Chapter 13
Understanding the Role of Microbes and Plants in the Management of Heavy Metal Stress: A Current Perspective

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Abstract Significant amount of heavy metals is regularly added to the soils globally due to various natural and anthropogenic activities. The heavy metal uptake and accumulation in crops lead to yield losses. A common consequence of heavy metal toxicity to plants is an excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG). Both molecules may cause peroxidation of lipids, oxidation of protein, DNA damage, inactivation of enzymes, and/or affect other vital plant constituents. Higher plants have evolved a balanced antioxidant defence system and a glyoxalase system to scavenge ROS and MG. Besides plants, microbes can also be used to remove heavy metals from polluted soils. This chapter highlights the suitability of various strategies adopted by useful soil microbiota and plants to eradicate heavy metal toxicity and consequently to enhance crop production in metal stressed soils.

13.1 Introduction

13.1.1 Heavy Metal Pollution and Its Agro-Ecological Impact: An Overview

Heavy metal pollution has emerged as a global challenge in developed and developing countries, which limits the economic growth and causes human health problems via food chain (Tchounwou et al. 2012; Khalid et al. 2017). Classically, heavy metals which are biologically and industrially important refer to a group of toxic elements having densities greater than 5 g cm^{-3}. Recently the term has also been used for metals and semimetals with potential human or environmental toxicity (Saunders et al. 2013; Mohmand et al. 2015). In soils, metals may be added through transport of continental dusts, emissions from volcanoes, and by weathering of metal-enriched rocks (Algreen et al. 2012). Natural inputs are unlikely to add

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considerably higher amount of HM to soils but due to rapid industrialization, there has been massive addition of heavy metals emanating from anthropogenic activities which has resulted in a greater public concern (Shaheen et al. 2017). Arsenic, lead, cadmium, and mercury are some of the notable soil heavy metals. Despite metal pollution arising from sewage application, sewage has primarily served as an important supplementary source to combat agricultural irrigation crisis in areas deficient in good quality waters (Li et al. 2017). Irrigation by wastewater in agriculture practices is common in many countries including China, India, Pakistan, Mexico, and Iran. The use of wastewater for irrigation has been found economical and to a certain extent has also solved the problem of effluent disposal (Navarro et al. 2015). Recent findings suggest that 35.9 Mha of irrigated crop lands are located in the vicinity of wastewater treatment plants and out of 82% of the agricultural fields, 75% are irrigated by wastewater (Thebo et al. 2017). Despite these benefits, use of wastewater for irrigation can pose substantial risks to the plants (Marrugo-Negrete et al. 2017). Although soil has some ability to clear and degrade pollutants via microbial metabolism and transformation but it is not enough to control heavy metal pollutants that accumulate in groundwater or soil solution due to continuous release of untreated pollutants or changes in soil pH. Hence, build-up of heavy metals in soils can restrict soil functioning (fertility) and result in toxicity to plants and physiological activities of microbes which in turn affect the quality and safety of foods severely and cause eventually human health problems (Meng et al. 2016).

13.1.2 Metal Toxicity to Human Health

The non-biodegradability and lethal effect of heavy metals is a serious problem for human health worldwide (Azimi et al. 2017). Though metals like Zn, Cu, Ni, Co and Cr at small quantities play a pivotal role in metabolic and physiological processes of plants (Singh et al. 2016), humans (Yamada 2013), and microorganisms (Boer et al. 2014). For instance, they affect redox processes, regulate osmotic pressure, and stabilize molecules through electrostatic interactions and act as cofactors for numerous enzymes and electron transport systems (Emamverdian et al. 2015). In contrast, there are non-essential heavy metals like Ag, As, Cd, Pb, and Hg which do not have any biological importance to living organisms and are very toxic even at very low concentrations. Heavy metals usually enter the human body via different food chains, inhalation, and ingestion. Once inside the human body, they stimulate the immune system and may cause nausea, anorexia, vomiting, gastrointestinal abnormalities, and dermatitis (Megido et al. 2017). Metals like Cr, As, Zn, Ur, Se, Au, and Ni may also adversely affect the quality of soil, crop production as well as public health (Venkanna and Karthikeyan 2017). These pollutants in general are major cause of life-threatening degenerative diseases such as Alzheimer's disease, atherosclerosis, cancer, Parkinson's disease, etc. Due to the detrimental effects of heavy metals, there is urgent need to find strategies to effectively eradicate HM from the environment and stabilize the ecosystem.

13.1.3 Metal–Microbe–Plant Interactions: A General Perspective

13.1.3.1 Heavy Metals and Rhizospheric Microflora

Soil microorganisms play a key role in maintaining soil fertility through various activities such as organic matter disintegration, adsorption, and pH balance and hence optimize crop production (Shahbaz et al. 2017). However, soil microbiota are greatly influenced when exposed to stress factors like heavy metals (Gutierrez-Ginés et al. 2014), high pH (Wu et al. 2017), salinity (He et al. 2017), extreme temperature (Akkermans et al. 2017), and chemical pollution (Gianfreda and Rao 2017). Among microbial communities, the bacterial communities in general have been reported to be the most severely affected by high HM concentration as compared to fungal population (Rajapaksha et al. 2004). The beneficial or detrimental effect of HMs onto microbial cells however depends on concentration, speciation, and duration of exposure of metals.

