

Chapter 2

Microbial-Assisted Phytoremediation: A Convenient Use of Plant and Microbes to Clean Up Soils



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2.1 Introduction

Environmental pollution by metal(loid)s (e.g., heavy metals—HMs) is a severe problem worldwide, as soils and aquatic resources became increasingly contaminated, threatening land ecosystems, surface and groundwater, as well as food safety and human health [1]. The primary sources contributing to this extended pollution are anthropogenic inputs related to the burning of fossil fuels, mining and continued industrial activities, disposal of municipal solid wastes and wastewater discharges or use for irrigation, and excessive utilization of fertilizers and pesticides [1–9]. A consequence of these anthropogenic activities is an increase of contaminated areas, which should be remediated to prevent or mitigate transfer of contaminants into terrestrial, atmospheric, or aquatic environments. Point and diffuse contamination by organic and inorganic pollutants causes wide concerns, and intentional or accidental introduction of these substances in the environment may represent serious impacts on public health.

Soil contamination is an important issue across the European Union (EU). About 3.5 million sites in the EU were estimated to be potentially contaminated

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with 0.5 million sites being highly contaminated and in need of remediation. About 400,000 polluted sites were already identified in Germany, England, Denmark, Spain, Italy, the Netherlands, and Finland. Sweden, France, Hungary, Slovakia, and Austria have at least 200,000 contaminated sites. Greece and Poland reported 10,000 contaminated land areas, while Ireland and Portugal reported fewer than 10,000 contaminated sites [10, 11]. Soils act as a final acceptor of toxic substances/trace elements (e.g., HMs; nonessential metal(loid)s), and these inorganic pollutants can limit development and growth of plants and pose a health hazard to humans and animals because some may be bioaccumulated and bio-magnified along the whole food chain [3, 12–15].

When the concentration of these pollutants is above a defined legal standard value, water, air, and soils are described as polluted, and environmental remediation becomes fundamental to decrease the potential risk of food chain contamination and other associated health risks [13, 16, 17]. Thus, it is necessary to use efficient soil cleanup techniques to restore heavy metal-polluted soils. Over the last decades, several physical, chemical, and biological approaches have been attempted to achieve this goal. Conventional treatments include excavation and transfer of soil to landfills, soil washing with water and solubilizing agents, building a physical cover, solidification through use of stabilizing agents, vitrification at high temperatures, electrochemical separation, etc. which may rapidly remediate soils, but irreversibly damage the ecological environment [18–21].

Between the different approaches for the reclamation of metal(loid)s contaminated soils, special attention is drawn to the technologies of phytoremediation (green and clean technologies) [10]. Phytoremediation is based on efficient, inexpensive, and eco-friendly rehabilitation strategies that use plants and associated soil microorganisms to absorb, accumulate, immobilize, or biodegrade organic and inorganic pollutants, present in different environmental matrices (air, soil, and water), through physical, chemical, and biological processes. Phytoremediation is proposed as a relatively recent technology with sustainable costs [13, 22, 23] that improves the native microflora and the physical, chemical, and biological properties, thus enhancing soil health and fertility [1, 20, 21, 23–27]. Moreover, phytoremediation appears to benefit plant growth and carbon sequestration, because harvested biomass can be used to produce renewable energies like biofuel production [13, 23, 28–30].

Phytoremediation is therefore a suitable option to clean metal(loid)s soil contamination. However, metal(loid)s are immutable, and therefore several of the low-cost phytoremediation options that are available for the remediation of organic contamination, such as phytodegradation and rhizodegradation, are not applicable to metal(loid)s-contaminated soils [31, 32]. Moreover, another phytoremediation technique, phytovolatilization, can only be used for some metals like mercury (Hg) and selenium (Se) which have volatile forms [24, 31]. Remediation options that remain are phytostabilization and phytoextraction [31, 32]. Based on economic implications, the aim of phytoremediation can be (1) plant-based extraction of metals with financial benefit (phytoextraction), (2) risk minimization (phytostabilization),

and (3) sustainable soil management in which phytoremediation steadily increases soil fertility allowing growth of crops with added economic value [10, 32].

It can be an effective strategy for in situ or ex situ stabilization, removal, or biodegradation of a great range of pollutants in the different environmental compartments, including trace elements (HMs and nonessential metal(loid)s), radionuclides, excess nutrients, salts, and recalcitrant organic pollutants, like petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated solvents, and explosives (e.g., 2,4,6-trinitrotoluene) [1, 33]. Phytoremediation includes a range of plant-based remediation processes, and the most usual are presented in Table 2.1.

Phytoremediation reduces the risks of pollutants dispersion, and it is applicable for the decontamination of soils or wastewaters with mixed pollutants [13, 34]. 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 [13, 35].

The plants considered more efficient for phytoremediation are the metallophytes. These are able to survive and reproduce on metal-polluted soils [31, 36]. However, a great number of known metallophytes have small biomass and slow growth, characteristics that are not advantageous for phytoremediation technologies [31, 37]. Some metallophytes can be further classified as metal hyperaccumulators [31, 36]. Content of specific metal(loid)s in these plants exceeds levels that are usually required for normal growth and development. Hyperaccumulators belong to distantly related families, but share the ability to grow on metalliferous soils and accumulate metals in levels far in excess of those found in the majority of species, without suffering phytotoxic effects [1]. Three basic hallmarks distinguish hyperaccumulators from related non-hyperaccumulating plants: a strongly enhanced rate of metal(loid)s uptake, a faster root-to-shoot translocation, and a greater ability to detoxify and sequester metals in leaves [1].

These plants can be used in phytoextraction applications, but in most cases, hyperaccumulator plants are only able to accumulate one metal, while metal-polluted soils often contain a mixture of metals [31, 32, 36, 38]. This mixed pollution is not only challenging for phytoremediation with hyperaccumulator plants but also for metal-tolerant plants in general as plant metal tolerance mechanisms are

Table 2.1 Overview of most employed phytoremediation techniques (Adapted from [1])

Technique	Description
Phytostabilization	Immobilization of pollutants in the root zone while stabilizing the soil, thus reducing metal leaching and aerial dispersion of contaminated soil particles
Phytoextraction	Uptake of pollutants by plant roots and their translocation and subsequent accumulation in aboveground tissues
Rhizodegradation	Breakdown of organic pollutants through rhizospheric microbes
Phytodegradation	Plant metabolism transforms, breaks down, stabilizes, or volatilizes organic compounds from the soil and groundwater into harmless by-products
Phytovolatilization	Uptake of pollutants by plant roots and their transformation into volatile forms that transfer into the atmosphere

usually metal-specific. Another group of metallophytes, the metal excluders, are also considered appropriate for phytoremediation. These plants accumulate metals from the soil into their roots, but restrict metal(loid)s transport inside the plant. Such plants cannot be used for phytoextraction, rather they can be effective in phytostabilization techniques [31, 39]. Other plants used for phytoextraction of metals are high biomass-producing non-hyperaccumulator plants [32]. In comparison with hyperaccumulators, these plants accumulate a lower concentration of metal(loid)s but produce higher aboveground biomass, removing similar quantities of metals as hyperaccumulators [24, 31]. In conclusion, there are a large number of different plant species and ecotypes referred in the literature with the potential for phytoremediation purposes (phytoextraction or phytostabilization).

The success of phytoremediation is strongly determined by the amount of plant biomass present and the concentration of metal(loid)s in plant tissues. Therefore, high uptake and an efficient root-to-shoot transport system combined with enhanced metal tolerance provide hyperaccumulators with a high potential detoxification potential. However, high levels of metal(loid)s are toxic to most plants and can impair cell metabolism, reduce plant growth, and restrict metal phytoextraction. Physiological mechanisms that may be affected include enzymatic activity, protein structure, water balance, respiration and ATP content, photosynthesis, plant division, and morphogenesis [1, 5]. Phytoremediation has some disadvantages: naturally occurring hyperaccumulators grow slowly, the remediated area is only that close to the root, harvestable aboveground biomass is low, and numerous species cannot be planted in places strongly polluted. So genetic engineering approaches to develop transgenic plants with more favorable characteristics such as high biomass production, more metal accumulation, tolerance against metal toxicity, and well adapted to a variety of climatic conditions might be more beneficial in this respect [40].

Further research is needed in the field of genetic engineering to improve the phytoremediation abilities of transgenic plants and to understand the mechanisms and effectiveness of phytoremediation techniques in order to make these technologies more effective, timesaving, and economically feasible [40]. Accordingly, it is necessary to develop other phytoremediation strategies for metal(loid)s-contaminated soils. Strategies to improve phytoremediation efficiency are the use of soil amendments (to increase or decrease metal availability) and the use of the plants' associated microorganisms. The rhizospheric environment is an essential habitat for different microbes including protozoa, algae, fungi, and bacteria. Such microorganisms exhibit a diversity of associations with plants [41, 42]. Microbes have the ability to synthesize and sense signaling molecules that trigger microbial populations to form a biofilm around the root surface and induce a related response. A number of plant-associated microorganisms are favorable because they can enhance the bioavailability of nutrients and mitigate the negative effects of metal(loid)s on plants. Rhizospheric microorganisms possess potential to biodegrade organic pollutants, through rhizodegradation, biotransformation, and volatilization [41].

Numerous studies have demonstrated the adverse effect of different metal(loid)s on the soil microbial diversity and their disastrous interaction with plants in polluted soils. Excessive concentrations of metal(loid)s in soils can affect the growth,

morphology, and metabolism of microorganisms mainly through destruction of cellular membranes and organelles, enzyme denaturation, and functional or conformational disturbance [41, 43]. To optimize the results of the application of phytoremediation techniques, many researchers have analyzed the dynamics between plants, microorganisms, and metal(loid)s in rhizospheric environments [43–54]. Recently, inoculation of plants with selected and acclimatized microbes (bioaugmentation) has attained salience for phytoremediation of metal(loid)s polluted soils, and assisted phytoremediation techniques are starting to be used to decontaminate polluted soils on large scales [40, 48, 50, 52, 55].

The interface between microbes and plant roots in the rhizosphere is believed to vastly influence the growth and survival of plants. The biotechnological potential of microorganisms to resist and/or remove metals directly from polluted media and their beneficial effects on plant growth may lead to environmental-friendly and cost-effective strategies toward reclamation of polluted soils. Rhizosphere microbes can determine metal availability and change speciation of HMs by producing biosurfactants, organic ligands via microbial degradation of soil organic matter, and exudates (e.g., metabolites, microbial siderophores), which can modify HMs bioavailability and uptake by plants [25, 44, 56–58]. Thus, microbial activities strongly influence metal speciation and transport in the environment. Different organisms exhibit diverse responses to toxic ions, which confer upon them a certain range of metal tolerance.

In conclusion, microbe-assisted phytoremediation has emerged as a sustainable soil cleanup technology with reduced soil disturbance, low maintenance, and overall low costs [25]. Recent studies have demonstrated that microorganisms play an important role in phytoremediation technology. Some species of microorganisms, including biodegradative bacteria, plant growth-promoting bacteria (PGPB), and filamentous fungi, appear to be beneficial in phytoremediation by changing rhizospheric environment, increasing biomass production and bioavailability or stabilization of HMs, and reducing the respective toxicity [23, 59]. Therefore, they can be used in soil amelioration. Microorganisms can produce organic compounds that solubilize and/or stabilize HMs by changing pH and oxidation-reduction potential of their soil environment.

Some polysaccharides secreted by microorganisms can easily bind soil particles, thereby improving the formation of soil aggregates. For example, glomalin and other glycoproteins released by arbuscular mycorrhizal fungi (AMF) may improve soil structure by increasing particle aggregation and aggregating stabilization against wind and water erosion. In addition, some soil bacteria are able to biodegrade toxic organic compounds, including solvents, produced in mineral processing [23].

Microorganisms further play an important role in removing or detoxifying HMs during the phytoremediation process [23]. Although phytoremediation is a sustainable and inexpensive technology for the removal of pollutants from the environment by plants, it is nevertheless a slow process.

Thus, it is important to improve the efficiency and increase the level of the stabilization or removal of toxic metal(loid)s from soils by plants. For this reason, greater attention has been paid to the role that fungi play in plants grown on

metal-contaminated soils that have poor nutrients, low water-holding capacity, and adverse physical conditions [60].

2.2 Phytoremediation of Metals Assisted by Fungi

The microbiota can contribute to plant growth, productivity, carbon sequestration, and phytoremediation. Fungi are generally more tolerant to metals than bacteria [44, 61]. Fungi can efficiently explore soil microsites that are not accessible for plant roots due to the small diameter of the mycelia and can compete with other microorganisms for water and metal uptake, protect the roots from direct interaction with the metals, and hinder metal transport through increased soil hydrophobicity [44, 58]. Fungi have been defined as eukaryotic, heterotrophic, and absorptive organisms, which typically develop a branched, tubular body called a mycelium and reproduce by means of sporulation. Furthermore, the ability of fungi to form extended mycelial networks makes them well suitable for bioremediation processes. The application of filamentous fungi can be a promising alternative or a valuable complement in situations of bacterial malfunction [46, 47, 62]. Indeed, they play an important role in organic and inorganic transformation, element cycling, rock and mineral transformations, bio-weathering, mycogenic mineral formation, fungal-clay interactions, and metal-fungal interactions. The extensive hyphal networks in the soil can also significantly contribute to stabilization of soil aggregates; they modify the chemical composition of root exudates and soil pH and control metal(loid)s bioavailability in the soil [60].

Many fungi, such as *Trichoderma*, *Aspergillus*, and the arbuscular mycorrhizal fungi (AM), have shown the potential to improve phytoremediation processes in metal-contaminated soils [45, 63], because they have high ability to immobilize toxic metals by either the formation of insoluble oxalate, biosorption, or chelation onto melanin-like polymers. In fact, vascular plants host a great variety of fungi. In addition to being susceptible to soilborne pathogens, plant roots are also colonized by nonpathogenic or mutualistic fungi, such as AM fungi, ectomycorrhizae (EM), and dark septate endophytes (DSE). The AM fungi comprise about 150 species of zygomycetous fungi, and EM fungi include about 6000 species that are primarily basidiomycetes along with a few ascomycetes and zygomycetes [44].

The AM fungi are associated with most of herbaceous plants and with various woody plant families, while the EM fungi are mainly associated with a limited number of woody plant families [44, 64]. The mycorrhizal fungi facilitate the absorption of nutrients from the soil and help their translocation to host plants, sequester potentially deleterious metal(loid)s, and can stimulate soil microbial activity contributing to the overall biodegradation of soil pollutants [3, 40, 44, 65, 66]. Some DSE have been found as fungal symbionts in members of the *Cruciferae* species, although these are known as non-mycorrhizal plants [44]. The DSE are broadly classified as conidial and sterile septate fungal endophytes, which form melanized structures, such as inter- and intracellular hyphae and microsclerotia, in plant roots. The DSE

fungi have been found worldwide and coexist often with different mycorrhizal fungi [44]. They have been reported from more than 600 plant species, including plants that are considered non-mycorrhizal [44, 64].

