growth and phytoremediation of HM-contaminated sites. Therefore, this chapter has mainly focused on the microbe-assisted phytoremediation, illustrates various mechanisms elicited for plant growth promotion and heavy metal phytoremediation (accumulation/detoxification), and discusses drawbacks and future challenges with recommendations for further research.

6.2 Heavy Metals: Environmental Pollution and Toxicity Profile

Heavy metals (HMs) can be introduced into the environment either by natural or anthropogenic processes. Natural processes are geological activities, for instance, mineral weathering, erosion, volcanic eruptions, and continental dust. Anthropogenic activities include industrial operations such as mining, smelting, electroplating, and industrial effluent discharge as well as agricultural practices like the use of pesticides and phosphate fertilizers and release of agricultural wastes (Ali et al. 2013; Mahar et al. 2016; Antoniadis et al. 2017). Industrial activities are the major source of HM pollution (water and soil) in the environment. If HMs enter the food chain, they may bioaccumulate and/or biomagnify at higher trophic levels resulting in severe health threats and thus are of serious ecotoxicological concern.

The indiscriminate discharge of toxic metal-rich industrial effluents is one of the major sources of environmental pollution. The effluent discharged from metal-based industries, especially leather industries (Cr used in leather tanning), causes serious soil and water pollution, and hence its treatment and management is a key challenge to pollution control authorities (Sahu et al. 2007; Saxena et al. 2016). A high concentration of HMs has been reported in sediments of Ganga River and its tributaries receiving Cr-loaded tannery effluent (Beg and Ali 2008). In addition, HM beyond the permissible limits also deteriorates water quality and makes it unfit for drinking and irrigation purpose (Nazeer et al. 2014). The effluent released from electroplating and distillery industries also constitutes a highly rich source of HMs and hence is considered as hazardous to living beings (Venkateswaran et al. 2007;

Chandra et al. 2008). Furthermore, effluent released from domestic activities is also responsible for HM pollution and thus is of serious ecotoxicological concerns (Bhardwaj et al. 2017).

In an aquatic ecosystem, HM adversely affects gamete production, sperm quality, and embryonic development; delays hatching; causes physical deformities in fishes; and ultimately leads to the death of newly hatched larvae (Segura et al. 2006; Jezierska et al. 2009; Fatima et al. 2014). HM also causes endocrine disruption, oxidative stress, and genotoxicity in fishes (Jezierska et al. 2009; Luszczek-Trojnar et al. 2014; Javed et al. 2016). Further, HM also causes a reduction in hematological parameters and glycogen reserve and thus makes the fishes weak, anemic, and vulnerable to diseases (Javed and Usmani 2015).

The soil is a nonrenewable resource for sustainable agriculture and acts as a major sink for HMs. The contamination of agricultural soil with toxic metals affects its physicochemical and biological properties and reduces land usability for agricultural farming leading to food insecurity and thus creating land tenure problems (Wuana and Okieimen 2011). Moreover, the coexistence and persistence of HMs in soil is also responsible for the entry of toxic metals into the food chain and thus leads to severe health hazards in living beings (Khan et al. 2008).

HM inhibits several microbial metabolic processes such as respiration, denitrification, and enzymatic activity and, hence, retards the bioremediation processes (Zhuang et al. 2007; Sobolev and Begonia 2008). HM also causes a reduction in the number of specific microbial populations and a shift in the microbial community structure. For instance, Ding et al. (2017) evaluated the effect of Cd and Cr on the microbial community structure in the rhizospheric soil of rice plant during a pot experiment. Results revealed that the relative abundance of a bacterial genus *Longilinea* was significantly higher in the control soil than in Cd- and Cr-treated soils, whereas the relative abundance of the genus *Pseudomonas* was significantly higher in the Cd-treated soils than in the Cr-treated and control soils. However, the relative abundance of a genus *Sulfuricurvum* was also significantly higher in the Cd-treated soil than in the Cr-treated and control soils, whereas the relative abundance of the genus *Bellilinea* was significantly higher in the Cr-treated soil than in the other treated soils. HMs also inhibit the cell division, transcription process, and denaturation of protein and adversely affect the cell membrane distribution in microbes (Jacob et al. 2018). Hexavalent chromium (Cr^{6+}) is also reported to cause DNA damage by exerting oxidative stress in soil bacteria and thus leads to genotoxic effects (Quievryn et al. 2003).

The irrigation of food crops in the agriculture field with water contaminated with toxic metal-rich industrial effluents is a common practice in many developing countries. It may provide a chance for the movement of potentially toxic metals from contaminated soil to edible crops, ultimately reaching into the human/animal body via consumption and, thus, rendering severe toxic effects. HM affects various metal-sensitive enzymes in plants such as alcohol dehydrogenase, nitrogenase, nitrate reductase, and amylase and hydrolytic (phosphatase and ribonuclease) and carboxylating (phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase) enzymes (Nagajyoti et al. 2010; Yadav 2010). Hence, HM disrupts several

biochemical/physiological processes in plants such as seed germination, enzymatic activities, nitrogen metabolism, electron transport system, transpiration, CO₂ assimilation, antioxidant defense system, photosynthesis, photophosphorylation, cellular metabolism, nitrogen fixation, water balance, mineral nutrition, and cellular ionic homeostasis and ultimately leads to plant death (Yadav 2010; Lajayar et al. 2017). Irrigation of agricultural crops with heavy metal-loaded industrial effluents also disrupts several cytological processes in plants such as root growth and elongation, cell membrane permeability, mitotic activity, and the stability of genetic material and also creates chromosomal abnormalities (Nagajyoti et al. 2010; Yadav 2010). For example, the irrigation of agricultural crops with the HM-rich distillery and tannery effluent has been reported to cause a reduction in root/shoot growth and biomass, seed germination, and seedling growth and also induce chlorosis and photosynthetic impairment (Chandra et al. 2009).

HMs may cause oxidative stress by forming reactive oxygen species (ROS), which disrupt the antioxidant defense system and lead to cell damage in humans/animals, and in extreme cases can be fatal (Jaishankar et al. 2014). For instance, hexavalent chromium (Cr⁶⁺) has been reported to cause cancer in humans and damage cellular components during its reduction into trivalent chromium (Cr³⁺), leading to the generation of free radicals that cause DNA damage (Mishra and Bharagava 2016). Therefore, the remediation of HM-contaminated sites is of utmost important for environmental safety.