AM fungi of the Glomeromycota are the most common soil microorganisms in natural and agricultural soils [3, 67]. Approximately 160 AM fungal taxa of the order *Glomales* (Glomeromycota) have been described to date. Based on the morphological and molecular identification methods, the AM fungi were divided into 12 genera (*Acaulospora*, *Ambispora*, *Archaeospora*, *Diversispora*, *Entrophospora*, *Kuklospora*, *Geosiphon*, *Gigaspora*, *Glomus*, *Intraspora*, *Paraglomus*, *Scutellospora*) with the *Glomus* species as dominant [60, 68–70]. AM fungi are ubiquitous and form symbiotic associations with the majority of terrestrial plants [71, 72]. Consequently, they represent an important part of the soil microbiome and provide their hosts with benefits including increased access to nutrients through the enlargement of soil volume that can be assessed by roots/mycelia [3, 40], especially phosphorus; they also improve water acquisition and reinforce pathogen resistance [73, 74]. Therefore, mycorrhizal plants invest less energy into the extension of the root system than non-mycorrhizal plants and are more resistant to drought stress and pathogen attacks [31].

Mycorrhizal fungi keep the number of pathogens low through acidification of the rhizosphere and/or increased production of antibiotics [31, 75]. Mycorrhizal fungi can also increase plant's resistance at a physiological level. It has been shown that AMF can lead to greater resistance to herbivores through the action of jasmonic acid [31, 75]. Moreover, mycorrhizal associations could promote a faster closure of the leaf stomata, preventing plant wilting [31, 75], and boost plant osmolyte levels such as proline [31, 76]. Studies carried out by Sarwat et al. [77] showed that AM fungi inoculation in mustard (*Brassica juncea*) raised osmolyte content as proline and mitigate overall symptoms of drought stress caused by sodium chloride.

Mycorrhiza can assist in phytoremediation either by making metal(loid)s more bioavailable for uptake by plants or by reducing metal toxicity in their host plants [12, 31, 37, 78]. However, the phytoremediation efficiency is extremely dependent on fungal species and ecotype [31, 37, 78–80]. The selection of the fungal isolate to use in phytoremediation determines which plant species can be chosen for the application. Obviously, to be able to exert a beneficial effect on phytoremediation effectiveness, mycorrhizal fungi must first establish a mycorrhizal symbiosis. Therefore, the ability of the fungus to survive in metal(loid)s contaminated areas is a prerequisite for its use in phytoremediation applications.

To ensure their survival in metal(loid)s-contaminated soils, mycorrhizal fungi can use different extracellular and intracellular defense mechanisms. Extracellular mechanisms, such as chelation and cell wall binding or biosorption, may be used to prevent metal uptake. Intracellular mechanisms, including binding to nonprotein thiols and transport into intracellular compartments, can reduce the concentration in the cytosol [31, 37]. Intracellular mechanisms depend on transporter proteins and intracellular chelation (e.g., by metallothioneins, glutathione, organic acids, amino acids, and compound-specific chaperones) [31, 81]. Metal transporter proteins can alleviate metal stress by subcellular compartmentation via transporters into the vacuole or

other internal cell compartments and/or vacuolar compartmentation of a complex (e.g., the GSH-M complex). Once chelated, these metal complexes can be transported as well. Furthermore, uptake/efflux of metals via specific transporter systems located in the plasma membrane can be downregulated [31, 81].

Additionally, antioxidative defense processes to detoxify reactive oxygen species (ROS) and mechanisms that focus on the repair of metal-damaged biomolecules can be present [31, 37]. Chelation is a first defense mechanism of mycorrhizal fungi against high metal(loid)s concentrations, which is both metal- and species-dependent. In accordance with the literature, a large range of different chelating agents, excreted by fungi, can be able to chelate metals, but there are also a number of studies reporting the opposite, metal solubilization from metal-containing chelates due to the exudation of organic compounds by mycorrhizal fungi [31, 37, 39, 82, 83]. Mutualistic fungi can also play a role in the protection of roots from metal(loid)s toxicity by mediating the interactions between the metals and the plant roots. The ability of mycorrhizal associations to attenuate metal toxicity for higher plants has been demonstrated [60, 71, 72, 78, 84, 85]. Several mechanisms explain why AM fungi can alleviate the stress of metal(loid)s. Mycorrhizal plants have larger biomass that can dilute the metal concentration [3, 86], and the metal(loid)s can be immobilized and compartmentalized in AM hyphal cells [3, 86, 87]. In particular, some native mycorrhizae surviving at contaminated sites may cause precipitation of metal oxalates in the intracellular spaces of the fungi or the host plant and thus restrict apoplastic transport by the Casparian strip [60, 88]. Alternatively, mycorrhizal fungi can directly protect the plant from the buildup of phytotoxic concentrations of certain pollutants by secreting specific detoxifying compounds (e.g., organic acids) or by binding the pollutants into fungal tissues associated with the roots, thus creating a physical barrier against toxic metal translocation [60, 89].

In addition, AM fungi can produce fungal polyphosphates, metallothioneins, and glycoproteins as glomalin, which have high binding capacities for metal(loid)s [3, 90]. Glomalin is a component of spore and hyphal cell wall of AM fungi and has the ability to sequester metal(loid)s. Driver et al. [91] found that glomalin is tightly bound in AM fungi hyphal and spore walls, and small amounts (<20%) of glomalin are released by AM fungi into the soil environment. Glomalin, though still not biochemically defined, is an N-linked glycoprotein composed of 3–5% N, 36–59% C, 4–6% hydrogen, 33–49% oxygen, and 0.03–0.1% P, and it also contains 0.8–8.8% Fe, which may be responsible for the reddish color of glomalin [92]. Apart from the Glomeromycota, no other fungal group produces this glycoprotein in significant amounts [92].

A number of studies have reported the contributions of glomalin to phytoremediation [92]. While examining the roles of glomalin in metal(loid)s sequestration of two polluted soils, González-Chávez et al. [93] stated the potential of glomalin to reduce availability and toxicity of “potentially toxic elements” such as Cu, Cd, and Pb. Furthermore, Cornejo et al. [94] reported that glomalin-related soil proteins (GRSP) bound to about 28% of the Cu and 6% of the Zn present in a soil highly polluted by these metals. From their study, it appears that the higher the concentration

of the pollutants, the higher the ability of GRSP to bind them and make the pollutants unavailable.

Although the mechanisms by which metal(loid)s are sequestered by glomalin are not clear at all, Malekzadeh et al. [92] proposed that mycelium of AM fungi play a major role in the sequestration of metals. As a result, glomalin may be involved in metal(loid)s sequestration due to its presence on the cell wall of the hyphae. González-Chávez et al. [95] showed that the hyphae of AM fungi sequester Cu as previously illustrated by using transmission electron microscopy and scanning electron microscopy linked to an energy dispersive X-ray spectrometer. This sequestration occurs not only in the mucilaginous outer hyphal wall zone and the cell wall but also inside the hyphal cytoplasm. In conclusion glomalin may reduce toxic elements bioavailability via their stabilization and may decrease their toxicity risk to microorganisms and plants in metal(loid)s-polluted sites [92].

AM fungi may often lower metal(loid) mobility and toxicity either by increasing soil pH [20, 21, 96, 97] or by sequestering inside extraradical mycelium [21, 98]. The phytotoxicity of metal(loid)s and level of plant tolerance are closely related to the stored forms of metal(loid)s and their mobility in plant tissues [20, 21, 99, 100]. Therefore, the distribution of chemical forms could be one of the most important metal(loid)s detoxification mechanisms in plants. It has been reported that AM fungi might enhance the tolerance to Cd of *Medicago sativa* L. by altering Cd chemical forms in different plant tissues [20, 21, 96, 101]. AM fungi can also alter the gene expression that relates to metal tolerance of host plants [3]. AM fungi have also been frequently reported in hyperaccumulators growing in metal-polluted soils indicating that these fungi have evolved a heavy metal tolerance and that they may play important roles in the phytoremediation of these sites [14, 80, 86, 102, 103].

Recently, it has been demonstrated that AM fungi can increase the metal(loid) translocation factor, biomass, and trace element concentration of hyperaccumulators [104–106]. Hyperaccumulators combined with AMF have advantages over the independent use of hyperaccumulators and have been proposed as one of the most promising green remediation techniques [36, 104, 105]. However, it is important to note that reductions [104, 105, 107, 108], increases [104, 105, 109, 110], or no changes of metal(loid)s concentrations in plants following mycorrhizal inoculation have all been observed, depending on the fungal-plant association [104, 105, 111–113].

Sheikh-Assadi et al. [114] reported a higher accumulation of Pb and Cd in the roots of inoculated *Limonium sinuatum* in a pot experiment. Plants inoculated with a mixture of *G. mosseae* and *G. intraradices* and exposed to different Cd and Pb concentrations accumulated Cd and Pb in the roots and translocated very little to the shoots. Total Cd and Pb accumulated in the roots was nearly two to three times higher in AM fungi-inoculated plants compared to non-mycorrhized plants. Furthermore, it was observed that mycorrhized plants had a higher metal tolerance. Therefore, it was concluded that mycorrhization alleviated metal toxicity in the plants and that inoculated *L. sinuatum* could be useful as a Cd or Pb controlling agent for phytoremediation. A good understanding of AM fungal communities under natural metal(loid)s stress can contribute greatly to the recognition of interactions

between fungi, hosts, and metal(loid)s and further their rational utilization in metal(loid)s-polluted sites. Compared with AM fungi, endophytic fungi are ubiquitous and comprise a diverse group of fungi also showing potential to enhance phytoremediation [46, 47, 115].

Endophytic microorganisms (including bacteria and fungi) are likely to interact closely with their hosts and are more protected from adverse changes in the environment. Exploiting endophytic microorganisms to reduce metal(loid) toxicity to plants has been investigated to improve phytoremediation efficiencies. Therefore, endophyte symbiosis can counteract metal(loid) stress that exerts negative effects on plant growth.

The endophytic fungi could increase resistance of host plants to multimetal contamination. They possess suitable metal sequestration or chelation systems to increase their tolerance to metal(loid)s, and their higher biomass is also suitable for bioremediation [44, 116]. Furthermore, the presence of metal-resistant endophytic microorganisms may be valuable for host plants because they can enhance both nutrient assimilation and metal bioavailability, through the exuded metabolites in the plant rhizosphere, improving the phytoextraction processes [44, 54]. The metabolic processes operated by endophytes make them relevant resources for phyto(bio)remediation, helping on the phyto(bio)remediation of pollutants and biotransformation of recalcitrant organic compounds through their own degradative capabilities (phytostimulation or rhizodegradation) [44, 48, 117, 118].

Endophytic yeasts *Cryptococcus* sp. CBSB78 and *Rhodotorula* sp. CBSB79 have been isolated from canola roots (*B. chinensis*) in multimetal-contaminated soils and show resistance to Cd, Pb, Zn, and Cu [44, 119, 120]. Multiple HMs hyperaccumulating plants of the genus *Portulaca* contain endophytic fungi such as *Trichoderma*, *Fusarium*, *Aspergillus*, *Paecilomyces*, *Penicillium*, *Paecilomyces*, *Cladosporium*, and *Lasioidiplodia* [44, 46, 47]. *Penicillium* spp. and *Trichoderma* spp. were the most frequently isolated fungal taxa that can counteract HMs stress [44, 121–123]. Recent studies highlight the possible role of fungal endophytes harbored inside *S. nigrum*, which are able to promote host plant growth and enhanced metal extraction, improving the efficiency of phytoremediation in the cleanup of Cd-contaminated soils [56, 57]. In fact, the inoculation of Cd-resistant endophytic fungi with *S. nigrum* increased the plant's tolerance to the high concentrations of Cd, and the parameters related to the biosorption of Cd, including translocation factor, bioconcentration factor, and Cd tolerance index, were significantly enhanced.

In addition, some filamentous fungi such as *Aspergillus niger*, *Mucor rouxii*, and *Rhizopus arrhizus* can be used as sorbents because of their capacity to sorb metal ions such as Cu^{2+} , Co^{2+} , Cd^{2+} , Zn^{2+} , and Pb^{2+} [23].

Table 2.2 summarizes the published studies on microbial effects on plants under metal stress.

Table 2.2 Microbial-mediated metal(loid)s stress tolerance

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Glomerales</i> species <i>Rhizophagus</i> (25.4%), <i>Funneliformis</i> (19.6%), <i>Claroideoglossum</i> (10.7%)	Sb	<i>Lactuca sativa</i> <i>Daucus carota</i>	Significant increase on its accumulation in carrots (all organs) with higher accumulation in roots In lettuce, accumulation appeared to be dependent on the Sb chemical species Moreover, it was observed for the first time that AM fungi changed the human bioaccessible fraction of Sb in edible organs	Pierart et al. [124]
<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i> , <i>Claroideoglossum lamellosum</i>	Cr(III), Cr(VI)	<i>Ricinus communis</i>	Decreased Cr(VI) concentration in soils	Gil-Cardesa et al. [125]
AM fungi	Pb, As, Cd	<i>S. melongena</i>	AM fungi application improved growth, biomass, and antioxidative defense response of plants against metal(loid)s stress The biomass and metal(loid)s uptake increased with AM fungi inoculation	Chaturvedi et al. [73]
<i>Funneliformis mosseae</i> , <i>Rhizophagus intraradices</i>	Cd	<i>S. nigrum</i>	Significantly enhanced shoot biomass and Cd shoot concentration	Li et al. [65]
<i>Rhizophagus intraradices</i>	Cd	<i>Oryza sativa</i>	Significant effects on root biomass, straw, and root Cd concentration	Luo et al. [21]
<i>G. intraradices</i> BEG140, <i>G. mosseae</i> BEG95, <i>G. etunicatum</i> BEG92, <i>G. claroideum</i> BEG96, <i>G. microaggregatum</i> BEG56, <i>G. geosporum</i> BEG199	Hg	<i>Zea mays</i> L.	Played an important role in the biogeochemical cycle of Hg in terrestrial ecosystems, indicating that AM fungi can alter Hg ligand environment and Hg soil to root mobility	Kodre et al. [126]
<i>Rhizophagus irregularis</i>	Cd	<i>Phragmites australis</i>	AM fungi improved Cd tolerance by promoting growth and changes in the distributions of elements in the treated plants For the first time, this study determined that <i>P. australis</i> inoculated by AM fungi could be a Cd-tolerant species	Huang et al. [127]

(continued)

Table 2.2 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Glomerella truncata</i> PDL-1, <i>Phomopsis fukushii</i> PDL-10	Cd	<i>Solanum nigrum</i>	Significantly improved shoot and root length, chlorophyll content, and dry weight. The results of this study highlight the possible role of fungal endophytes harbored inside <i>S. nigrum</i> , which have the potential to improve the efficiency of phytoremediation or phytostabilization in the cleanup of Cd-contaminated soils	Khan et al. [57]
<i>Funneliformis mosseae</i>	Sb	<i>Cynodon dactylon</i>	Plant biomass was significantly increased by the symbiosis. Compared to uninoculated controls, mycorrhizal colonization significantly increased shoot and root Sb concentrations under all Sb treatment levels. Bioconcentration and translocation factors were elevated by mycorrhizal colonization. The fungus served an important role in Sb transport and fate in soil-plant systems	Wei et al. [128]
<i>Claroideoglossum claroideum</i> , <i>Funneliformis mosseae</i>	Cd, Pb	<i>Calendula officinalis</i> L.	Stimulated accumulation of important secondary metabolites (total phenols, flavonoids, carotenoids) in marigold flowers and, therefore, enhanced the antioxidant capacity. The highest b-carotene values and lycopene were found in the marigold- <i>F. mosseae</i> association	Hristozkova et al. [129]
<i>Rhizophagus irregularis</i>	Pb	<i>Trifolium repens</i> L.	Significant increase in glomalin production at all levels of Pb. Fungal symbiont seems to change the distribution pattern of Pb in organs including extraradical hyphae and roots	Malekzadeh et al. [92]