6.3 Current Remediation Technologies: Status and Drawbacks

Rapid industrialization and urbanization around the world has led to the recognition and understanding of the relationship between environmental contamination and public health. Industries are the key players in the national economies of many developing countries; however, unfortunately, they are also the major polluters of the environment. Among the different sources of environmental pollution, industrial wastewater discharged from different industries is considered the major source of environmental pollution (soil and water). Industries use a variety of chemicals for the processing of raw materials to obtain good-quality products within a short period of time and economically. To obtain good-quality products within a short period of time, industries generally use cheap and poorly or nonbiodegradable chemicals, and their toxicity is usually ignored. However, in the public domain, there are many reports available that confirm the presence of a variety of highly toxic chemicals in industrial wastewaters.

Industrial wastewaters contain a variety of organic and inorganic pollutants that cause serious environmental pollution and health hazards (Maszenan et al. 2011; Megharaj et al. 2011). During production processes, a variety of chemicals with large volumes of water are used to process raw materials in industries. This generates large volumes of high-strength wastewater, which is a major source of environmental pollution (Saxena et al. 2016). The wastewater generated from

pollution-causing industries is characterized by high chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDSs), total suspended solids (TSSs), and a variety of recalcitrant organic and inorganic pollutants. Organic pollutants include phenols, chlorinated phenols, endocrine-disrupting chemicals, azo dyes, polyaromatic hydrocarbons, polychlorinated biphenyls, and pesticides, whereas inorganic pollutants include a variety of toxic heavy metals such as cadmium (Cd), chromium (Cr), arsenic (As), lead (Pb), and mercury (Hg). The high concentration and poor biodegradability of recalcitrant organic pollutants and nonbiodegradable nature of inorganic metal pollutants in industrial wastewaters pose a major challenge for environmental safety and human health protection; thus, it is required to adequately treat industrial wastewater before its final disposal in the environment. Although a number of physicochemical methods are applied for the treatment of industrial wastewaters, all of these are costly, use a large amount of chemicals, and generate a large amount of sludge after treatment, which also acts as a secondary pollutant in the environment. Alternatively, biological treatment methods using an array of microorganisms have diverse metabolic pathways and, hence, are regarded as environmentally friendly, cost-effective methods for wastewater treatment with simple structural setup, wider application, operational ease, and less sludge production compared to physicochemical methods (Mendez-Paz et al. 2005; Pandey et al. 2007). Biological methods using microbes are becoming much more popular for the treatment of industrial wastewaters in wastewater treatment plants. Further, most chemical compounds are degraded by acclimated microorganisms during wastewater treatment at wastewater treatment plants; however, some of the chemical compounds are not properly degraded/detoxified due to their recalcitrant nature during wastewater treatment and are discharged along with wastewaters, causing serious environmental pollution (Maszenan et al. 2011). Hence, the application of bioremediation technology using potential microorganisms and their consortia or of phytoremediation technology (use of green plants in constructed wetlands) is required for the degradation and detoxification of such types of recalcitrant industrial wastewaters prior to safe disposal in the environment.

Phytoremediation is considered as the most applicable remediation technology at contaminated sites. Phytoremediation is the engineered use of green plants with associated soil beneficial microbes to remove toxic pollutants via degradation and detoxification mechanisms from contaminated soil and water/wastewaters (Bharagava et al. 2017; Mukhopadhyay and Maiti 2010; Ali et al. 2013). It is an eco-friendly, nonintrusive, and aesthetically pleasing remediation technology that removes metal pollutants from the contaminated sites (Lee 2013; Chandra et al. 2015; Chirakkara et al. 2016). The aim of phytoremediation can be (a) plant-based extraction of metals with financial benefit (phytoextraction), (b) risk minimization (phytostabilization), and (c) sustainable soil management in which phytoremediation steadily increases soil fertility allowing growth of crops with added economic value (Mahar et al. 2016; Vangronsveld et al. 2009). Phytoremediation includes a range of plant-based remediation processes. Phytoremediation reduces the risks of pollutant dispersion, and it is applicable for the decontamination of soils or wastewaters with mixed pollutants (Mahar et al. 2016; Mudhoo et al. 2010). Mechanisms

and efficiency of phytoremediation depend on several factors such as the pollutant class, its bioavailability especially in soils, physical and chemical characteristics of the matrix (soil, water, and wastewaters), and plant species (Mahar et al. 2016; Sreelal and Jayanthi 2017). The plants considered more efficient for phytoremediation are the metallophytes. These are able to survive and reproduce on metal-polluted soils (Coninx et al. 2017; Alford et al. 2010). However, a great number of known metallophytes have small biomass and slow growth, characteristics that are not advantageous for phytoremediation technologies (Coninx et al. 2017; Cabral et al. 2015). Further, longtime frame required for phytoremediation and physiological damage to remediating plants under toxic metal stress is a major issue. Therefore, plant–microbe interactions (PMIs) could be exploited to enhance the plant growth and phytoremediation of HM-contaminated sites.

The root-/rhizosphere-colonizing, plant growth-promoting rhizobacteria (PGPR) have been reported to enhance host plant growth in toxic metal-contaminated sites (Yuan et al. 2013; Ma et al. 2015, 2016a). PGPR produces growth hormones such as auxins (IAA, indole-3-acetic acid), cytokinins, gibberellins, and ethylene (Rajkumar et al. 2012; Ma et al. 2015). The mechanisms of plant growth promotion may vary from bacterial strain to strain and depend on various secondary metabolites produced (Ma et al. 2011; Backer et al. 2018). PGPR also produces some other beneficial compounds such as enzymes, osmolytes, biosurfactants, organic acids, metal-chelating siderophores, nitric oxide, and antibiotics (Rajkumar et al. 2012; Ma et al. 2015). These beneficial compounds reduce ethylene production *via* synthesis of ACC (1-aminocyclopropane-1-carboxylate) deaminase that prevents the inhibition of root elongation, lateral root growth, and root hair formation and also improves the mineral (N, P, & K) uptake in acidic soil (Babu et al. 2013; Ma et al. 2015). These compounds also suppress phytopathogens, provide tolerance to abiotic stress, and help in associated nitrogen fixation (Rajkumar et al. 2012; Babu et al. 2013; Ma et al. 2015). Hence, PGPRs are applied in sustainable agriculture development. Besides these, PGPR can lower the metal toxicity to remediating plants through biosorption/bioaccumulation as bacterial cells have an extremely high ratio of surface area to volume (Ma et al. 2016b; Li et al. 2018). PGPR could adsorb high metal concentration by either a metabolism-independent passive or metabolism-dependent active processes. Hence, using PGPR in environmental bioremediation could be a useful strategy for plant survival in the stressed environment. PGPRs reported for the enhanced HM phytoremediation with associated benefits have been reviewed in the past (Ma et al. 2011; Rajkumar et al. 2012; Ullah et al. 2015). Some updated examples from recent studies are summarized in Table 6.1.

Endophytes are the microbes (bacteria/fungi) that reside in the inner tissues of plants without causing harm to host. They also help in plant growth promotion and development under biotic or abiotic stressed environment and exert many beneficial effects than rhizobacteria (Luo et al. 2011; Ma et al. 2011, 2015). They are able to tolerate high metal concentration and hence lower phytotoxicity to remediating plants as well as help in growth promotion enhancing through biocontrol mechanism and induced systemic resistance against phytopathogens (Ma et al. 2011, 2015). They produce phytohormones, organic acids, siderophores, biosurfactants,

Table 6.1 Some studies on microbe-assisted phytoremediation of heavy metal-contaminated soils

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
<i>Enterobacter</i> sp. LC1, LC4, & LC6; <i>Kocuria</i> sp. LC2 & LC5; and <i>Kosakonia</i> sp. LC7	<i>Solanum nigrum</i>	As	Soil	IAA and P-solubilization	Mukherjee et al. (2018)
<i>Pseudomonas libanensis</i> and <i>Pseudomonas reactans</i>	<i>Brassica oxyrrhina</i>	Cu, Zn	Soil	IAA, ACC deaminase, siderophores	Ma et al. (2016a)
<i>Pseudomonas putida</i> , <i>Rhodopseudomonas</i> sp.	<i>Cicuta virosa</i> L.	Zn	Soil	Metal-chelating compounds	Nagata et al. (2015)
<i>Rhizobium leguminosarum</i>	<i>Brassica juncea</i>	Zn	Soil	Metal chelation	Adediran et al. (2015)
<i>Photobacterium</i> spp.	<i>Phragmites australis</i>	Hg	Soil	IAA, mercury reductase activity	Mathew et al. (2015)
<i>Bacillus pumilus</i> E2S2 and <i>Bacillus</i> sp. E1S2	<i>Sedum plumbizincicola</i>	Cd	Soil	IAA, ACC deaminase, siderophores, P-solubilization	Ma et al. (2015)
<i>Pseudomonas</i> sp. LK9	<i>Solanum nigrum</i>	Cd	Soil	Biosurfactants, siderophores, organic acids	Chen et al. (2014)
<i>P. aeruginosa</i>	<i>Triticum aestivum</i>	Zn	Soil	Antioxidative enzymes (catalase, peroxidase, superoxide dismutase)	Islam et al. (2014)
<i>Mesorhizobium Amorphae</i>	<i>Robinia pseudoacacia</i>	Cu, Zn, Cr	Soil	IAA, induced stress Tolerance	Hao et al. (2013)
<i>Acinetobacter</i> sp.	<i>Cicer arietinum</i>	As	Soil	IAA production	Srivastava and Singh (2014)
<i>Enterobacter</i> sp. JYX7 and <i>Klebsiella</i> sp. JYX10	<i>Polygonum pubescens</i>	Cd	Soil	IAA, siderophores, ACC deaminase, P-solubilization	Jing et al. (2014)
<i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>B. megaterium</i>	<i>Orychophragmus violaceus</i>	Cd	Soil	IAA production	Liang et al. (2014)

(continued)

Table 6.1 (continued)

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
<i>Phyllobacterium myrsinacearum</i> RC6b	<i>Sedum plumbizincicola</i>	Cd, Zn, and Pb	Soil	ACC deaminase, IAA, siderophores, P solubilization	Ma et al. (2013)
<i>Staphylococcus arlettae</i> NBRIEA G-6	<i>B. juncea</i>	As	Soil	IAA, siderophores, ACC deaminase	Srivastava et al. (2013)
<i>Rahnella</i> sp.	<i>Amaranthus hypochondriacus</i> , <i>A. mangostanus</i> , and <i>S. nigrum</i>	Cd	Soil	IAA, siderophores, ACC deaminase, P-solubilization	Yuan et al. (2013)
<i>Paenibacillus macerans</i> NBRFT5, <i>Bacillus endophyticus</i> NBRFT4, and <i>Bacillus pumilus</i> NBRFT9	<i>Brassica juncea</i>	Ni	Mix. of fly ash and press mud	Siderophores, organic acids, protons, and other nonspecified enzymes	Tiwari et al. (2012)
<i>Pantoea agglomerans</i> Jp3-3 and <i>Pseudomonas thivervalensis</i> Y1-3-9	<i>Brassica napus</i>	Cu	Quartz sand	IAA, siderophores, ACC deaminase, P-solubilization	Zhang et al. (2011)
<i>Azotobacter chroococcum</i> and <i>Rhizobium leguminosarum</i>	<i>Zea mays</i> L.	Pb	Soil	IAA production increased and soil pH decreased	Hadi and Bano (2010)
<i>Bacillus subtilis</i> , <i>B. cereus</i> , <i>Flavobacterium</i> sp., and <i>Pseudomonas</i> sp.	<i>Orychophragmus violaceus</i>	Zn	Soil	ACC deaminase, IAA, siderophores	He et al. (2010)
<i>Achromobacter xylosoxidans</i> Ax10	<i>Brassica juncea</i>	Cu	Soil	ACC deaminase, IAA, phosphate solubilization	Ma et al. (2009)
<i>Burkholderia</i> sp. J62	<i>Zea mays</i> and <i>Lycopersicon Esculentum</i>	Pb, Cd	Soil	IAA, siderophores, ACC deaminase, P solubilization	Jiang et al. (2008)
<i>Burkholderia</i> sp. J62	<i>B. juncea</i>	Zn, Pb, Cu	Soil	P, K solubilization	Wu et al. (2006)
<i>Brevibacillus brevis</i>	<i>Trifolium repens</i>	Cd, Ni, Pb	Soil	IAA production	Vivas et al. (2006)
Endophytes					