(continued)

Table 2.2 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Acaulospora</i> , <i>Glomus</i> , <i>Rhizophagus</i>	Pb	<i>Vetiveria zizanioides</i> <i>Ricinus communis</i>	Higher tendency for Pb absorbed by the roots to be transported to the shoots	Schneider et al. [63]
<i>Glomus fasciculatum</i>	Cd, Zn	<i>Helianthus annuus</i> L.	Increased the dry biomass of the plant and accumulation of Zn and Cd in roots and shoots	Mani et al. [130]
<i>Claroideoglomus claroideum</i> , <i>Funneliformis mosseae</i>	Cd, Pb	<i>Calendula officinalis</i> L.	Accumulation of secondary metabolites (phenols, flavonoids, carotenoids) and enhanced antioxidant capacity	Hristozkova et al. [129]
<i>Glomus viscosum</i> , <i>Glomus constrictum</i> , <i>Glomus intraradices</i> , <i>Rhizophagus intraradices</i>	Mn	<i>Phytolacca americana</i>	Accumulated much higher concentrations of Mn, but no obvious correlations with AM fungi	Wei et al. [105]
<i>Glomus etunicatum</i>	Pb	<i>Calopogonium mucunoides</i>	Promoting plant nutrient (P, S, and Fe) acquisition, attenuating the negative effects of Pb on membranes, and contributing to the reduction of ROS generation	De Souza et al. [131]
<i>Glomus mosseae</i>	Cd, Pb	<i>Cajanus cajan</i> (L.) Millsp.	Reduced metal translocation from root to shoot. Exposure to Cd and Pb significantly increased the levels of PCs and GSH. The metal contents were higher in roots and nodules when compared with that in shoots. The results indicated that PCs and GSH might function as potential biomarkers for metal toxicity, and microbial inoculation showed bioremediation potential by helping pigeon pea to grow in multimetal-contaminated soils	Garg and Aggarwal [132]

(continued)

Table 2.2 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Aspergillus niger</i> , <i>Penicillium bilaiae</i> , <i>Penicillium</i> sp.	Ni, Cu, Zn, Pb	NA	Main organic acids identified were oxalic acid (<i>A. niger</i>) and citric acid (<i>P. bilaiae</i>). Exudation rates of oxalate decreased in response to Pb exposure, while exudation rates of citrate were less affected The release of metals was related to the production of chelating acids, but also to the pH decrease. This illustrates the potential to use fungi exudates in bioremediation of contaminated soil	Arwidsson et al. [133]
<i>Sclerotoderma citrinum</i> , <i>Amanita muscaria</i> , <i>Lactarius rufus</i> L.	Zn, Cd, Pb	<i>Pinus sylvestris</i> L.	Reduced translocation of Zn, Cd, or Pb from roots to shoots in pine seedlings	Krupa and Kozdrój [134]
<i>Beauveria caledonica</i>	Pb	NA	Solubilized Pb from pyromorphite and accumulated the highest water-soluble fraction and total Pb concentration in the mycelium	Fomina et al. [135]
<i>Glomus mosseae</i>	Cu, Pb, Cd	<i>Sorghum</i>	Immobilized Cu, Pb, and Cd and accumulated metals in a nontoxic form leading to increased plant growth	González-Chávez et al. [136]
<i>Oidiodendron maius</i>	Zn	NA	Mobilized insoluble inorganic zinc compounds (ZnO and Zn ₃ (PO) ₂). Induction of organic acids (malate and citrate) by the metal compounds was at least in part responsible for metal solubilization	Martino et al. [137]

M metal(loid)s, NA not applicable

2.3 Phytoremediation of Metals Assisted by Bacteria

Between the microorganisms involved in soil metal(loid)s phytoremediation, the rhizosphere bacteria deserve special attention because they can directly improve the phytoremediation process by changing the metal bioavailability via soil pH adjustment, release of chelators, induced redox changes, etc. [50, 54, 58, 61, 102, 138]. It

was further demonstrated that some kinds of microorganisms are able to take up metal(loid)s from contaminated soils by reducing them to a lower redox state [23]. For example, some bacteria, such as *Bacillus arsenicoselenatis*, *Chrysiogenes arsenatis*, and *Sulfurospirillum arsenophilum*, have been found to have a large capacity for mobilizing As in mine tailings, wherein they can rapidly achieve microbial reduction of As(V) [23].

Combining increased rhizospheric plant bioavailability and reduced internal bioavailability of metal(oid)s should allow plants to bioaccumulate higher amounts of metal(oid)s, without increases on phytotoxicity [46, 47, 139]. Further, since the bacterial cells (approximately 1.0–1.5 μm^3) have an extremely high ratio of surface area to volume, they could sorb a greater quantity of metal(loid)s than inorganic soil components (e.g., kaolinite, vermiculite, mica) either by an independent passive metabolism or by a metabolism-dependent active process [50]. Several authors have pointed out that bacterial biosorption/bioaccumulation mechanisms, together with other plant growth-promoting features, accounted for improved plant growth in metal(loid)s-contaminated soils [50, 140, 141].

Plant growth-promoting (PGP) bacteria are so named due to their potential to enhance plant growth. These PGP bacteria may develop symbiotic/mutualistic associations with plants and may be found as free living rhizospheric or endophytic bacteria. Genera including *Gluconacetobacterium*, *Flavobacterium*, *Beijerinckia*, *Klebsiella*, *Erwinia*, *Enterobacter*, *Burkholderia*, *Pseudomonas*, *Serratia*, and *Bacillus* are among the beneficial PGP bacteria able to control plant growth [41, 142, 143]. Some studies have found that plant growth-promoting rhizobacteria (PGPR) have the capacity to alleviate metal-induced phytotoxicity and enhance biomass production of plants when grown in metal(loid)s-contaminated soils. Although the role of PGPR on plant growth and metal(loid)s phytoremediation potential in polluted soils has been studied extensively, the dynamics of plant-endophytic bacteria and their potential role in phytoremediation are only beginning to be described in the literature in recent years [25, 52, 144–147].

The metal-resistant bacteria on roots and in the rhizosphere can benefit from root exudates, but some bacteria enter the plant as endophytes that do not cause harm and can establish a mutualistic association [148, 149]. However, endophytic populations, as rhizospheric populations, can be restricted by biotic and abiotic factors [149–151]. The endophytic bacteria may be more protected from the effects caused by biotic and abiotic stresses than rhizospheric bacteria [149]. In accordance with their life strategies, endophytic bacteria can be classified as “obligate” or “facultative.” Obligate endophytes are strictly dependent on the host plant for their growth and survival, and their transmission to other plants occurs vertically or via vectors. Facultative endophytes have a stage in their life cycle in which they exist outside host plants. Endophytic bacteria enter plant tissues usually through the root zone; however, aerial parts of plants, such as flowers, stems, and cotyledons, may also be used for this entry [152]. The bacteria penetrate the plant tissues via germinating radicles [153], secondary roots [154], stomates [155], or as a result of foliar damage [156]. Endophytes, once inside the plant, may either become placed near the

entrance area or spread throughout plant tissues. These microorganisms can be present within cells, in the intercellular spaces, or in the vascular system.

Although many reports attest the importance of PGPR in microbial-assisted phytoremediation, the bacterial endophytes offer several advantages over PGPR. For instance, colonization of the plant roots with PGPR is often problematic, because application of the inoculant should be at the exact time required for the plant. Moreover, being in natural biocenosis, they lose competitiveness over endemic bacteria [53]. The survival and colonization potential of PGPR depends also on various factors, such as intrinsic physiological characteristics of the organisms and abiotic and biotic soil factors [53]. The endophytic bacteria may derive significant competitive advantage over PGPR from their close contact with plants. In addition, reinoculation of endophytic bacteria does not affect the indigenous endophyte population in plants [53, 157]. Furthermore, localization within the plant may provide endophytic bacteria with the ability to recolonize the plant surface and tolerate some biotic and abiotic stress situations in the soil [53, 149].

Although this field of research is at an early stage, the available literature suggests that metal-resistant endophytic bacteria cannot only protect plants from metal(loid)s toxicity but also enhance the metal bioaccumulation in plant tissues with concurrent stimulation of plant growth. These beneficial effects exhibited by endophytic bacteria, together with the suggested interrelationship between microbial metal(loid)s tolerance and plant growth-promoting efficiency, indicate that inoculation with endophytic isolates might have significant potential to improve phytoremediation efficiency in polluted soils [53]. Endophytic bacteria reside in plant tissues beneath the epidermal cell layers, from where they can colonize the internal tissues and form a range of different lifestyles with their host including symbiotic, mutualistic, commensalistic, and trophobiotic [52, 158]. They are ubiquitous in a large diversity of plant species and can colonize a particular host with highest densities in root and less from stems to leaves [52, 159].

In general, most endophytes originate from the epiphytic bacterial communities in the rhizosphere or phyllosphere or other plant parts; however, some may be transmitted through the seed or damaged foliar tissues ([160, 161]). The long-term coevolution of plants and endophytic bacteria resulted in an intimate ecosystem, which helps plants to adapt/survive in both biotic and abiotic stress conditions (e.g., pathogen infection, drought, salinity, and contaminants) and enhance the ecological balance of the natural system [52, 162]. Although bacterial endophytes exist in plants variably and transiently [50, 163], they are often capable of triggering physiological changes that promote the growth and development of the plant [50, 164]. In general, the beneficial effects of endophytes are more extensive than those of many rhizobacteria [50].

PGP bacteria can also enhance plant development by acting as biofertilizers (increasing the availability of essential nutrients through, e.g., N_2 fixation and phosphate and iron solubilization), as organic contaminant biodegraders, as phytostimulants (producing plant growth regulators and hormones, such as indoleacetic acid—IAA; cytokinins; and other auxins), as stress controllers (by decreasing ethylene production through the synthesis of 1-aminocyclopropane-1-carboxylic acid

deaminase—ACCD), and as plant defense inducers against phytopathogens (by producing siderophores, antibiotics, or fungicidal compounds) [25, 148, 165–170].

Moreover, a number of other beneficial effects on plant growth have been attributed to endophytes which include osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals, and change on nitrogen accumulation and metabolism [148]. In recent years, phytoremediation assisted by bacterial endophytes has been highly recommended for cleaning up of metal(loid) s-polluted soils since 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 plants [50, 52].

Endophytic bacteria improve plant growth in metal-polluted soils in two different ways: (1) directly by producing plant growth beneficial substances including solubilization and bioconversion of mineral nutrients (phosphorus, nitrogen, and potassium) and production of phytohormones, siderophores, and specific enzymes and (2) indirectly by controlling plant pathogens or by inducing a systemic resistance of plants against pathogens. Besides, they also change metal bioaccumulation ability in plants by excreting metal immobilizing extracellular polymeric substances (EPS), as well as metal mobilizing organic acids and biosurfactants [52]. Further, the extracellular polymeric substances secreted by endophytic bacteria, consisting mainly of polysaccharides, proteins, nucleic acids, and lipids, also play a significant role in metal complexation, thereby reducing their bioaccessibility and bioavailability [43, 52].

Joshi and Juwarkar [171] assessed the ability of *Azotobacter* spp. to produce EPS able to form complexes with Cd and Cr decreasing metal uptake by *Triticum aestivum*. Further, the biosurfactants produced by endophytic bacteria seemed to increase the bioavailability of poorly soluble metals and to improve phytoremediation rates [52, 160]. Biosurfactants are low-molecular-weight amphiphilic molecules consisting of a hydrophilic and a hydrophobic moiety, comprising a wide range of chemical structures, such as mycolic acid, glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids, etc. [52, 172]. These molecules are able to decrease the surface tension, critical micelle concentration, and interfacial tension, thus affecting the distribution of the metals among the phases (Ma 2016). Endophytic bacteria produce the biosurfactants and release to the host as root exudates.

The secreted biosurfactants initially interact with complex insoluble metals on the interface of rhizosphere soil particles and then desorb metals from soil matrix, leading to the change of metal mobility and bioavailability in the soil solution [52, 53]. The interactions between endophytic bacteria, metals, and biosurfactants can be explained from a functional perspective, considering that the key role attributed to biosurfactants is their involvement in facilitating metal uptake [52, 160]. With regard to indirect effects on plant growth promotion, several plant growth-promoting endophytic (PGPE) bacteria are known to diminish the stress effects in plants by suppressing phytopathogen damage [52] either via biological control of pathogens or induced systemic resistance (ISR) of plants against pathogens.

The endophytic bacteria as natural biocontrol agents may have numerous competitive advantages over plant growth-promoting rhizobacteria [52, 53]. Some

endophytic bacteria can produce substances that may effectively limit phytopathogens, such as antibiotics, siderophores, a variety of hydrolytic enzymes (such as chitinases, proteases, and glucanases), and antimicrobial volatile organic compounds [52, 173]. In many biocontrol systems, one or more antibiotics have been shown to play a role in disease suppression. For instance, the endophytic bacterial strains *Bacillus megaterium* BP 17 and *Curtobacterium luteum* TC 10 effectively suppressed the burrowing nematode (*Radopholus similis* Thorne) by the synthesis of antibiotics [52, 174]. Recently, Bacon et al. [175] also reported that a patented strain of *Bacillus mojavensis* produced the biosurfactant C-15 surfactin, which was able to control the maize mycotoxic fungus *Fusarium verticillioides*. Additionally, endophytic bacteria can also be effective as competitors of pathogens for colonization niches and bioavailable nutrients, indirectly promoting plant productivity ([176]; Ma 2016).

Induced systemic resistance (ISR) refers to the state of systemically enhanced resistance to a broad spectrum of pathogens [52]. The ISR to various diseases caused by chemicals and plant growth-promoting bacteria is highly beneficial in agroecosystem protection. ISR induced by endophytic bacteria has been demonstrated against various fungal, bacterial, and viral pathogens in many plant taxa ([176]; Ma 2016). Priming plants with bacterial endophytes induced a plant defense system, which pathogens must overcome to colonize the host [52]. Once the defense genes are expressed, ISR activates multiple potential defense mechanisms that include the increased activity of chitinases, β -1,3-glucanases, superoxide dismutase, guaiacol, catalase (CAT), and peroxidases (POS) [52, 177]. The activity of these enzymes is responsible for reactive oxygen species production, and protection of cell organelles against oxidative stress may change as a result of the activity of endophytic bacteria ([178]; Ma 2016).

In addition, Wan et al. [178] and Zhang et al. [179] found that endophytic bacteria could modulate the activity of plant antioxidant enzymes (such as POS, CAT, SOD, glutathione peroxidase, ascorbate peroxidase) and lipid peroxidation (malondialdehyde formation).