(continued)

Table 6.1 (continued)

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
<i>Bacillus thuringiensis</i> GDB-1	<i>Alnus firma</i>	As	Mine tailing waste	ACC deaminase, IAA, siderophores, P-solubilization	Babu et al. (2013)
<i>Pseudomonas koreensis</i> AGB-1	<i>Miscanthus Sinensis</i>	As, Cd, Cu, Pb, and Zn	Soil	ACC deaminase activity, IAA	Babu et al. (2015)
<i>Staphylococcus</i> , <i>Curtobacterium</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Microbacterium</i> , <i>Arthrobacter</i> , <i>Leifsonia</i> , <i>Paenibacillus</i>	<i>Alyssum bertolonii</i>	Ni, Co, Cr, Cu, and Zn	Soil	Production of siderophores	Barzanti et al. (2007)
<i>Serratia nematodiphila</i> LRE07, <i>Enterobacter aerogenes</i> LRE17, <i>Enterobacter</i> sp. LSE04 <i>Acinetobacter</i> sp. LSE06	<i>Solanum nigrum</i> L.	Cd	Soil	Production of IAA, siderophores, ACCD, and solubilization of P	Chen et al. (2010)
<i>P. monteilii</i> PsF84, <i>P. plecoglossicida</i> PsF610	<i>Pelargonium graveolens</i>	Cr	Soil	Production of IAA and siderophores, solubilization of P	Dharni et al. (2014)
<i>Rahnella</i> sp. JN6	<i>Brassica napus</i>	Pb	Soil	IAA, ACC deaminase, siderophores, P-solubilization	He et al. (2014)
<i>Actinobacterium</i>	<i>Salix caprea</i>	Cd and Zn	Soil	Production of siderophores and ACCD	Kuffner et al. (2010)
<i>Burkholderia cepacia</i> L.S.2.4, <i>Herbaspirillum seropedicae</i> LMG2284	<i>Lupinus luteus</i> L	Cu, Cd, Co, Ni, Pb, and Zn	Soil	ND	Lodewyckx et al. (2001)

(continued)

Table 6.1 (continued)

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
<i>Pseudomonas fluorescens</i> VI8L1, <i>Bacillus pumilus</i> VI8L2, <i>P. fluorescens</i> II8L4, <i>P. fluorescens</i> VI8R2, <i>Acinetobacter calcoaceticus</i> II2R3	<i>Sedum alfredii</i>	Zn and Cd	Soil	Production of IAA, siderophores, fixation of nitrogen, solubilization of ZnCO ₃ and Zn ₃ (PO ₄) ₂	Long et al. (2011)
<i>Serratia marcescens</i> LKR01, <i>Arthrobacter</i> sp. LKS02, <i>Flavobacterium</i> sp. LKS03, <i>Chryseobacterium</i> sp. LKS04	<i>Solanum nigrum</i> L.	Zn, Cd, Pb, and Cu	Soil	Production of IAA, siderophores, ACCD, and solubilization of P	Luo et al. (2011)
<i>Serratia</i> sp. LRE07	<i>S. nigrum</i> L	Cd, Cr, Pb, Cu, and Zn	Soil	Production of IAA, siderophores, and solubilization of P	Luo et al. (2011)
<i>Bacillus</i> sp. SLS18	<i>Sorghum bicolor</i> L.	Cd and Mn	Soil	Production of IAA, siderophores, and ACCD	Luo et al. (2011)
<i>Pseudomonas</i> sp. A3R3	<i>Alyssum serpyllifolium</i>	Ni	Soil	Production of IAA, siderophores, ACCD, and solubilization of P; excreted cellulase and pectinase	Ma et al. (2011)
<i>Methylobacterium oryzae</i> CBMB20, <i>Burkholderia</i> sp. CBMB40	<i>Lycopersicon esculentum</i>	Ni and Cd	Soil	ND	Madhaiyan et al. (2007)
<i>P. fluorescens</i> G10, <i>Microbacterium</i> G16	<i>Brassica napus</i>	Pb, Cd, Zn, Cu, and Ni	Soil	Production of IAA, siderophores, ACCD	Sheng et al. (2008)

(continued)

Table 6.1 (continued)

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
<i>Bacillus</i> sp. MN3-4	<i>Alnus firma</i> and <i>B. napus</i>	Pb, Cd, Zn, Ni, and Cu	Soil	Production of IAA and siderophores	Shin et al. (2012)
Endophytes belonged to <i>Firmicutes</i> , <i>Actinobacteria</i> , <i>Proteobacteria</i>	<i>Elsholtzia splendens</i> , <i>Commelina communis</i>	Cu	Soil	Production of IAA, siderophores, ACCD, and arginine decarboxylase	Sun et al. (2010)
<i>Microbacterium</i> sp. NCr-8, <i>Arthrobacter</i> sp. NCr-1, <i>Bacillus</i> sp. NCr-5, <i>Bacillus</i> sp. NCr-9, and <i>Kocuria</i> sp. NCr-3	<i>Noccaea caerulea</i> , <i>Thlaspi perfoliatum</i>	Ni	Soil	Production of IAA, siderophores, and ACCD	Visioli et al. (2014)
<i>Serratia nematodiphila</i> LRE07	<i>Solanum nigrum</i> L.	Cd	Soil	ND	Wan et al. (2012)
<i>Rahnella</i> sp. JN27	<i>Amaranthus hypochondriacus</i> and <i>A. mangostanus</i>	Cd	Soil	Production of IAA, siderophores, ACCD, and solubilization of P	Yuan et al. (2014)
<i>Burkholderia</i> sp. SaZR4, <i>Burkholderia</i> sp. SaMR10, <i>Sphingomonas</i> sp. SaMR12, and <i>Variovorax</i> sp. SaNR1	<i>Sedum alfredii</i> Hance	Cd and Zn	Soil	ND	Zhang et al. (2013)
Endophytes belonged to <i>Firmicutes</i> , <i>Proteobacteria</i> , and <i>Actinobacteria</i>	<i>Pteris vittata</i> and <i>P. multifida</i>	As	Soil	Production of IAA	Zhu et al. (2014)
Arbuscular mycorrhizal fungi					

(continued)

Table 6.1 (continued)

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
<i>Glomus mosseae</i>	<i>Trifolium subterraneum</i> , <i>Lolium perenne</i>	Cd, Zn	Soil	AMF adsorbed up to 0.5 mg Cd per gram of mycelia equivalent to threefold binding capacity of non-tolerant fungi or tenfold higher than reported for <i>Rhizopus arrhizus</i> (commonly used as biosorption organism)	Joner et al. (2000)
<i>Glomus intraradices</i>	<i>Helianthus annuus</i>	Cr	Soil	AMF increased fivefold root Cr concentration	Davies et al. (2001)
<i>Glomus mosseae</i> , <i>Glomus caledonium</i> , and <i>Glomus claroideum</i>	<i>Sorghum vulgare</i>	Cu	Soil	RM increased Cu-sorption from 2.3 to 13.8 mg Cu g ⁻¹ dry mycelium	
<i>Glomus mosseae</i>	<i>Trifolium pratense</i> L	Zn	Soil	22% of total Zn plant uptake linked to ERM	Chen et al. (2003)
<i>Gigaspora rosea</i> and <i>Glomus mosseae</i>	<i>Zea mays</i> and <i>Sorghum vulgare</i>	Cu	Soil	GRSP produced by <i>G. rosea</i> hyphae bound up to 28 mg Cu g ⁻¹ and <i>G. mosseae</i> ranged from 1.0 to 1.6 mg Cu g ⁻¹	Gonzalez-Chavez et al. (2004)
Mixed spores of mycorrhizal fungal species isolated from orchard soil	<i>Kummerowia striata</i> , <i>Ixeris denticulata</i> , <i>Lolium perenne</i> , <i>Trifolium repens</i> , and <i>Echinochloa crus-galli</i>	Pb	Soil	AMF inoculation increased the Pb root concentration from 7.6% to 57.2%	Chen et al. (2005)
Indigenous mycorrhizal populations from polluted soils	<i>Argemone subfusiformis</i> , <i>Baccharis linearis</i> , <i>Oenothera affinis</i> , <i>Polypogon viridis</i>	Cu, Zn	Soil	GRSP bound from 1.4% to 28% of total Cu in soil and from 1.4% to 5.8% of total Zn	Cornejo et al. (2008)

(continued)

Table 6.1 (continued)

Plant growth-promoting rhizobacteria (PGPR)					
Bacterial strain(s)	Host plant	Heavy metal	Medium	Beneficial effects	References
Indigenous mycorrhizal populations from polluted soils	Degraded ecosystem with presence of <i>Sesleria caerulea</i>	Pb, Zn	Soil	GRSP bound Pb attained until 23.4 mg g ⁻¹ , which represents about 16% of total soil Pb	Vodnik et al. (2008)

enzymes, and growth regulators that help in water and nutrient (P, N, & K) uptake, osmolyte accumulation, osmotic adjustment, stomatal regulation, and associated nitrogen fixation as additional benefits to host plants (Ma et al. 2011, 2016b). Thus, inoculating plants with endophytes could be an excellent strategy to enhance the phytoremediation of HM-contaminated sites. Endophytes applied to enhance HM phytoremediation with associated benefits have been recently reviewed by several researchers (Afzal et al. 2014; Ma et al. 2016b).

Arbuscular mycorrhizal fungi (AMF: colonize plant roots) have been also reported to protect their host plants against heavy metal toxicity through their mobilization from soil and thus help in phytoremediation (Marques et al. 2009; Meier et al. 2012; Khan et al. 2014). The possible mechanisms by which AMF protect their host plants through metal mobilization from soil include:

- (a) Immobilization by chelation;
- (b) Binding of metals to biopolymers in the cell wall;
- (c) Superficial immobilization in the plasmatic membrane once metals cross the cell wall;
- (d) Membrane transportation that mobilizes metals from the soil to the cytosol;
- (e) Intracellular chelation through MTs, organic acids, and amino acids;
- (f) Export of metals from cytosol by membrane transporters;
- (g) Sequestration of metals into vacuoles;
- (h) Transportation of metals by means of fungal hyphae;
- (i) Storage of metals in fungal spores; and
- (j) Exportation by the fungus and access into the plant cells, involving both active and passive transportation into the mycorrhizae (Meier et al. 2012; Cabral et al. 2015).

They confer resistance against drought, high salt, and toxic metal concentration and improve nutrient supply and soil physical properties (Khan et al. 2014). The exact mechanism of plant protection is still not fully understood, and further research is required to explore their role in the phytoremediation.

6.4 Microbe-Assisted Phytoremediation: Concepts and Mechanisms

Most plants growing in polluted environments are often characterized by relatively low growth caused by toxic effects of accumulated substances or their degradation products (Glick 2003). However, the negative effect of the environment can be alleviated by soil microorganisms. The soil is an environment settled by a wide range of genetically diverse microorganisms, which play crucial roles in nutrient cycling and in soil-forming processes (Ahemad and Khan 2013). They include both bacteria, which are the most numerous (9×10^7 in one gram of typical soil), and fungi (2×10^5) (Alexander 1991). Microorganisms inhabiting metalliferous soils often exhibit tolerance to high concentrations of heavy metals (HMs) in the environment. Many studies have confirmed that interactions between plants and metallo-tolerant microorganisms facilitate the recultivation of HM-polluted areas (e.g., Chen et al. 2014; Ma et al. 2015; Zloch et al. 2017). This synergism can accelerate the process of remediation by phytostabilization or phytoextraction of HMs but can also increase plant growth and development under adverse environmental conditions (Khan et al. 2009). The functioning of plant–microorganism associations in HM-contaminated soils depends on both the microorganisms and the plant host (Egamberdieva et al. 2016). The plant roots secrete exudates that are the source of nutrients for microorganisms and also increase the solubility of macro- and microelements affecting the activity of microorganisms associated with plant roots (Iqbal and Ahemad 2015). Plant-associated microorganisms can play significant roles in nutrient cycling, improving soil structure, detoxifying harmful contaminants, modulating plant defense responses to stress factors, and assisting in biological control of phytopathogens and plant growth (Elsgaard et al. 2001; Filip 2002; Giller et al. 1998).