Recent experiments with hyperaccumulator plants revealed that the inoculation of soils/seeds/seedlings with metal-resistant endophytic bacteria improved plant growth and accelerated the phytoremediation process in naturally and/or artificially metal(loid)s-contaminated soils by enhancing nutrient acquisition, cell elongation, metal bioaccumulation or stabilization, and alleviation of metal stress in plants [52, 104, 105, 108, 145, 147, 180–183]. Similarly, the colonization and propagation of plant growth-promoting endophytic bacteria are also well known for their role in the enhancement of soil fertility and stimulation of host plant development by providing a plethora of growth regulators [52, 184] and essential nutrients [52] or by synthesizing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase [52, 185], as well as by reducing disease severity by suppressing pathogens [52, 174]. Further, bioaugmentation with such endophytic bacteria possessing multiple plant growth-promoting traits, including metal resistance/detoxification/accumulation/biotransformation/sequestration, can reduce phytotoxicity and change the bioavailability of

metal(loid)s in contaminated soils, making them a perfect choice for microbial-assisted phytoremediation studies [50, 52, 53, 168, 169].

Although metal(loid)s negatively influence endophytic bacterial diversity in plants [52, 181], numerous studies have demonstrated that endophytic bacteria isolated from hyperaccumulator plants exhibit greater metal tolerance than those from non-hyperaccumulator plants [186]. This may be due to the adaptation strategy of endophytic bacteria to metal(loid)-containing environments [52, 187]. Numerous studies have demonstrated the effect of different metal(loid)s on endophyte diversity, biomass, and activity. However, it is well known that the bacteria isolated from polluted environments are tolerant to higher concentrations of metals than those isolated from unpolluted areas. Further, after the addition of metals, metal tolerance is increased in bacterial communities by the death of sensitive species and subsequent competition and adaptation of surviving bacteria [188]. Experimental results suggest that bacterial metal(loid) resistance can be a result of horizontal gene transfer (HGT) mediated by plasmids encoding metal resistance genes [52, 189]. For instance, some mercury-resistant endophytic bacteria express the *MerB* gene encoding organomercurial lyase that cleaves organomercurials into mercuric ion (Hg^{2+}) [52, 190] and *MerA* gene encoding mercuric reductase that converts the highly toxic ionic Hg^{2+} into the less toxic and volatile Hg^0 [52, 191], thus alleviating metal toxicity and improving the efficiency of phytovolatilization. The genes specifying the various functions needed for biodegradation of organomercurials and reduction of Hg^{2+} are organized in the mercury resistance (*Me*) operon, which is mostly found in gram-negative bacteria [52, 190].

Idris et al. [186] investigated the endophytic bacteria and rhizobacteria associated with the Ni hyperaccumulator plant *Thlaspi goesingense* using both cultivation-dependent and cultivation-independent techniques. The results showed that most of the endophytes were cultivation-independent and tolerated higher Ni concentrations than rhizobacteria. Furthermore, endophytic bacteria are thought to exhibit different multiple metal resistance through similar mechanisms described for rhizobacteria [50, 144]. Therefore, the beneficial endophytes have been proposed as potential natural resources to enhance phytoremediation of metal(loid)-contaminated soils, due to their biotechnological applications in metal bioremediation. The study of the diversity and structure of bacterial communities living in niches under metal stress is of paramount importance.

Like rhizobacteria, there are several mechanisms by which PGPE directly facilitate growth of their host plants. The mechanisms include nitrogen fixation, solubilization of minerals, and production of phytohormones, specific enzymes, and siderophores [52, 53, 192]. Bacteria that can fix nitrogen, i.e., convert stable atmospheric nitrogen gas into a biologically useful form, are known as diazotrophs. These organisms reduce dinitrogen to ammonia with the help of the enzyme nitrogenase [193]. Endophytic bacteria with strong associative nitrogen-fixing ability allow plants to survive in nitrogen-poor soil environments and play a major role in promoting plant health and growth, compared to other rhizospheric microbes [52, 194, 195]. Phosphorus (P) is one of the major essential plant nutrients as it plays a crucial role in the overall growth of plants by influencing various key metabolic

processes such as cell division and development, energy transport, signal transduction, macromolecular biosynthesis, photosynthesis, root development, and respiration of plants [52, 196, 197]. Phosphorus in soil is immobilized or becomes less soluble by absorption and chemical precipitation, so that the amount of readily bioavailable phosphorus is very low, compared with the total amount of phosphorus present. Therefore, P is often regarded as a limiting nutrient in agricultural soils [197, 198]. Under metal(loid)s stress conditions, some metal-resistant endophytic bacteria were found to solubilize precipitated phosphates in soil by acidification, ion exchange, and release of chelating agents [52, 199] or to mineralize organic phosphorus in soil by exuding extracellular acid phosphatase [52], thereby enhancing P availability to plants. It is well known that endophytic bacteria assimilate soluble P and prevent its subsequent sorption [52, 200]. Hence, these endophytic bacteria may serve as a sink for phosphorus by rapid microbial phosphorus mobilization even under phosphate-limiting conditions. Subsequently, these endophytes become a source of phosphorus to the plant hosts upon its release from bacterial cells.

Phosphate solubilizing bacteria are common in the rhizosphere, and secretion of organic acids and phosphatases facilitates the conversion of insoluble forms of P to bioavailable forms. Endophytes such as *Pseudomonas citronellolis*, *Pseudomonas oryzaehabitans*, *Enterobacter agglomerans*, *Pantoea* sp., *Enterobacteriaceae*, *Burkholderia*, *Ralstonia pickettii*, *Erwinia* sp., *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Acinetobacter* sp., *Acinetobacter calcoaceticus*, *Enterobacter sakazakii*, *Agrobacterium* sp., and *Caulobacter/Asticcacaulis* have been defined as efficient P solubilizers [53]. Iron (Fe) is one of the important elements for life, and almost all organisms, with the exception of certain lactobacilli, need iron-containing proteins involved in physiological activities and a series of enzymatic reactions [52]. However, in the aerobic environment of soils, iron exists mainly as Fe(III) and tends to form insoluble hydroxides and oxyhydroxides, making it largely unavailable to microorganisms. To acquire sufficient iron, bacteria had to develop strategies to solubilize this metal before uptake. In addition to rhizosphere bacteria, metal-resistant endophytic bacteria that also produce siderophores have been isolated from many different plant species.

Siderophores are produced by a diverse group of microbes ranging from animal and plant pathogens to free-living and symbiotic nitrogen-fixing microorganisms. However, siderophore production is most common among plant growth-promoting rhizosphere bacteria, which exhibit their optimum growth and siderophore production activity at extreme environmental conditions, including scarcity of nutrients or the presence of elevated concentrations of metal(loid)s and thus may be particularly useful for phytoremediation purposes. Iron deficiencies induced by excess of metal(loid)s have been reported in various plant species as interveinal chlorosis in younger leaves [54]. Their low iron content generally inhibits both chloroplast development and chlorophyll biosynthesis, thus affecting plant growth [54]. Under such conditions, siderophore-producing bacteria (e.g., rhizospheric, endophytic) might offer a biological rescue system that is capable of scavenging Fe³⁺ and making it available to iron-deficient plants.

In short, iron bioavailability to plant roots may be modified by the microbial production of chelating agents (e.g., siderophores), which can solubilize Fe under iron deficiency conditions. Siderophores are low-molecular-weight organic compounds (500–1500 Da) with an affinity for Fe³⁺ ions, which can also bind other metal ions that can be uptaken by plants [52, 53]. Iron acquisition takes place by two basic strategies. The first is by the uptake of Fe in Fe-siderophore complexes after root-mediated biodegradation of the chelate [52, 53]. The second strategy involves the solubilization of unavailable forms of iron by the release of phyto siderophores. Since microbial siderophores typically have higher affinity for iron than phyto siderophores, plants growing in metal-contaminated soils are able to accumulate high amounts of iron with the help of siderophore-producing bacteria. Hence, bacterial siderophores are assumed to serve as major sources of phytoavailable Fe for plants under metal stress conditions [50, 52].

Mechanisms of iron acquisition in higher plants can be grouped into strategy I and strategy II [54]. Although strategy II plants (*Poaceae*), similar to microorganisms, release (phyto)siderophores (e.g., mugineic acid in barley and avenic acid in oat) to enhance their Fe uptake, in metal-contaminated soils, these plants are unable to accumulate sufficient amounts of iron, unless bacterial siderophores are also present due to their higher affinity for iron [54]. Unlike strategy II plants, strategy I plants (dicots and monocots, except *Poaceae*) do not produce phyto siderophores themselves. Rather, their iron acquisition is achieved by other means, such as an enhanced Fe(III)-reductase activity, the release of reducing agents such as phenolics, and acidification of the rhizosphere [54]. However, this strategy is considered to be less efficient than that of strategy II plants [54]. Therefore, inoculation of plants with bacteria that are able to produce siderophores could help to prevent them from becoming chlorotic when they are grown in metal-polluted soils.

Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. In addition to iron, siderophores can also form stable complexes with other metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb, and Zn, as well as with radionuclides including U and Np [54, 201]. Currently, almost 500 different siderophores have been identified [54, 202]. Although they differ widely in their overall structure, the functional groups that coordinate the iron atom are not as diverse. In their metal-binding sites, siderophores have either α -hydroxycarboxylic acid, catechol, or hydroxamic acid moieties and thus can be classified as hydroxycarboxylate-, catecholate-, or hydroxamate-type siderophores [54]. The biosynthetic pathways of siderophores are tightly connected to aerobic metabolism involving molecular oxygen activated by mono-, di-, and N-oxygenases and acids originating from the final oxidation of the citric acid cycle, such as citrate, succinate, and acetate. Moreover, many siderophores are polypeptides that are synthesized by members of the non-ribosomal peptide synthetase multienzyme family, which is also responsible for the synthesis of the majority of microbial peptide antibiotics [54]. However, many of the hydroxamate- and α -hydroxy acid-containing siderophores are not polypeptides but are assembled instead from alternating dicarboxylic acid and either diamine or amino alcohol building blocks (which are nevertheless derived from amino acids) that are

linked by amide or ester bonds. Such siderophores are assembled by the non-ribosomal peptide synthetase-independent siderophore pathway, which is widely utilized in bacteria. Several recent reviews describe siderophore structure and biosynthesis mechanisms in more detail [54, 203].

Siderophores generally form 1:1 complexes with Fe^{3+} , which are then taken up by the cell membrane of bacteria, where the Fe^{3+} is reduced to Fe^{2+} and released from the siderophore into the cell. In some cases, the siderophore is destroyed during this reduction, but in other cases, it is recycled [54]. This mechanism of iron uptake from siderophores has already been identified in both gram-negative and gram-positive bacteria [54, 204]. Although siderophores are elicited primarily in response to iron deficiency, external pH is also important for the biosynthesis of microbial siderophores, as well as in their chemical stability in the environment [54]. Production of siderophores by different microorganisms appears to contradict the commonly acknowledged pH-dependence of Fe deficiency. Hydroxamate siderophores in general are prevalent in acidic soils and reflect the preference of low pH values for the synthesis of hydroxamate siderophores by microorganisms. In contrast, neutral to alkaline soils support the production of catecholate siderophores. In addition to pH, heavy metal concentrations in the surrounding environment could also influence microbial growth and siderophore biosynthesis.

Bacterial strains isolated from polluted environments were shown to be tolerant to higher concentrations of metals than those isolated from unpolluted areas [53, 54]. These metal-tolerant bacteria have evolved several mechanisms for survival under metal stress. Interestingly, several studies have found a stimulating effect of heavy metals on siderophore biosynthesis in various bacteria. Two different possible explanations have been suggested for the stimulating effect of heavy metals on siderophore production. Firstly, the heavy metal might be directly involved in the siderophore biosynthesis pathways or their regulation [54, 205]. Alternatively, the free siderophore concentration in the medium might be reduced by complex formation with metal ions. This process interferes with the complexation of siderophores with iron and thus decreases the soluble iron concentration. As iron deficiency stimulates siderophore production, more siderophores would then be produced [54, 206, 207].

Plants inoculated with siderophore-producing bacteria could then take up iron from siderophores via various mechanisms, such as chelate degradation and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction [54, 208, 209]. A variety of plant species were shown to acquire iron from Fe-siderophore complexes, including *Cucurbita pepo*, *B. juncea*, *Helianthus annuus*, *Medicago sativa*, and *Vigna unguiculata*, and, moreover, it was shown that this acquisition was the reason for their stimulated growth in metal-contaminated soils. Experiments with *Phaseolus vulgaris* revealed that the inoculation with the Pb- and Cd-resistant siderophore-producing bacteria strain *Pseudomonas putida* KNP9 significantly increased plant growth without showing any symptoms of lead and cadmium toxicity compared with the controls [54, 210]. This effect was attributed to the increased level of siderophores produced by the KNP9 strain that were able to provide iron to the plant in the presence of high levels of Pb and Cd.

Inoculation with other rhizobacteria, such as *Pseudomonas* sp. Ps29C and *Bacillus megaterium* Bm4C, which had been isolated from Ni-rich serpentine soils, has also been studied in detail [205, 211, 212]. These bacteria significantly reduced the toxicity of Ni in *B. juncea* and promoted plant growth. Although bacterial siderophores have the potential to sparingly mobilize soluble metals, the efficiency of siderophore-producing bacteria (SPB) to either mobilize or immobilize heavy metals from soils is dependent on several factors, such as the form of the heavy metals present, the charge of the siderophores, as well as the pH of the soil and its mineral composition and organic content [54, 205, 211, 212]. Endophytic bacteria are also able to supply essential vitamins and growth regulators (phytohormones) to plants, which can increase plant nutrient uptake through their effects on root growth dynamics [52, 213].

The phytohormone production by endophytes is believed to play an important role in plant-bacterial interactions and plant growth in metal-contaminated soils [214]. Recent studies investigating the role of phytohormones in protecting plants against metal(loid)s have demonstrated that the endophytic colonization often causes increases in nutrient uptake and plant biomass [52, 184, 213, 215]. These may suggest that metal(loid)s stress alleviation by endophytic bacteria results from a combination of nutritional and biochemical benefits. In general, the phytohormones comprise indole-3-acetic acid (IAA), cytokinins, gibberellins, abscisic acid, and ethylene, which may be either growth inhibitors or promoters depending upon the substance concentration [52, 213]. As a major auxin, IAA is involved in different physiological processes in plants, such as adjustment of plant development [52, 215], induction of plant defense systems [52], and as a cell-cell signaling molecule [52, 216]. IAA is transported downward causing a concentration gradient in different plant parts. Depending on its concentration, inhibition, or stimulation of growth, tissue differentiation may result [52, 217]. More recently, the amount of IAA released by endophytes is thought to play a vital role in modulating the plant-endophyte association and plant development in metal(loid)-contaminated soils [183].

Auxins and cytokinins were found to be produced by strains of *Pseudomonas*, *Enterobacter*, *Staphylococcus*, *Azotobacter*, and *Azospirillum*. These substances, together with gibberellins, may alter plant growth and development [53, 218]. For instance, *Azospirillum*-inoculated roots showed a stimulation of root cell membrane activity, as well as an increase in the levels of free IAA, indole-3-butyric acid, and the specific activities of both the tricarboxylic cycle and the glycolysis pathway [53, 219]. According to the IAA level, root elongation may change qualitatively. A low level of the phytohormone produced by bacteria promotes primary root elongation, whereas a high level of IAA stimulates lateral and adventitious root formation but inhibits primary root growth [220]. Thus, endophytes can facilitate plant growth by altering the plant hormonal balance. *P. fluorescens*, for instance, is generally regarded as being a rhizosphere bacterium that colonizes mainly the elongation and root hair zones of roots. Some *P. fluorescens* can also be endophytic, being found within the roots and stems of some hyperaccumulators [53, 221, 222]. The observed plant growth promotion, under Pb stress after inoculation of plant with *P. fluores-*

cens, is thought to be the consequence of bacterial IAA production and excretion [53, 222]. Therefore, any direct influence on phytohormone production by bacteria may in turn affect their phytostimulating efficiency.