To generalize, the activity of microorganisms inhabiting the roots (endophytes) or rhizosphere can increase the capacity of metalliferous soil phytoremediation as follows:

1. Directly: Plant-associated microorganisms directly increase the uptake and translocation of metals (facilitation of phytoextraction) or reduce the mobility/availability of metals within the rhizosphere (phytostabilization).
2. Indirectly: Microorganisms increase plant tolerance to HMs and/or promote plant biomass production in order to remove/stabilize contaminants. A general outline of plant–microbe–metal interactions for the phytoremediation of heavy metal-contaminated soils is shown in Fig. 6.1.

6.4.1 Direct Mechanisms

In most metalliferous soils, HMs are strongly adsorbed onto soil particles and are therefore hardly available for plant roots during phytoextraction (Gamalero and Glick 2012). Microorganisms can increase their solubility and availability via (a) auto- and heterotrophic leaching (associated with redox reaction), (b) secretion of

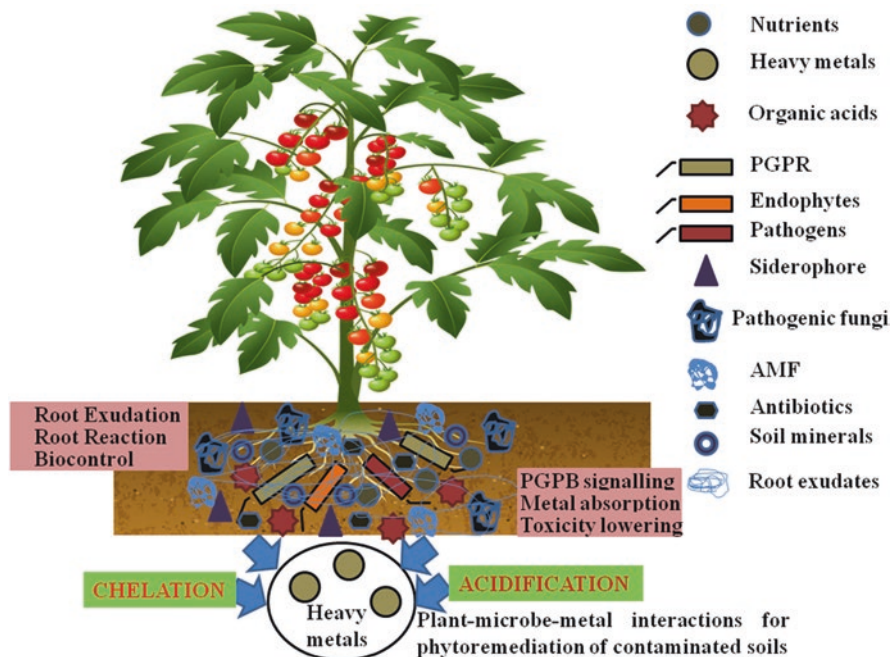


Fig. 6.1 Outline mechanism of plant–microbe–metal interactions for microbe phytoremediation of heavy metal-contaminated sites

organic acids and biosurfactants, and (c) release of siderophores (Gadd 2004; Wenzel 2008; Li et al. 2012). These processes can lead to the dissolution of minimally soluble metal–mineral compounds (including phosphates, sulfates, and more complex ores) as well as metal desorption from the surface of clay minerals or organic matter (Gadd 2004). Microorganisms can acidify the environment by releasing H^+ through the transmembrane H^+ -ATPase, maintaining the membrane potential or as a result of carbon dioxide accumulation generated during respiratory processes, which leads to the release of free metal cations from their complexes with anions *via* ion exchange occurring between H^+ and metals (Gadd 2004). In most cases, autotrophic leaching of metals is performed by acidophilic bacteria, which assimilate carbon dioxide and produce energy from Fe^{2+} oxidation or sulfur compound reduction (Rawlings 1997; Schippers and Sand 1999). Moreover, many studies have confirmed that rhizosphere bacteria such as *Thiobacillus thiooxidans* are interesting in the context of phytoextraction because they reduce rhizosphere pH through the conversion of reduced sulfur into sulfate, improving the availability of Cu, for example, to plants (Rawlings and Silver 1995; Shi et al. 2011). In recent years, much attention has been paid to the phenomenon of low-molecular-weight organic acids (LMWOAs, compounds with molecular weights ≤ 300 Da and containing one or more carboxylic groups) being secreted by plant-associated

microorganisms and their potential role in the regulation of HM solubility and mobilization of mineral compounds within the rhizosphere (Rajkumar et al. 2012).