Another way in which the endophytic bacteria might influence the host plant growth is the utilization of ACC as a sole N source. Endophytic bacteria that are generally beneficial to plants in situ, such as *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Enterobacter*, *Staphylococcus*, etc., are known to be involved with production of phytohormones [52, 160]. Luo et al. [20] reported that the enhanced growth of the Cd hyperaccumulator *S. nigrum* L. induced by bacterial endophytes under cadmium stress might be the consequence of both bacterial IAA and ACC deaminase. Ethylene, as a ubiquitous plant hormone, plays a vital role in plant response (growth and survival) to abiotic and biotic stresses including root initiation and nodulation, cell elongation, leaf senescence, abscission and fruit ripening, as well as auxin transport [52, 223]. Ethylene is synthesized in higher plants via the following pathway [49, 52]: (1) the enzyme *S*-adenosyl-L-methionine (SAM) synthetase catalyzes the conversion of methionine and adenosine triphosphate (ATP) to SAM; (2) ACC synthase mediates the hydrolysis of SAM to ACC and 5'-methylthioadenosine (MTA); and (3) ACC is finally oxidized by ACC oxidase to form ethylene, carbon dioxide, and hydrogen cyanide. It has been reported that metal(loid) stress in plants induces ethylene production, which causes the inhibition of root elongation, lateral root growth, and root hair formation. Under such conditions, certain endophytic bacteria might alleviate the stress-mediated impact in plants by enzymatic hydrolysis of ACC.

Ethylene is implicated in virtually all aspects of plant growth and development, ranging from seed germination to shoot growth and leaf abscission [53, 224]. Therefore, production of ACC deaminase is likely an important and efficient way for endophytes to manipulate their plant hosts. The enzyme ACC deaminase may hydrolyze ACC into α -ketobutyrate and ammonia, which can readily be metabolized by the bacteria as a source of nitrogen. Endophytic bacteria containing ACC deaminase are usually located inside plant roots in the apoplast. In this way, these bacteria act as a sink for ACC. By lowering ethylene levels, the bacteria increase the growth of plant roots and shoots and reduce the inhibitory effects of ethylene synthesis ([53, 225]; Ma 2016). A higher percentage of endophytes than of rhizosphere bacteria were able to utilize ACC as the sole N source. Experiments with *Methylobacterium oryzae* and *Burkholderia* sp. (isolated from rice tissue) showed the ability of these bacteria to reduce the level of ethylene, protecting tomato (*Lycopersicon esculentum*) from the toxicity of high concentrations of Ni and Cd [53].

The success of phytoremediation is widely dependent on the ability to overcome metal phytotoxicity. Therefore, different bacterial-mediated mechanisms are implicated on the endophyte-host coevolution process either by relieving metal toxicity or by enhancing plant metal tolerance [52, 53, 147]. Shin et al. [183] found that the endophytic bacterial strain *Bacillus* sp. MN3-4 evolved a better defined metal-resistant mechanism, e.g., active export via a P-type ATPase efflux pump, which can transport metal ions across biological membranes against the concentration gradient using energy released by ATP hydrolysis. Endophytic bacteria possessing specific

and remarkable metal bioaccumulation abilities can be used in plant-endophyte mutualistic systems to facilitate detoxification of metal(loid)s and improve the efficiency of phytoremediation [52, 183, 226]. It is well known that the transfer of metal(loid)s from soils to plants depends primarily on metal bioavailability which can be affected by several factors, such as soil particle size, nutrients, soil pH, redox potential, organic matter content, and the presence of other ions [48, 52, 55]. Numerous studies have suggested that endophytic bacteria possessing a metal resistance/sequestration pathway (e.g., *ncc-nre*) can alleviate the phytotoxicity and enhance the bioavailability of metal(loid)s through the release of metal chelating agents (e.g., siderophores, biosurfactants, and organic acids), acidification of soils, redox activity, and phosphate solubilization [50, 52].

Currently, the interactions between endophytes and hyperaccumulator plants have attracted the attention of several investigators due to the biotechnological applications for bioremediation and to study the composition of bacterial communities living on naturally contaminated environments. In general, hyperaccumulating plants accumulate huge amounts of metal(loid)s and can therefore provide a specific environment for bacterial endophytes adapted to survive in high metal concentrations. For instance, metal-resistant endophytic bacteria have been isolated from various hyperaccumulating plants such as *Alyssum bertolonii*, *Thlaspi caerulescens*, *Thlaspi goesingense*, and *Nicotiana tabacum* [186, 227–230]. Attempts have been made to characterize the endophytic bacterial communities in plant tissues, but comparisons between studies are difficult to make. An interesting finding was that isolates from shoot and root displayed different tolerances, suggesting that different microbial communities exist in different compartments of the plant [53].

The beneficial effects of endophytes on their hyperaccumulators appear to occur through similar mechanisms described for PGPR. This makes sense because most of the bacterial endophytes isolated from various plants can be considered to be facultatively endophytic and are capable of living outside plant tissues as rhizospheric bacteria. Additionally, many endophytic bacterial taxa from hyperaccumulators were reported to be common soil bacteria [186, 222, 231, 232]. Metal biosorption by bacteria comprises two steps:

1. Passive biosorption of metals by living and dead/inactive cells that essentially take place in the cell wall due to a number of metabolism-independent processes [52, 233]. In this process, metal ions are sorbed rapidly to the cell surface by reactions between metals and functional groups on the cell surface, such as hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, amide, and phosphonate [50, 52]. Various metal-binding mechanisms such as ion exchange, complexation, coordination, sorption, chelation, electrostatic interaction, or microprecipitation may be synergistically or independently involved.
2. Active biosorption (bioaccumulation) referring to the uptake of metals (transport into cells). This only occurs in living cells through a slower active metabolism-dependent transport of metals into bacterial cells [52, 234]. Once the metals are inside living cells, they may be bound, precipitated, accumulated, sequestered

within specific intracellular organelles, or translocated to specific structures, depending upon the organism and element concerned [50, 52].

Particularly, in order to circumvent metal stress, endophytic bacteria have evolved several types of mechanisms, through which they alleviate the toxicity of metal ions, including the efflux of metal ions from cells and subsequent extracellular precipitation [52, 144], intracellular accumulation [52, 183], biotransformation of toxic metal ions into less or nontoxic forms [52, 108], sequestration of metals on the cell surface or in intracellular polymers, and precipitation, adsorption/desorption, or biomethylation [43, 52]. Binding of metals to extracellular material can immobilize the metal and prevent its entry into the cell. For example, different metal(loid)s can bind to anionic functional groups (e.g., sulfhydryl, carboxyl, hydroxyl, sulfonate, amine, and amide groups) present on cell surfaces. Similarly, microbial extracellular polymers, such as polysaccharides, proteins, and humic compounds, can effectively bind heavy metals. These substances thus detoxify metals simply by complex formation or by forming an effective barrier surrounding the cell [54, 235].

In addition, many bacteria mediate reactions or produce metabolites that result in crystallization and precipitation of metals [54, 206, 207, 236]. Furthermore, a great number of bacteria are known to possess transporters to promote metal(loid)s efflux from cells, in case of toxicity or excessive concentration, via ATPase pumps or chemiosmotic ion/proton pumps [54, 237]. These types of transporters are characterized by a high substrate affinity, and they are therefore able to reduce the metal load in the cytosol. Several bacteria have developed a cytosolic sequestration mechanism for protection from metal(loid)s. Once inside the cell, metal ions might also become compartmentalized or being converted into more innocuous forms. This process can constitute an effective detoxification mechanism, and the respective microbes might be able to accumulate higher intracellular concentrations [54, 237].

Examples include the synthesis of metal-binding proteins such as metallothioneins. These are low-molecular mass cysteine-rich proteins with high affinities for cadmium, copper, silver, lead, mercury, etc. Their production is induced by presence of metals, and their primary function is metal detoxification. In addition, certain bacteria use methylation as a metal resistance or detoxification mechanism. However, this process is considered to be metal-dependent as only some metals are methylated. It involves the transfer of methyl groups to metals and metalloids. Selenium is an example of metalloids that has reduced toxicity when methylated [54, 238]. In the last 15 years, several studies focused on the use of actinobacteria for cleaning up the environment [239]. Strategies such as bioaugmentation, biostimulation, cell immobilization, production of biosurfactants, design of defined mixed cultures, and the use of plant-microbe systems were developed to enhance the capabilities of actinobacteria in bioremediation.

Actinobacteria exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes and the formation of a wide variety of secondary metabolites [239, 240]. This versatility in secondary metabolite production

makes them important tools for pharmaceutical, medical, and biotechnological applications such as bioremediation. The quantitative analysis of soil microbial populations through total culturable numbers showed a marked decrease of the different microbial groups for contaminated soil samples, in comparison with uncontaminated samples. However, actinobacteria showed less sensitivity than other culturable heterotrophic bacteria and asymbiotic nitrogen fixers. Culture-dependent methods have allowed the isolation and characterization of over 35 genera of actinobacteria tolerant to heavy metals [239].

Finally, actinobacteria have demonstrated their potential as tools for bioremediation of several contaminants including oil, rubber, plastics, pesticides, and heavy metals, among others, based on their physiological and metabolic versatility. The real worldwide problem is co-contamination. Environments contaminated with inorganic and organic compounds are considered difficult to bioremediate since metal(loid)s would inhibit biodegradation. Nevertheless, recent works highlighted that actinobacteria strains are able to remove HMs and pesticides simultaneously [239]. *Streptomyces*, *Rhodococcus*, and *Amycolatopsis* are among the most studied genera, although their bioremediation skills were never supported by integrated omic approaches. The relevance of the use of omic tools relies on the fact that this information may be used to enhance bioremediation processes of actinobacteria through pathway engineering techniques. On the basis of the existence of co-contaminated environments, engineering bio-tools resistant to organic and inorganic toxic compounds could be necessary.

Plant-bacteria associations in several metal(loid)s phytoremediation studies are presented on Table 2.3.

2.4 Phytoextraction

Phytoextraction, also known as phytoaccumulation, phytoabsorption, or phytosequestration, is the uptake of contaminants from soil, water, wastewater, or sediments by plant roots and their translocation and accumulation in aboveground biomass, i.e., shoots or any other harvestable plant parts [1, 5]. Plants able to accumulate metal(loid)s are grown on contaminated sites, and the metal-rich aboveground biomass is harvested, resulting in the removal of a fraction of the contaminant. Phytoextraction is the main and most useful phytoremediation technique for removal of heavy metals and metalloids from polluted soils [1, 24]. It is also the most widespread and promising alternative of soil reclamation for commercial applications. Metal translocation to shoots is a crucial biochemical process desirable for an effective phytoextraction because the harvest of root biomass is generally not feasible.

Phytoextraction has important advantages:

- It does not damage/change the landscape.

Table 2.3 Recent examples of bacterial-assisted phytoremediation studies

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Rhizobium</i> sp. strains E20-8 and NII-1	Cd	<i>Pisum sativum</i> L.	Overcame Cd toxicity by extracellular immobilization, periplasmic allocation, cytoplasmic sequestration, and biotransformation of toxic products Higher glutathione concentrations allowing cytosolic Cd complexation, reducing Cd deleterious effects, and rendering this strain more tolerant to Cd than the sensitive one Promoted plant growth, thus rendering phytoremediation a more efficient technology	Cardoso et al. [241]
<i>F. tricinatum</i> RSF-4L, <i>A. alternata</i> RSF-6L	Cd	<i>Solanum nigrum</i>	Increased plant growth and chlorophyll content in inoculated RSF-6L plants in comparison to non-inoculated plants. RSF-6L inoculation decreased uptake of Cd in roots and aboveground parts. Inoculation protected the host as shown by low peroxidase and polyphenol peroxidase activities and high catalase activity	Khan et al. [56]
<i>Microbacterium</i> sp. NE1R5, <i>Curtobacterium</i> sp. NM1R1, and <i>Microbacterium</i> sp. NM3E9	As, Cu, Pb, Zn	<i>Brassica nigra</i>	Facilitated root development and seed germination	Román-Ponce et al. [242]

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Micrococcus</i> sp., <i>Pseudomonas</i> sp., <i>Arthrobacter</i> sp.	Cd	<i>Glycine max</i> L.	Cadmium accumulation in roots, stems, and leaves of <i>G. max</i> L. was significantly enhanced by <i>Arthrobacter</i> sp. with nutrient biostimulation. A combined use of <i>G. max</i> L. and <i>Arthrobacter</i> sp. with nutrient biostimulation accelerated cadmium phytoremediation. In addition, cadmium was retained in roots more than in stems and leaves, and <i>G. max</i> had the lowest translocation factor at all growth stages, suggesting that <i>G. max</i> can be a phytostabilizing plant	Rojjamateeranj et al. [243]
<i>Thiobacillus thiooxidans</i>	Cd, Pb	<i>Gladiolus grandiflorus</i> L.	Promoted root length, plant height, dry biomass, and enhanced accumulation of Cd and Pb	Mani et al. [130]
<i>Bradyrhizobium japonicum</i>	Ni, Cu, Pb	<i>Lettuce</i>	Increased the shoot and root lengths	Seneviratne et al. [244]
<i>Pseudomonas putida</i>	Cd, Zn	<i>Helianthus annuus</i> L.	Promoted biomass of the plant, accumulation of Zn and Cd in roots and shoots	Mani et al. [130]
<i>Pseudomonas brassicacearum</i> , <i>Rhizobium leguminosarum</i>	Zn	<i>Brassica juncea</i>	Induced metal chelation and toxicity attenuation	Adediran et al. [245]
<i>Pseudomonas</i> spp. Lk9	Cd, Zn, Cu	<i>Solanum nigrum</i> L.	Improved soil Fe, P, and metal availability, shoot biomass, and uptake of Cd, Zn, and Cu. Production of siderophores and organic acids that induced growth and metal uptake	Chen et al. [145]

(continued)

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Enterobacter</i> sp. JYX7, <i>Klebsiella</i> sp. JYX10	Cd, Pb, and Zn	<i>Polygonum pubescens</i>	Production of IAA, siderophores, ACC deaminase, and solubilized inorganic phosphate improved phytoremediation efficiency	Jing et al. [246]
<i>Bacillus thuringiensis</i> GDB-1	Cd, Ni, As, Cu, Pb, Zn	<i>Alnus firma</i>	Production of phytohormones, siderophore, ACC deaminase, and solubilization of phosphorus. Increased biomass, chlorophyll content, nodule number, and accumulation of metal(loid)s (As, Cu, Pb, Ni, and Zn)	Babu et al. [180]
<i>Rhodococcus</i> sp. TS1, <i>Delftia</i> sp. TS33, <i>Comamonas</i> sp. TS37, <i>Delftia</i> sp. TS41, <i>Streptomyces lividans</i> sp. PSQ22	As	<i>Pteris vittata</i>	Reduced As(VI) to As(III) and enhanced As uptake by <i>Pteris vittata</i>	Yang et al. [247]
<i>Serratia</i> sp. MSMC541	As, Cd, Cu	<i>Lupinus luteus</i>	Reduced translocation of As, Cd, and Cu from roots to shoots	Aafi et al. [248]
<i>Consortium of sulfur-oxidizing bacteria</i>	Cu	<i>Oryza sativa</i>	Increased bioavailability of Cu	Shi et al. [249]
<i>Pseudomonas</i> sp., <i>Sanguibacter</i> sp.	Cd, Zn	<i>Nicotiana tabacum</i>	Improved biomass production, as well as total plant Cd	Mastretta et al. [229]
<i>Arthrobacter</i> sp. MT16, <i>Microbacterium</i> sp. JYC17, <i>Pseudomonas chlororaphis</i> SZY6, <i>Azotobacter vinelandii</i> GZC24, <i>Microbacterium lactium</i> YJ7 (EN)	Cu	<i>Brassica napus</i>	Root length promotion, ACCD, siderophore, IAA, P solubilization	He et al. [250]