Chelators are mainly known to enhance the solubility of HMs and include citric, lactic, malic, oxalic, malonic, 5-ketogluconic, tartaric, succinic, and formic acids (Panhwar et al. 2013). Commonly synthesized oxalates and citrates are known for their ability to form stable complexes with many HMs; furthermore, citrates are highly mobile and highly resistant to degradation (Francis et al. 1992). Saravanan et al. (2007) observed that during secretion of 5-ketogluconic acid by an endophytic bacterium of *Gluconacetobacter diazotrophicus*, various Zn^{2+} sources (e.g., ZnO , $ZnCO_3$, or $Zn_3(PO_4)_2$) are dissolved, which increases the pool of Zn^{2+} readily available for roots. Moreover, Han et al. (2006) revealed stimulatory effects of acetic and malic acid on the Cd^{2+} accumulation in the roots of corn (*Zea mays* L.). Similar observations were noticed in the case of, for example, increased uptake of Cd^{2+} and Zn^{2+} by *Sedum alfredii* due to secretion of formic, acetic, tartaric, succinic, and oxalic acids by rhizosphere bacteria (Li et al. 2010) as well as stimulation of Cd^{2+} uptake by wheat in the presence of citric acid (Panfili et al. 2009). Regarding synthesis of LMWOAs, particularly oxalate, by fungal strains, it has also been suggested that the release of metal ions *via* enhanced mineral weathering plays an important role and leads to the uptake of HMs by plants and microorganisms (Jones 1998; Gadd and Sayer 2000). Such an ability was noted for *Beauveria caledonica*, *Aspergillus niger*, *Penicillium bilaiae*, or *Oidiodendron maius* in the case of cadmium, copper, lead, nickel, or zinc mineral solubilization (Martino et al. 2003; Fomina et al. 2005; Arwidsson et al. 2010). Another important class of metabolites with great potential to increase metal mobility and stimulate the phytoremediation process is the microbial surface-active substances called biosurfactants (Rajkumar et al. 2012). Biosurfactants are amphiphilic molecules consisting of long nonpolar parts (hydrophobic) and polar/ionic (hydrophilic) heads. Their hydrophilic parts consist of mono-, oligo-, or polysaccharides, peptides, and proteins, while their hydrophobic parts usually contain saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols. Siderophores are low-molecular-weight organic compounds (500–1500 Da) with high specificity and affinity for Fe^{3+} chelation (Miethke and Marahiel 2007), which release iron from minerals or organic matter in order to facilitate iron uptake when its availability in the environment is limited (Li et al. 2012). Despite the substantial diversity of chemical structures of siderophores (over 500 diverse siderophores described to date), they can be divided into several groups depending on the presence of metal-binding ligands: (a) hydroxamates, (b) catecholates, (c) phenolates, (d) carboxylates, and (e) mixed (Essen et al. 2006; Saha et al. 2013; Wang et al. 2014; Pluhacek et al. 2016). While the key role of siderophores in iron homeostasis in microorganisms has been well known for over 60 years, there is increasing evidence for the activation of siderophore synthesis by bacteria in the presence of toxic metals, which indicates their potential role in HM homeostasis (Schalk et al. 2011; Złoch et al. 2016). It was suggested that siderophores may form stable complexes with ions such as Ag^+ , Zn^{2+} , Cu^{2+} , Co^{2+} , Cr^{2+} , Mn^{2+} , Cd^{2+} , Pb^{2+} , Ni^{2+} , Hg^{2+} , Sn^{2+} , Al^{3+} , In^{3+} , Eu^{3+} , Ga^{3+} , Tb^{3+} , and Tl^+ . Enhanced siderophore synthesis by bacteria (so-called siderophore-producing bacteria, SPB) can protect them

from the toxic effects of HMs by, for example, extracellular sequestration, thereby preventing metals from entering into the cells (Saha et al. 2013). Similar observations were noted for fungi; however, the relatively weak ability of fungal siderophores (mainly hexadentate hydroxamate) to chelate HMs other than Fe(III) (Enyedy et al. 2004; Farkas et al. 2008) makes their potential in HM bioremediation rather limited (Pocsi 2011). On the other hand, increased siderophore synthesis can improve the phytoextraction capacity of plants by increasing the mobility of metals and thus their availability for roots (Glick 2003; Rajkumar et al. 2010).

6.4.2 Indirect Mechanisms

The most important mechanisms, and those confirmed so far in the scientific literature, are (a) the synthesis of phytohormones and enzymes (primarily indole-3-acetic acid [IAA], 1-aminocyclopropane-1-carboxylate [ACC] deaminase), (b) increased nutrient uptake (nitrogen fixation, phosphorus, and iron mobilization), and (c) tolerance to biotic (pathogen control) and abiotic (drought, salinity, contamination) stress conditions (Hryniewicz and Baum 2012; Ma et al. 2016b). The specific response of nitrogen-fixing legumes in response to Cd, like an overproduction of reactive oxygen species (ROS) in the nodules and its mitigation by PGPB (e.g., by the release of siderophores), was reviewed by Gomez-Sagasti and Marino (2015). IAA is one of the most important phytohormones and regulates many physiological and morphological functions of plants (Glick 2012). In addition to stimulation of root growth, alleviating salt stress, participating in plant–pathogen interactions, and eliciting induced systemic resistance (ISR) against various diseases, IAA is primarily involved in stimulating the proliferation of lateral roots. IAA-synthesizing microorganisms can indirectly increase the extraction of metals and nutrient supplementation of plants by inducing root proliferation and increasing their uptake surface (Glick 2010). Apart from IAA, soil microorganisms demonstrate the ability to synthesize other phytohormones (cytokinins, gibberellins). However, fungi are also known for their ability to secrete compounds similar to phytohormones such as auxins, cytokinins, gibberellic acids, or ethylene (Chanclud and Morel 2016). Ethylene is a crucial phytohormone that regulates plant cell elongation and metabolism (Ping and Boland 2004), and its overproduction induced by stress factors, such as HMs, may inhibit processes involved in plant development (i.e., root elongation, lateral root growth, and formation of root hairs) (Mayak et al. 2004). Microbial ACC deaminase causes the hydrolysis of 1-aminocyclopropane-1-carboxylic acid (an ethylene precursor) to α -ketobutyric acid and ammonia, which can be used as a source of carbon and nitrogen by microorganisms. Thus, inoculation of plants with strains synthesizing ACC deaminase indirectly affects root growth and proliferation and positively influences the plant biomass and efficiency of HM phytoremediation (Gleba et al. 1999; Agostini et al. 2003; Arshad et al. 2007). ACC deaminase-containing bacteria are relatively common in soil (typically free-living pseudomonads) (Glick 2005, 2014), while among fungi, this activity is less frequently observed (although it has been reported in *Penicillium*