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Sanguibacter</i> sp., <i>Enterobacter</i> sp., <i>Pseudomonas</i> sp.	M Cd, Fe	<i>Nicotiana tabacum</i>	In several cases, inoculation with endophytes resulted in improved biomass production under conditions of Cd stress, as well as in higher plant Cd concentration and total plant Cd content compared to non-inoculated plants	Mastretta et al. [229]
<i>Cellulosimicrobium cellulans</i> KUCr3	Cr	Chilli plants	Reduced the mobile and toxic Cr(VI) to nontoxic and immobile Cr(III) and decreased Cr uptake by chilli plants	Chatterjee et al. [251]
<i>Streptomyces tendae</i> F4	Cd	<i>Helianthus annuus</i>	Promoted plant growth, facilitated soil metal solubilization, enhanced Cd, and Fe uptake	Dimkpa et al. [209]
<i>Achromobacter xylosoxidans</i> Ax10	Cu	<i>Brassica juncea</i>	Increased root and shoot length and biomass; ACC deaminase, IAA, and phosphate solubilization	Ma et al. [252]
<i>Enterobacter aerogenes</i> , <i>Rahnella aquatilis</i>	Ni, Cr	<i>Brassica juncea</i>	Stimulated plant biomass and enhanced phytoextraction of metals (Ni and Cr). Concurrent production of siderophores, ACC deaminase, IAA, and phosphate solubilization	Kumar et al. [140]
<i>Acidithiobacillus thiooxidans</i> , <i>A. ferrooxidans</i> , <i>Leptospirillum ferrooxidans</i>	Cu, Cd, Hg, Zn	NA	Increased the mobility of Cu, Cd, Hg, and Zn	Beolchini et al. [253]
<i>Streptomyces acidiscabies</i> E13	Al, Cu, Fe, Mn, Ni, U	<i>Vigna unguiculata</i>	Protected plants from metal toxicity; enhanced uptake of Al, Cu, Fe, Mn, Ni, and U	Dimkpa et al. [208]

(continued)

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Azotobacter</i> spp.	Cd, Cr	<i>Triticum aestivum</i>	Immobilized Cd and Cr and decreased their uptake by <i>Triticum aestivum</i>	Joshi and Juwarkar [171]
<i>Bacillus edaphicus</i> NBT	Pb	<i>Brassica juncea</i> L. Czern	Promoted plant growth (root and shoot biomass) and Pb uptake	Sheng et al. [222]
<i>Bacillus licheniformis</i> , <i>B. biosubtyl</i> , <i>B. thuringiensis</i>	Se, Cd, Cr	<i>Brassica juncea</i>	Increased metal uptake depending on specific metal-bacteria combinations	Hussein [254]
<i>Streptomyces acidiscabies</i> E13	Ni	<i>Vigna unguiculata</i>	Simultaneous inhibition of Ni uptake and solubilization and supply of Fe to plants. Hydroxamate siderophores promoted growth under Ni contamination by binding Fe and Ni, thus playing a dual role of supplying Fe and protecting against Ni	Dimkpa et al. [206]
<i>P. putida</i> HS-2	Ni	<i>Brassica napus</i>	Increased seed germination and plant biomass, siderophores, IAA, ACC, deaminase	Rodriguez et al. [255]
<i>Pseudomonas</i> sp. 29C, <i>Bacillus megaterium</i> 4C	Ni	<i>Brassica juncea</i>	Increased aboveground biomass, protected plants against the inhibitory effects of Ni, probably due to the production of IAA, siderophore, and solubilization of phosphate	Rajkumar and Freitas [211]
<i>Pseudomonas aeruginosa</i> MKRh3	Cd	<i>Vigna mungo</i>	Growth increment, ACCD, siderophore, auxin synthesis, P solubilization. Reduced Cd toxicity and uptake	Ganesan [256]
<i>Pseudomonas</i> sp., <i>Janthinobacterium lividum</i> , <i>Serratia marcescens</i> , <i>Flavobacterium</i> sp., <i>Streptomyces</i> sp., <i>Agromyces</i> sp.	Cd, Zn	<i>Salix caprea</i>	Increased plant leaf biomass, siderophore, IAA. Decreased Zn and Cd uptake	Kuffner et al. [257]
<i>Bacillus</i> sp. J119 Biosurfactant-producing bacterial strain <i>Bacillus</i> sp. J119	Cd	<i>Solanum lycopersicum</i> , <i>Zea mays</i> , <i>Brassica napus</i>	Increased Cd content in aboveground tissues of all plants	Sheng et al. [231]

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>Glunacetobacter diazotrophicus</i> PA15	Zn	NA	Solubilized ZnO 5-Ketogluconic acid, a derivative of gluconic acid was the major organic acid produced. This organic anion may be an important agent that helped in the solubilization of insoluble Zn compounds	Saravanan et al. [258]
<i>Methylobacterium oryzae</i> CBMB20 <i>Burkholderia</i> sp. CBMB40	Ni, Cd	<i>Lycopersicon esculentum</i>	Reduced Ni and Cd uptake and their translocation to shoots ACCd activity, phytohormone production	Madhaiyan et al. [141]
<i>Rhodococcus erythropolis</i> MtCC	Cr	<i>Pisum sativum</i>	Bacterial metal detoxification mechanism. Increased plant growth in the presence of Cr(VI) at low temperature	Trivedi et al. [259]
<i>Rhizobium</i> sp. RP5	Ni, Zn	<i>Pisum sativum</i>	Increased nitrogen fixation, growth promotion, and the ability to reduce the toxicity of Ni and Zn	Wani et al. [260]
<i>P. putida</i> ARB86	Ni	<i>Arabidopsis thaliana</i>	Increased biomass and chlorophyll content. Nickel influx into plants was decreased by bacterial sorption in the rhizosphere	Someya et al. [261]
<i>Pseudomonas aeruginosa</i> BS2	Cd, Pb	NA	Mobilized Cd and Pb	Juwarkar et al. [262]
<i>Pseudomonas</i> sp. PsA4, <i>Bacillus</i> sp. Ba32	Cr	<i>Brassica juncea</i>	Protected the plants against the inhibitory effects of Cr; probably due to the production of IAA, siderophores, and solubilization of phosphate	Rajkumar et al. [263]
<i>Pseudomonas fluorescens</i>	As	<i>Helianthus annuus</i>	Increased plant growth	Shilev et al. [264]

(continued)

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
<i>P. putida HS-2</i>	Ni	<i>Brassica napus</i>	Increased biomass and total Ni per plant	Farwell et al. [265]
<i>Pseudomonas</i> sp. RJ10, <i>Bacillus</i> sp. RJ16	Cd	<i>Brassica napus</i>	Increased root and shoot weight and an increase in Cd content varying from 16% to 74%, compared to the non-inoculated control	Sheng and Xia [266]
<i>P. putida 06909</i>	Cd	<i>Helianthus annuus</i>	Increased Cd uptake and decreased toxicity; the bacterium expresses a metal-binding peptide	Wu et al. [267]
<i>Brevibacillus</i> sp. B-1	Zn	<i>Trifolium repens</i>	Decreased the concentration of Zn in shoot tissues	Vivas et al. [268]
<i>P. fluorescens, P. putida</i>	Ni	<i>Brassica napus</i>	Increased seed germination and plant growth	Ashour et al. [269]
<i>Pseudomonas putida KNP9</i>	Cd, Pb	<i>Phaseolus vulgaris</i>	Reduced Cd and Pb accumulation in <i>Phaseolus vulgaris</i>	Tripathi et al. [210]
<i>Pseudomonas asplenii</i> AC	Cu	<i>Brassica napus</i>	Increased biomass; IAA	Reed and Glick [270]
<i>Variovax paradoxus</i> , <i>Rhodococcus</i> sp., <i>Flavobacterium</i> sp.	Cd	<i>Brassica juncea</i>	Increased root length, IAA, siderophores, and ACC deaminase	Belimov et al. [271]
<i>Pseudomonas putida KNP9</i>	Pb, Cd	<i>Vigna radiata</i>	Increased biomass and siderophore production. Decreased metal uptake	Tripathi et al. [210]
<i>Pseudomonas tolaasii</i> RP23, <i>Pseudomonas fluorescens</i> RS9	Cd, Zn, Ni	<i>Graminaceae</i>	Have plant growth-promoting characteristics that can potentially support heavy metal uptake and reduce stress symptoms in plants	Dell' Amico et al. [272]
<i>Stenotrophomonas maltophilia</i>	Se	<i>Astragalus bisulcatus</i>	Reduced soluble and harmful Se(IV) to insoluble and unavailable Se(0) and thereby decreased plant Se uptake	Di Gregorio et al. [273]

Table 2.3 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress	References
Rhizosphere bacteria colonizing <i>T. goesingense</i>	Ni	<i>Thlaspi goesingense</i>	Increased siderophore production	Idris et al. [186]
<i>Pseudomonas fluorescens</i> Avm, <i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i> CPMex46	Cu, Fe	<i>Medicago sativa</i>	Improved Cu and Fe translocation from roots to shoots	Carrillo-Castañeda et al. [274]
<i>Microbacterium arabinogalactanolyticum</i>	Ni	<i>Alyssum murale</i>	Increased Ni uptake	Abou-Shanab et al. [275]
<i>Brevibacillus</i> sp.	Pb	<i>Trifolium pratense</i>	Decreased Pb uptake; IAA	Vivas et al. [276]
<i>Enterobacter cloacae</i> CAL2	As	<i>Brassica napus</i>	Increased biomass; ACC deaminase	Nie et al. [277]
<i>Burkholderia cepacia</i> with <i>ncc-nre</i> <i>ncc-nre</i> nickel resistance system <i>Herbaspirillum seropedicae</i> with <i>ncc-nre</i> nickel resistance system	Ni	<i>Lupinus luteus</i>	Facilitated bioaccumulation of Ni in roots	Lodewyckx et al. [278]
<i>Microbacterium saperdae</i> , <i>Pseudomonas monteilii</i> , <i>Enterobacter cancerogenus</i>	Zn	<i>Thlaspi caerulescens</i>	Increased Zn uptake	Whiting et al. [279]
<i>Kluyvera ascorbata</i> SUD165, SUD165/26	Ni, Pb, Zn	<i>Brassica napus</i> , <i>Lycopersicon esculentum</i> , <i>Brassica juncea</i>	Increased biomass, ACC deaminase, siderophores	Burd et al. [280]
<i>Kluyvera ascorbata</i> SUD165 <i>Enterobacteriaceae</i>	Ni	<i>Brassica napus</i>	Increased biomass and ACC deaminase	Burd et al. [281]

M metal(loid)s, NA not applicable

- It preserves the ecosystem.
- It is the main technique of phytoremediation for the removal of heavy metals from soil, sediments, and water.
- It is also considered as the most commercially promising technique because it is inexpensive.

However, although it presents several advantages, there are some factors that limit metal phytoextraction [13, 282]:

- A low bioavailability of metals in the rhizosphere
- A low uptake rate
- A low translocation rate confining the metals in the roots

The amount of metal removed depends on concentration in aboveground plant materials and the plant biomass produced [283, 284]. However, the small biomass and slow growth of many (hyper)accumulators, as well as a low soil metal bioavailability, can limit the effectiveness of phytoextraction [53, 284]. A large number of plant species have the ability to hyperaccumulate metal(oid)s in their tissues. Throughout the years a general increase in pollution and the necessity to find reliable methods for the restoration of contaminated sites have led to an increased interest on hyperaccumulation, phytostabilization, or phytoextraction. Pertinent problems arise when trying to define plants as either hyperaccumulators or as suitable for phytostabilization or phytoextraction events. Plant(part)/soil and plant part/plant part ratios have been described, and new terms have been created, i.e., a particular ratio has been defined by several different names and acronyms. So, in the bibliography different terms have been attributed to the same ratio, and this often represents an overlap in terminology. On the other hand, the same term corresponds to several different ratios, and this could create confusion and misinterpretation in data comparison. Recently, Buscaroli [285] reviewed the various factors, coefficients, and indexes developed so far to evaluate terrestrial plant performance in respect to phytoremediation.

Some important hyperaccumulator families are Brassicaceae, Fabaceae, Caryophyllaceae, Flacourtiaceae, Euphorbiaceae, Asteraceae, Lamiaceae, Poaceae, Violaceae, and Scrophulariaceae [1, 5, 193]. The hyperaccumulator species (e.g., *Thlaspi caerulescens*, *Alyssum bertolonii*, *Arabidopsis halleri*) are able to accumulate contaminants but produce little biomass, and therefore it is possible to use species that accumulate less but which produce more biomass like *Brassica* spp., *Arundo donax*, and *Typhas* spp. [1, 13, 286–289]. An ideal plant for trace element phytoextraction should possess the following characteristics: (a) tolerance to the trace element accumulated, (b) fast growth and highly effective trace element accumulating biomass, (c) accumulation of trace elements in the aboveground parts, and (d) easy to harvest [230].

A typical trace element phytoextraction protocol consists of the following steps: (a) cultivation of the appropriate plant/crop species on the contaminated soil, (b) removal of harvestable trace element-enriched biomass from the site, and (c) post-harvest treatments (i.e., composting, compacting, thermal treatments) to reduce vol-

ume and/or weight of biomass for disposal as a hazardous waste or for its recycling to reclaim the elements that may have an economic value. The storage, treatment, and placement of the contaminated plant biomass are of great concern. Compaction and composting of the plant biomass decrease its volume and transport costs, but increase leaching of dissolved metal organic compounds [10, 290]. One of the most economical approaches to deal with the contaminated biomass is its commercial use as a source of energy [10]. Gasification and combustion are important trends in the production of thermal and electric energies. Direct combustion should be accomplished under control with a reduction in the biomass to 3–6% and the correct placement of the ash [10]. Thermal processing or thermos chemical gasification of the plant biomass into biogas (gaseous fuel) is a complex and high-tech process proceeding at 800–1300 °C. The ash obtained can be used as biore, and this process is termed as phytomining [10, 291]. Another effective method is thermochemical processing of the plant biomass (pyrolysis), which is a process of thermal decomposition of organic compounds in an oxic condition at relatively low temperatures (500–800 °C). This process allows the collection of fluid oils (biofuel, pyrofuel, resins), gases, and coke. The coke retains the heavy metals and is useful as a fuel in industry [10, 292]. To apply phytoextraction techniques, metal(loid)s must be bioavailable and ready to be absorbed by roots. The speciation of metal(loid)s in soils can include [13, 293]:

1. Free metal ions
2. Oxides, hydroxides, and carbonates
3. Integration into the structure of silicate/aluminum minerals
4. Soluble metal complexes
5. Associations with soil organic matter

Metal speciation regulates metal uptake by plant roots and consequently phytoextraction efficiency. Indeed, it is known that low bioavailability of soil metal(loid)s may be a rate-limiting factor for metal uptake by plants. The major limitations of most metal phytoextraction processes are (1) the bioavailability of the target metal(loid)s and (2) the ability of various plants to bioaccumulate metals within their aboveground biomass [48, 294]. Metal bioavailability can be defined as the fraction of metal in the soil that can interact with a biological target. In the soil solution, elements are present as free uncomplexed ions, ion pairs, ions complexed with organic anions, and ions complexed with organic macromolecules and inorganic colloids. The most important metal pools in the solid phase include the metals complexed by organic matter, sorbed onto or occluded within oxides, and clay minerals coprecipitated with secondary pedogenic minerals (e.g., Al, Fe, Mn oxides, carbonates and phosphates, sulfides) or as part of the crystal lattices of primary minerals [44, 61].

Current phytoextraction practices employ either hyperaccumulators or fast-growing high biomass plants, and the phytoextraction process may be enhanced by addition of soil amendments that can increase trace element bioavailability in the soil [230]. When bioavailability of metal(loid)s in the soils is insufficient for active root sorption, transport in solution and mobilization can be promoted using chelating

agents [295], such as organic and mineral acids, elemental sulfur, and ammonium fertilizers. This chelant-assisted accumulation of heavy metals by plants that do not concentrate them under normal conditions was termed as chelant-induced hyperaccumulation. The application of chelating agents, for instance, synthetic aminopolycarboxylic acids, is efficient. Hydroxyethylene diamine triacetic acids (HEDTA), ethylenediamine dihydroxyphenylacetic acid, and ethylenediaminetetraacetic acid (EDTA) are potential acidifying chelants [295]. These agents can enhance the phytoextraction of Cu, Cd, Pb, Zn, and Ni [296].

There are a few disadvantages when using chelating agents, i.e., adverse effects on soil microorganisms, possible contamination of groundwater, and slow (several weeks or months) decomposition of the synthetic organic acids [296–298]. Following the application of chelate-forming agents, the removal of metals may continue for a long time. There are some rapid decomposing natural organic acids which are considered alternatives to synthetic chelating agents, i.e., ethylenediamine-*N,N'*-disuccinic (EDDS) acids and nitrilotriacetic acid (NTA) [296, 297]. In addition, products of plant metabolism and low-molecular organic compounds, i.e., tartaric [299], acetic [297], and malic acids, putrescine (polyamine), and citric and oxalic acids [300], can also be used for the same purpose. In recent years, different biotechnological approaches have also been used to increase biomass and growth rate of hyperaccumulator plants through genetic engineering tools, i.e., synthesis of various metal-binding peptides [10, 301]. Along with engineering, metal(loid)s accumulation by plants can be enhanced using conventional agronomic practices, i.e., fertilization, irrigation, seed control, shorter growth cycle, and liming [12].

2.4.1 Assisted by Fungi

There is increasing evidence that plant-microbe interactions/dynamics can define the efficiency of metal phytoextraction. Inoculation of the plant rhizosphere with microorganisms is an established route to improve phytoextraction efficiency. In general, microorganisms can improve phytoextraction by increasing the bioavailability of metal(loid)s to the plant and by increasing plant biomass [302].

A frequently utilized strategy to improve phytoextraction is the inoculation of beneficial microorganisms into the plant rhizosphere. Microorganisms may increase plant trace element uptake by three specific mechanisms: (1) they may increase root surface area and hair density, (2) increase element bioavailability, and/or (3) increase soluble element transfer from the rhizosphere to the plant [168]. Furthermore, enhanced plant biomass production can boost an increase on the efficiency of trace element phytoextraction [230]. Increased trace element uptake can be attributed to a microbial modification of the absorptive properties of the roots such as increased root length and surface area and numbers of root hairs or by increasing the bioavailability of trace elements in the rhizosphere and the subsequent translocation to shoots via beneficial effects on plant growth, trace element complexation, and alleviation of phytotoxicity [230].

Regardless of whether trace elements are mainly accumulated in roots or in shoots, internal tolerance mechanisms are the basis for efficient detoxification of the trace elements. These internal detoxification mechanisms are extensively described in the previous sections. Among various microbe-assisted strategies that have been proposed to date, AM fungi are known to benefit their host plants by affecting biological regulation of their phytoextraction efficiency [127, 303, 304]. AM fungi are thought to be one of the most important soil microbial groups that affect metal uptake by plants and metal immobilization in soils [74, 305] and are commonly introduced into soil for land reclamation [74, 127, 306]. Previous studies reported that both host plant biomass and metal concentrations could be increased via AM fungi inoculation [127, 307], thus resulting in increased uptake of metal(loid)s [127, 308]. Different host plants and fungal species as well as environmental conditions could potentially affect response strategies of plants ([88, 127]). The functional diversity is significantly reflected on plant growth, element uptake, and enzymatic activity [127, 309, 310]. In some cases, AM fungi contributed to a significant storage of metal(loid)s at the root level, instead of the aboveground tissues of the host plants (phytostabilization). However, in other cases AM fungi contributed to enhanced uptake and translocation to shoots promoting phytoextraction success [86, 127].

An important point about treating polluted soil with mycorrhizal plants is the selection of appropriate AM species [12]. The species selected from areas polluted with metal(loid)s are the most efficient species which have the ability to survive under metal stress conditions and hence may act more efficiently relative to other AM species [12, 14]. These communities are metabolically and taxonomically diverse, containing microorganisms that are preadapted to conditions in situ and are capable of performing metabolic activities that can alter metal(loid)s bioavailability and promote plant growth [252, 302, 311–314]. Generally, species of the genus *Glomus* are predominant in the rhizosphere of plants growing in metal(loid) s-contaminated soils [12, 315, 316]. AM fungi can colonize hyperaccumulator roots extensively in metal(loid)-contaminated soils forming symbiotic/mutualistic relationships with plants. In general, mycorrhizal hyperaccumulators produce considerably more biomass and grow faster than non-mycorrhizal plants, and because of the ability of AM fungi to survive in severely contaminated soils, they are able to play a role on phytoremediation processes enhancing the capacity of plants to withstand soil phytotoxicity [60, 71, 72]. AM fungi promote plant establishment in metal(loid) s-contaminated soils, strengthening plant defense systems, and promoting its growth [73, 127].

Several biological and physical mechanisms have been proposed to explain metal tolerance of AM fungi and their contribution to metal tolerance of host plants. These tolerance mechanisms are numerous, e.g., extracellular metal sequestration and precipitation, metal binding to the fungal cell walls, intracellular sequestration and complexation, compartmentation, and volatilization, and are broadly described in previous sections. Many fungi can survive and grow with high concentrations of toxic metals [44, 317]. The ability of mycorrhizal associations to decrease metal toxicity to higher plants has been shown for ericoid mycorrhizas, ectomycorrhizas,

and arbuscular mycorrhizas, while some ectomycorrhizal fungi accelerate mineral weathering. The mycorrhizal mycelia provide an efficient system for the uptake and direct transport of mobilized essential nutrients to their host plants, which are large sinks [44, 318]. Mineral weathering can also increase the concentrations of phytotoxic aluminum in the soil solution. Some ectomycorrhizal fungi increase Al tolerance through an enhanced exudation of oxalate [44, 319]. Furthermore, AM fungi inoculation can improve plant performance under metal(oid)s stress due to a number of mechanisms involving antioxidant enzymes, lipid peroxidation, and soluble amino acid profile changes caused by the intimate relationship between fungi and the host plant [73, 320, 321]. In fact, when exposed to metal(loid)s such as Cu, Fe, Pb, Cd, Cr, As, Hg, Cr, and Zn, plants enhance their level of reactive oxygen species (ROS) [73, 322, 323].

The contribution of AM fungi, such as *Funneliformis mosseae*, to enhanced Cd and Zn uptake and translocation by *S. nigrum* in heavy metal-contaminated sites has been noted without deleterious effects on plant growth [65, 324–326]. Fiorentino et al. [287] observed increases on Cd phytoextraction by *Arundo donax* assisted by the fungal microorganism *Trichoderma harzianum* in both, leaves (+20%) and rhizomes (+30%), while Chen et al. [327], observed an increase on Pb uptake and consequent accumulation in *Kummerowia striata*, *Ixeris denticulate* and *Echinochloa crusgalli*.

It has been discovered recently that adding indigenous AM fungi can boost the uptake and accumulation of As and the biomass of *P. vittata* [60]. It was also shown that plants from an As mine site, colonized by AM fungi, accumulated more As than the non-colonized plants. An increase on growth, promoted by the activation of the phosphate transport system in colonized plants, may justify these results.

The process of translocation may be assumed to vary with metal(loid)s concentrations in the substrate, even if they grow with the same AM inoculation. Consequently, whether AM fungi enhance the transport to shoots (phytoextraction) or immobilize in the roots (phytostabilization) depends on metal(loid)s stress in the substrate [127]. AM fungi play different roles to cope with Cd toxicity in *P. australis*. With a low Cd stress, AM enhanced plant growth, and this acted as an accumulator due to the growth dilution effect. However, with high Cd stress, the AM symbiosis leads the plant to act as an excluder [127]. Consequently, in the presence of AM fungi, phytoextraction played the predominant role at low Cd stress, while phytostabilization occurred with high Cd stress [127]. The AM *Glomus intraradices* was shown to enhance growth of *Helianthus annuus*, and as a result, the total Ni is extracted. It also increased the activity of glutamine synthetase, indicating an enhanced Ni tolerance [230, 328].

A stimulation on the biomass of *B. coddii* in mycorrhizal plants led to a higher total Ni content (and hence phytoextraction) [80]. The diverse endophytic fungi isolated from *S. nigrum* showed the symbiotic association of these microbes with the host plant and improved our understanding regarding plant fitness under extreme conditions [56, 57]. The inoculation of *S. nigrum* with both *Glomerella truncata* PDL-1 and *Phomopsis fukushii* PDL-10 increased its tolerance to high concentrations of Cd. As a result, the parameters related to the biosorption of Cd, including

translocation, bioconcentration factors, and tolerance index, were significantly enhanced. The strongest evidence for the presence of endophytes was the apparent promotion of plant growth and enhanced biochemical content of chlorophyll as compared to those of non-inoculated control plants [56, 57]. Endophytic fungi may increase host fitness and competitive abilities by increasing successful germination and growth rate or enhancing the uptake nutritional elements by the host [44, 116]. Furthermore, due to possessing suitable degradation pathways, metal sequestration, or chelation systems, fungal endophytes are able to increase host plant tolerance to metal(loid)s and assist the host survival in contaminated soils [44, 116].

2.4.2 Assisted by Bacteria

Plant-associated bacteria can potentially improve phytoremediation by altering the solubility, bioavailability, and transport of metal(loid)s and nutrients by altering soil pH, release of chelators (e.g., siderophores, organic acids, biosurfactants, glycoproteins), methylation, P solubilization, or redox changes [50, 54, 61, 138, 193, 284, 329]. Therefore, plant-associated bacteria can be exploited to improve the efficiency of the phytoextraction processes [48, 168, 169, 222, 230, 232, 257, 330–332]. Bacterial populations associated with plants growing in metalliferous soils have a high diversity. These communities might also have important functions in relation to plant growth under these adverse conditions as well as in improving uptake of trace elements [230]. Plant growth promotion plays a major role in the extraction and removal of trace elements since a simple improvement in biomass results in an increase in the overall trace element yield (phytoextracted trace elements) [230]. The Ni-resistant PGPB strain *Psychrobacter* sp. SRS8 originally isolated from the rhizosphere of the Ni hyperaccumulator *A. serpyllifolium* was found to effectively promote the growth and phytoextraction potential of the energy crops *Ricinus communis* and *Helianthus annuus* in artificially Ni-contaminated soils [50, 230].

The Ni hyperaccumulator *A. serpyllifolium* subsp. *lusitanicum* grown in an ultramafic soil showed a significantly higher translocation and shoot Ni concentration after inoculation with a Ni-resistant rhizosphere bacteria *Arthrobacter nitroguajacolicus* [333]. In both hydroponically and soil-grown plants, inoculating the Cd/Zn hyperaccumulator *S. alfredii* with the metal(loid)-tolerant rhizobacterial strains belonging to the genera *Burkholderia* improved plant tolerance, biomass production, and Cd (and Zn) uptake and extraction [334, 335]. Bacteria can acidify their environment by pumping protons to maintain the electrochemical gradient of membranes. These replace trace element cations at sorption sites and dissolve minerals such as phosphates. Acid-producing rhizosphere bacteria have been intensely studied due to their capacity to release phosphorus from insoluble phosphates. For the purpose, bacteria can produce and secrete an array of organic acids, such as gluconic, 2-ketogluconic, lactic, and acetic acids [302]. The associated decrease in soil pH can also increase the solubility of some metal(loid)s [336].

Recent studies investigating the role of endophytes in metal(loid)s uptake by plants have demonstrated that the production of specific bacterial organic acids (e.g., citric, oxalic, acetic acids, etc.) may result in increased nutrient and metal(loid)s uptake, probably as a result of the decrease in soil pH and their solubilizing power, improving the efficiency of phytoextraction processes [52, 145]. The different metabolites released by PGPB (e.g., siderophores, osmolytes, nitric oxide, antibiotics, bio-surfactants, organic acids, and plant growth regulators) can change metal(loid)s uptake either directly through their effects on plant growth or indirectly through acidification, chelation, precipitation, immobilization, and oxidation-reduction reactions in the rhizosphere [50]. Organic chelators scavenge trace element ions from sorption sites and mineral lattices and protect them from resorption [138, 230]. To date two groups of bacterially produced natural chelators are known. These are carboxylic acid anions and siderophores. Among a large variety of carbon compounds, oxalic, malic, and citric acids are some of the most important organic acids identified in roots and in microbial exudates [230, 337].

As the first pK_a values of most carboxylates are below 3.5 and the cytosolic pH of root cells typically ranges from 7.1 to 7.5, carboxylic acids are typically present in soil solution as fully or partially dissociated forms [162, 230]. In plant cells, complexation with carboxylic acids, particularly malate, citrate, but also with the basic amino acid histidine, is a powerful mechanism for trace element detoxification [230, 338]. In addition to plant growth promotion, bacteria were reported to have a beneficial effect on plant stress tolerance. This may be achieved by the enzyme ACC deaminase leading to a reduction of stress-induced ethylene levels in the plant [230, 280, 339].

For instance, experiments assessed by Sheng et al. [232] have shown the solubilizing potential of the Pb-resistant endophytic bacteria *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 on Pb uptake by *Brassica napus*. The results showed that both endophytes enhanced Pb bioavailability, thus increasing Pb accumulation in plant shoots from 76% to 131% (*P. fluorescens*) and from 59% to 80% (*Microbacterium* sp.), compared to the respective control. A possible explanation might be the production of siderophores or organic acids that induce solubilization of Pb.