citrinum and *Trichoderma asperellum* T203) (Jia et al. 2000; Viterbo et al. 2010) and has not been investigated in detail. The presence of elevated amounts of HMs often affects the supplementation of plant roots with Fe, P, Mg, or Ca, leading to plant growth retardation (Ouzounidou et al. 2006; Parida et al. 2003). Under such conditions, plant-associated microorganisms facilitate the uptake of nutrients by increasing their availability for plant roots (Rajkumar et al. 2012). Examples include the bacteria reported by Nautiyal et al. (2000), which demonstrate the ability to increase P availability for plants through phosphate precipitation by acidification of the soil solution, complexation, secretion of organic acids, and ion-exchange reactions or through mineralization of organic phosphorus compounds secreting acid phosphatase (van der Hiejden et al. 2008). Among P-solubilizing microorganisms, fungal strains belonging to *Aspergillus* and *Penicillium* are known for their strong ability to release P from insoluble inorganic compounds, primarily by producing organic acids and preventing the precipitation of P with metals (Jones 1998; Mendes et al. 2014). A similar effect is observed for iron, which is present in the Earth's crust in large quantities; however, iron is found mostly as insoluble hydroxides and oxyhydroxides that are not readily available to plants (Budzikiewicz 2010; Rajkumar et al. 2010). Moreover, plants growing in metalliferous soils are very often exposed to iron deficiency, which produces a decreased photosynthesis rate and consequently a decline in their growth and development (Nagajyoti et al. 2010a, b). In such cases, inoculation of plants with SPB can be a promising method to mitigate iron deficiency (Iqbal and Ahemad 2015). Many studies have confirmed that SPB successfully increased chlorophyll concentration and improved other plant growth parameters in the presence of HM contamination in the soils by facilitating iron uptake (Burd et al. 1998, 2000, Carrillo-Castaneda et al. 2003, Barzanti et al. 2007). It has also been observed that the synthesis of siderophores may stimulate plant growth in metalliferous areas via the following activities: (a) involvement in maintaining an appropriate level of IAA through binding of HMs, thereby reducing the inhibitory effect of metals on the IAA biosynthesis pathways, and through decreased production of reactive oxygen species (ROS), which can degrade IAA molecules; (b) mitigation of oxidative stress by stimulation of peroxidase activity; and (c) phytopathogen control via chelation of iron ions within the rhizosphere and decreasing the availability of iron for pathogens (Dimkpa et al. 2008; Rajkumar et al. 2009).

6.5 Microbe-Assisted Phytoremediation of Heavy Metal-Contaminated Sites

It has been well demonstrated that the inherent ability of endophytic bacteria may help host plants adapt to unfavorable soil conditions and enhance the efficiency of phytoremediation by promoting plant growth, alleviating metal stress, reducing metal phytotoxicity, and altering metal bioavailability in soil and metal translocation in plant (Ma et al. 2011; Ozyigit and Dogan 2015). Overall, the plant-associated microbes promote phytoremediation process in metal-polluted soils by two distinct

means, i.e., enhancement of plant metal tolerance and growth and alteration of metal accumulation in plants, as discussed in above sections. Some important studies on the phytoremediation of heavy metal-contaminated soils assisted by plant growth-promoting rhizobacteria, endophytes, and arbuscular mycorrhizal fungi have been summarized in Table 6.1.

6.6 Challenges and Future Perspectives

The success of phytoextraction depends on interactions among soil, metals, and plants. Many plants are not capable of gaining sufficient biomass for noticeable rates of remediation when elevated levels of pollutants are present (Harvey et al. 2002; Chaudhry et al. 2005). The remediation process of contaminated soils is limited and slowed because of their poor nutrient nature. Soil microbes are thought to exert positive effects on plant health via mutualistic relationships between them. However, microbes are sensitive to pollution, and depletion of microbial populations, both in terms of diversity and biomass, often occurs in such contaminated soils (Shi et al. 2002). Biotic or abiotic stress through a small change in the physicochemical–biological properties of rhizosphere soils can cause a dramatic effect on plant–microbe interaction. Further, isolation and characterization of suitable plant-associated beneficial microbes is a time-consuming process. It also requires the analysis of more than thousands of isolates, and thus identification of specific biomarkers may help to select the effective plant–microbe interactions for microbe-assisted phytoremediation (Rajkumar et al. 2012). Further, to ameliorate metal toxicity, plant growth promotion, and metal sequestration, extensive research efforts are also required to explore novel microbial diversity, their distribution, and functions in the autochthonous and allochthonous soil habitats for microbe-assisted phytoremediation of HM-contaminated sites.

6.7 Conclusions and Recommendations

- (a) HM pollution in the environment and associated toxicity in living beings is of serious eco-environmental concern.
- (b) Inoculation of plants with associated microbes (such as PGPRs, endophytes, and arbuscular mycorrhizal fungi) exhibiting multiple traits could be an excellent strategy to enhance metal detoxification in the rhizosphere. A clear-cut understanding of plant–microbe–metal–soil interactions is crucial for microbe-assisted phytoremediation of HM-contaminated soils.
- (c) The effectiveness of co-inoculation of PGPB and AMF in response to multiple biotic and/or abiotic stresses must be assessed for better applicability at field.
- (d) Identification of functional genes of beneficial microbes responsible for growth enhancement and metal detoxification should be identified.

- (e) Trials for the commercial production of bioinoculants for use in metal decontamination should be performed to make a positive remark toward their field applicability.
- (f) Genetic engineering of metal-accumulating plants and associated microbes with required traits could be a very useful strategy for the enhanced phytoremediation, but associated risks should also be considered before field application.
- (g) A detailed and accurate characterization of target metal(loid)-contaminated soils is needed before the inoculation of microbes, as well as adequate strategies to enhance inoculant performance by using efficient carrier materials.
- (h) The complexity and heterogeneity of soils contaminated with multiple metals and organic compounds requires the design of integrated phytoremediation systems that combine different processes and approaches.
- (i) Field trials are required to document time and cost data to provide recommendations and convince regulators, decision-makers, and the general public about the low-cost applicability of microbe-assisted phytoremediation of heavy metal-contaminated sites and for better acceptance in remediation industries.

Conclusively, microbe-assisted phytoremediation technology holds great promise in gaining the sustainable agricultural production in conjunction with phytoremediation of heavy metal-contaminated sites for environmental sustainability.

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