These effects of inoculation were reported also by Mastretta et al. [229], who reported that the inoculation of *N. tabacum* with the Cd-resistant endophyte *Sanguibacter* sp. increased the concentration of Cd in shoot tissues and consequently the phytoextraction capacity of *N. tabacum*, by approximately threefold compared with the respective uninoculated control. These studies suggest that it should be possible to improve the metal phytoextraction potential of accumulating or hyperaccumulating plants, through the inoculation of seeds or rhizosphere soil with beneficial metal-resistant endophytic bacteria. Although several conditions, such as soil properties, environmental conditions, and microbial activity, must be optimized for any phytoextraction event to become effective, the bioavailability of metals in the rhizosphere is considered to be a critical requirement for metal uptake to take place [54]. Braud et al. [340] reported that inoculating soils with *P. aeruginosa* significantly increased the concentrations of bioavailable Cr and Pb com-

pared with uninoculated controls. Furthermore, they also observed that *P. aeruginosa* significantly enhanced Cr and Pb accumulation in maize shoots by a factor of 4.3 and 3.4, respectively. In this case, metal(loid)s uptake could be correlated with the increased production of siderophores, in particular of pyoverdine and pyochelin [54]. These studies highlighted the potential of inoculating soils or plants with metal(loid)s-resistant siderophore-producing bacteria to further improve their phytoextraction potential.

Siderophores play a significant role in metal mobilization and accumulation, and siderophore-producing microbes are believed to play an important role in the phytoextraction of metal(loid)s [54, 209], as these compounds produced by PGPB may solubilize unavailable forms of metals but also form complexes with bivalent metal(loid)s ions that can be assimilated by root-mediated processes [50, 274, 340]. Siderophores are secreted, and Fe(III)-siderophore complexes are recognized and scavenged from the environment by membrane receptor proteins. They are too large to pass membrane porins [230]. All siderophores possess higher affinity for Fe(III) than for Fe(II) or any other trace element. However, complexes of lower stability are also formed with other trace elements [230, 341]. Divalent cations (e.g., Fe^{2+} , Zn^{2+} , Cu^{2+} , Cd^{2+}) form less stable complexes due to their reduced charge density (charge/size ratio). Addition of trace elements to bacterial cultures induces siderophore synthesis and leads to the formation of siderophore-metal complexes [206–208, 230]. Extracellular complexation by siderophores is considered to be a mechanism of bacterial trace element resistance [230, 332]. Siderophore synthesis was shown to simultaneously increase iron uptake and to reduce cadmium uptake in *Streptomyces* [208, 209, 230]. In contrast, siderophore-mediated uptake of trivalent trace element cations (Al^{3+}) has been demonstrated in iron-depleted cultures [230]. Synthesis of several siderophores varying in trace element affinity, preferences, and inductivity may convey competitive advantage in trace element contaminated environments [208, 209, 230].

The biosurfactants produced by PGPB also enhance metal(loid)s mobilization and improve phytoextraction on contaminated soils [50, 342]. Biosurfactants can desorb metal(loid)s from the soil matrix and hence increase metal solubility and bioavailability [193, 231, 343]. Certain nitrogen-fixing bacteria produce molybdate-binding tetradentate catecholates, which also function as siderophores [230, 341]. The pigment melanin, which is produced by many fungi and *Streptomyces*, can bind trace elements to its carboxylic groups and was shown to be involved in trace element sorption and trace element tolerance of *S. scabies* [230, 344]. In addition to beneficial effects on growth by improved plant nutrition, microorganisms can also enhance plant growth directly via the production of phytohormones, including IAA, cytokinins, and gibberellins. These compounds can stimulate germination, growth, and reproduction and protect plants against both biotic and abiotic stress [50, 345]. Indeed, the mechanism most often cited to explain the various direct effects of PGP on plants is the production of phytohormones, and most of the attention has focused on the role of the phytohormone auxin [48, 346, 347]. In addition to the well-characterized effects of microbial auxin and ethylene on plant growth, a number of plant growth-promoting bacteria synthesize cytokinins, which can stimulate the

growth of different crops under both stressed and non-stressed conditions helping the phytoextraction processes [48, 348].

In the last years, it has been found that a number of PGPB contain the enzyme ACC deaminase, which can cleave the plant ethylene precursor ACC and thereby lower the level of the phytohormone ethylene in a developing or stressed plant [302].

After working on microbe-assisted Cd phytoextraction, Wei et al. [104, 105] reported that the endophytic bacterial strain *Rahnella* sp. JN27, isolated from roots of *Zea mays*, enhanced Cd bioavailability in the soil and solubilized phosphate, producing indole-3-acetic acid, siderophores, and ACC deaminase. Multiple reports showed that foliar application of IAA or other phytohormones can improve the phytoextraction of metals, including Ni, Pb, and Cd [302, 349, 350]. The proliferation of specific microorganism strains, able to aggressively colonize the root surface, can promote growth and healthier plants, improving root development, and/or enhance plant tolerance to different environmental stresses, strengthening the phytoextraction processes [48, 351]. Accordingly, metal phytoextraction (as well as plant growth) can be helped by soil microorganisms associated with plant roots [48, 351]. Finally, a few studies reported that plant growth and/or trace element accumulation has been improved using combinations of plant-associated microorganisms. In a hydroponic study, a combination of seven As-resistant rhizobacteria (identified as *Pseudomonas* sp., *Comamonas* sp., and *Stenotrophomonas* sp.) enhanced As uptake by the As hyperaccumulator *Pteris vittata*. Microbial exudation of pyochelin-type siderophores, together with root exudates, solubilized As from the growth media spiked with insoluble FeAsO_4 and AlAsO_4 minerals [352].

In soil-grown plants, inoculation of *Salix caprea* with *Streptomyces* sp. in combination with the fungus *Cadophora finlandica* led to an increase in phytoextraction of Cd and Zn (Table 2.4) [230, 365].

2.5 Conclusions and Future Prospects

Phytoremediation techniques, based on interactions between plants and microorganisms, have been proposed as eco-friendly methods to clean polluted soils. Soil microorganisms can improve pollutant mobilization and respective uptake by plants. The success of phytoextraction depends on several factors, including the concentration of soil pollutants, metal bioavailability for root uptake, and the capability of plants to intercept, sorb, and accumulate metal(loid)s in their tissues. Ultimately, the success of phytoextraction depends on interactions among soil, metals, and plants. However, low bioavailability of metals, low biomass of most hyperaccumulators, and restricted metal translocation to the shoots limit the efficiency of phytoextraction. In order to solve these restrictive factors, some strategies such as advanced agricultural practices, genetic engineering, and chelate treatments need to be adopted to improve phytoextraction performance. The use of natural chelators to

Table 2.4 Results of phytoextraction studies assisted by plant-associated microbes

Microorganisms	M	Test plant	Microbial effects on plants under metal stress, phytoextraction	References
<i>Microbacterium</i> , <i>Variovorax</i> , <i>Micrococcus</i> , <i>Pseudomonas</i>	Zn	<i>Noccaea caerulescens</i> , <i>Rumex acetosa</i>	Increase in the growth of both plants, as well as higher values of Zn phytoextraction	Burges et al. [284]
<i>Bacillus pumilus</i> E2S2, <i>Bacillus</i> sp. E1S2	Cd, Zn, Pb	<i>Sedum plumbizincicola</i>	<i>B. pumilus</i> E2S2 significantly increased root and shoot length, biomass, and plant Cd uptake, whereas <i>Bacillus</i> sp. E1S2 significantly enhanced the accumulation of Zn. Results demonstrated the potential to improve phytoextraction of soils contaminated with multiple heavy metals by inoculating metal hyperaccumulating plants with their own selected functional endophytic bacterial strains	Ma et al. [147]
<i>Streptomyces</i> sp., <i>Cadophora finlandica</i>	Cd, Zn	<i>Salix caprea</i>	Increased phytoextraction of Cd and Zn	Sessitsch et al. [230]
<i>Firmicutes</i> sp., <i>Actinobacteria</i> sp., <i>Proteobacteria</i> sp.	Cu	<i>Brassica napus</i>	Increased root and aboveground tissues weight and shoot Cu concentration	Sun et al. [353]
<i>Bacillus subtilis</i> , <i>B. cereus</i> , <i>Flavobacterium</i> sp., <i>Pseudomonas aeruginosa</i>	Zn	<i>Orychophragmus violaceus</i>	Increased root length, biomass of root, stems, and leaves, and Zn uptake	He et al. [354]
<i>Burkholderia cepacia</i>	Zn, Cd	<i>Sedum alfredii</i>	Higher ability to mobilize Cd and Zn and tolerate high concentrations of soluble Zn. Increased the soluble Zn concentration in the medium from insoluble zinc oxide and zinc carbonate. Oxalic, tartaric, formic, and acetic acids had a significant correlation with the concentrations of Cd and Zn being mobilized	Li et al. [355]
<i>Pseudomonas</i> sp. RJ10, <i>Bacillus</i> sp. RJ16	Cd, Pb	<i>Lycopersicon esculentum</i>	Increased root length, aboveground biomass and aboveground metal content, siderophores, IAA, ACC deaminase production	He et al. [356]

(continued)

Table 2.4 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress, phytoextraction	References
<i>Psychrobacter</i> sp. SRA1 and SRA2, <i>Bacillus cereus</i> SRA10	Ni	<i>Brassica juncea</i> , <i>Brassica oxyrrhina</i>	Significantly increased the accumulation of Ni in the root and shoot tissues reinforcing the efficiency of phytoextraction	Ma et al. [313]
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Ralstonia metallidurans</i>	Cr, Pb	<i>Zea mays</i>	Enhanced Cr and Pb uptake by plants through their mobilization	Braud et al. [340]
<i>Pseudomonas fluorescens</i> G10, <i>Microbacterium</i> sp. G16	Pb	<i>Brassica napus</i>	Increased plant weight, ACCD activity, IAA production, P accumulation, and Pb translocation to shoots	Sheng et al. [232]
<i>Bacillus edaphicus</i> NBT	Pb	<i>Brassica juncea</i> L. Czern	Promoted plant growth (root and shoot biomass) and Pb uptake	Sheng et al. [222]
<i>Pseudomonas tolaasii</i> ACC23, <i>P. fluorescens</i> ACC9, <i>Mycobacterium</i> sp. ACC14	Cd	<i>Brassica napus</i>	The strains did not influence Cd concentration in the root or shoot, but they increased plant biomass and consequently the total Cd extracted	Dell'Amico et al. [312]
<i>Enterobacter</i> sp. NBRI K28	Ni, Zn, Cr	<i>Brassica juncea</i>	Stimulated plant biomass and enhanced phytoextraction of Ni, Zn, and Cr Production of siderophores, IAA, and phosphate solubilization. The strain also exhibited ACC deaminase activity	Kumar et al. [357]
<i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Pseudomonas pseudoalcaligene</i> , <i>Brevibacterium halotolerans</i>	Cu, Cr, Pb, Zn	<i>Zea mays</i> , <i>Sorghum bicolor</i>	Increased metal availability in soil, thus enhancing Cr, Pb, Zn, and Cu accumulation by <i>Z. mays</i> and <i>S. bicolor</i>	Abou-Shanab et al. [358]
<i>Burkholderia</i> sp. J62	Pb, Cd	<i>Brassica juncea</i> , <i>Zea mays</i> , <i>Lycopersicon esculentum</i>	Increased biomass and metal uptake	Jiang et al. [359]
<i>B. weihenstephanensis</i> SM3	Cu, Zn	<i>Helianthus annuus</i>	Increased the plant biomass and the accumulation of Cu and Zn in the root and shoot systems	Rajkumar et al. [205]

(continued)

Table 2.4 (continued)

Microorganisms	M	Test plant	Microbial effects on plants under metal stress, phytoextraction	References
<i>P. putida</i> ARB86	Ni	<i>Arabidopsis thaliana</i>	Increased biomass and chlorophyll content. Nickel influx into plants was decreased by bacterial sorption in the rhizosphere	Someya et al. [261]
<i>Burkholderia cepacia</i>	Cd, Zn	<i>Sedum alfredii</i>	Enhanced plant growth, metal uptake, and translocation of metals from root to shoot	Li et al. [335]
<i>P. marginalis</i> Dp1, <i>Rhodococcus</i> sp. Fp2	Cd	<i>Pisum sativum</i>	Increased Cd concentration in shoots	Safronova et al. [360]
<i>Pseudomonas monteilii</i>	Cd	<i>Sorghum bicolor</i>	Enhanced plant biomass, Cd uptake, and translocation to shoots	Duponnois et al. [361]
<i>Azotobacter chroococcum</i> HKN-5, <i>Bacillus megaterium</i> HKP-1, <i>Bacillus mucilaginosus</i> HKK-1	Cd, Cu, Pb, Zn	<i>Brassica juncea</i>	Stimulated plant growth and protected the plant from metal toxicity It did not influence metal concentrations in plant tissues, but led to greater aboveground biomass, thus resulting in much higher metal removal. It also influenced speciation of metals in the soil and consequently altered the bioavailability of metals	Wu et al. [362]
<i>Sinorhizobium</i> sp. Pb002	Pb	<i>Brassica juncea</i>	Stimulated biomass production and, hence, phytoextraction of Pb	Di Gregorio et al. [363]
<i>B. subtilis</i> SJ-101	Ni	<i>Brassica juncea</i>	Exhibited the capability to produce IAA and to solubilize inorganic phosphate. Promoted plant growth and decreased soluble soil Ni by biosorption and bioaccumulation	Zaidi et al. [364]
<i>Brevibacillus</i> sp. B-1	Zn	<i>Trifolium repens</i>	Decreased the concentration of Zn in shoot tissues	Vivas et al. [268]

enhance metal bioavailability and inoculation of microorganisms, including bacteria and fungi, may facilitate the phytoextraction appliance at a commercial scale.

A number of bacteria and fungi have been studied by researchers which are able to enhance metal accumulation by plants and the metal(loid)s phytoextraction rate. Fungi are generally more tolerant to metal(loid)s than bacteria. Furthermore, AM fungi can efficiently explore the soil microsites that are not accessible for plant

roots. In this context, a combination of plant-associated microorganisms could be more effective in enhancing reclamation of polluted soils than a single microorganism. It has been shown that endophytes (bacteria and fungi) can be a more reliable source of natural biocenosis because of their intimate association with plants. However, an understanding of the mechanisms enabling endophytic microbes to interact with host plants growing in metal-contaminated soils is essential to fully accomplish the biotechnological applications of efficient plant-microbe partnerships. Furthermore, there is ample experimental evidence that metal-resistant SPB are able to survive in adverse environmental conditions, where they carry out a variety of beneficial interactions that increase plant growth and metal(loid)s uptake. The beneficial effects exhibited by SPB indicate that the inoculation with metal-resistant strains may contribute to increase phytoextraction potential in metal-contaminated soils.

However, 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. In addition, inoculation of mixtures of ecologically diverse microbes instead of single strains might represent a highly successful strategy. In this way, beneficial functions might be expressed more continually in a soil or rhizosphere system, even under ecologically distinct conditions. Furthermore, application of genetic engineering may enhance phytoextraction efficiency. After the identification of novel genes, transgenic plants may be produced with superior extracting capacity involving metal(loid)s hypertolerance, raised uptake and translocation to shoots, and highly efficient detoxification mechanisms. Genes for metal chelators production, metal homeostasis, transporters, biodegradative enzymes, metal uptake regulators, and biotic and abiotic stresses relievers are important candidates for making recombinant microbes.

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. It is obvious that the complexity of interactions in the plant-microbe-soil-pollutant systems requires substantial further research efforts to improve our understanding of the rhizosphere processes involved.

Fortunately, modern biotechnology has opened up new possibilities concerning the application of beneficial microbes to improve plant growth, biological control, as well as metal(loid)s phytoremediation.

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