13.1.3.2 Impact of HM on Soil Microbial Composition and Function

Soil microbial community structure serves as an important marker for polluted soil since long-term exposure to pollutants changes the microbial compositions and functions. On the contrary, the long-term exposure to metals may also help microorganisms to adapt to the stressed environment (Kaci et al. 2016). However, various in-depth analysis have shown that metal pollution had significant impact on bacterial community structure causing changes in the relative abundance of specific bacterial taxa, but not bacterial taxon richness and community composition revealing their resilient nature in the ecosystem. Therefore, various metagenomic studies have focused on identifying the microbial communities and their relationship to the changing soil properties (Azarbad et al. 2015). In a study, Zhang et al. (2017) using 16S rRNA gene sequencing observed a general reorganization of soil microbial communities persistently exposed to metals specifically Cd and Pb in rhizosphere. On the other hand, Azarbad et al. (2015) assessed functional and potential microbial diversity (using Geo Chip 4.2) along two different gradients of metal polluted sites in Southern Poland. It was found that metal pollution caused negative impact on the relative abundance of specific bacterial genera including *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes, Firmicutes,* and *Proteobacteria*. Also, there were significant correlations between a group of metal-resistance genes and among the bacterial genera with metal concentrations in soil. Due to prolonged exposure to high metal concentrations, majority of the microflora that were found sensitive and unable to tolerate high doses of metals became extinct, while certain community members with different functional roles such as denitrification and metal resistance adapted and survived to form the basis for the emergence of other new community. Another important marker that indicates normal soil functionality is the soil microbial biomass that shows changes in soil properties due to complex environmental modifications (Hornick and Buschmann 2018; Rai et al. 2018). Yuan et al. (2015) reported a negative correlation between microbial viability and extended exposure to Pb. Similarly, Yao et al. (2017) studied the biological attributes of heavy metal-contaminated soils and established relationships between environmental variables and community composition. The microbes that were already tolerant became more competitive and thus were more in number. In a similar study, reduction in bacterial community, fungi, and actinobacteria in a heavy metal poisoned site located in one of the municipality of Brazil under the influence of a Votorantim Metal Company was reported by Dos Santos et al. 2016. Apart from composition and density, HMs have also been found to negatively affect the soil enzymes activity like cellulase, alkaline phosphatase, invertase, arylsulfatase, dehydrogenase, β-glucosidase, etc. (Burges et al. 2015).

13.1.3.3 Heavy Metal Toxicity to Physiological Processes of Microbes: A Brief Account

In general, high concentrations of heavy metals or metals above certain threshold level cause discrete and apparent injuries to microbial cells due to oxidative stress, protein dysfunction, or membrane damage (Olaniran et al. 2013). Essentially, metal ions have variable targets within microbial cells and hence affect various microbial activities (Table 13.1).

13.1.3.4 HMs-Induced Phytotoxicity and Physicochemical Changes in Plants

Although plants possess several strategies to offset metal toxicity but beyond certain limits such mechanisms often become ineffective and hence the survival of plant is compromised (Clemens and Ma 2016) (Table 13.2). The toxicity of heavy metals to plants varies with plant genotypes, metals species and its concentration, and soil characteristics (Topcuoğlu 2016).

13.2 Plant Defence Mechanisms Against Heavy Metal

13.2.1 Antioxidant Defence System

Plants have developed a number of strategies to overcome the adverse impacts imposed by heavy metals. Heavy metal toxicity also leads to the over production of ROS, and in turn causes peroxidation of many vital cell constituents. In this way, plants have an efficient defence system comprising of a set of enzymatic and non-enzymatic antioxidants.

Table 13.1 Effect of heavy metals on microbial growth and activities

Metal	Effects on microorganisms	Reference
Cadmium	Damage nucleic acid, denature protein, inhibit cell division and transcription, inhibit C and N mineralization, oxidative damage	Thomas and Benov (2018)
Chromium	Elongation of lag phase, inhibit growth and oxygen uptake, damage DNA	Thorgersen et al. (2017), Fathima and Rao (2018)
Copper	Disrupt cellular function, inhibit growth and enzyme activities, oxidative stress	Warnes and Keevil (2011), Saphier et al. (2018), Águila-Clares et al. (2018)
Mercury	Decrease population size, denature protein, disrupt cell membrane, inhibit enzyme function	Mahbub et al. (2017), LaVoie and Summers (2018)
Lead	Growth inhibition, denature nucleic acid and protein, inhibit enzymes activities and transcription, membrane damage	Adam et al. (2014)
Nickel	Reduce lipid content, disrupt cell membrane, inhibit enzyme activities, oxidative stress	Gupta and Karthikeyan (2016)
Silver	Cell lysis, inhibit cell transduction and growth	Westersund (2018), Choi et al. (2018)
Zinc	Decrease biomass, inhibit growth, DNA damage, membrane destruction	Ishida (2018)

Table 13.2 Heavy metals affecting plant health

Metal	Effects on plants	Reference
Chromium	Chlorosis, delayed, senescence, wilting, biochemical lesions, reduced biosynthesis germination, stunted growth, oxidative stress	Kabir (2016), Anjum et al. (2017)
Copper	Chlorosis, oxidative stress, retarded growth	Li et al. (2018)
Mercury	Affect photosynthesis and antioxidative system, enhance lipid peroxidation, induced genotoxic effect, inhibit plant growth, nutrient uptake and homeostasis, oxidative stress	Mishra et al. (2016)
Lead	Reduce growth, affect photosynthesis chlorosis, inhibit enzyme activities and seed germination, oxidative stress	Venkatachalam et al. (2017), Silva et al. (2017); Ahmad et al. (2018)
Nickel	Inhibit growth, decrease chlorophyll content, inhibit enzyme activities and growth, reduced nutrient uptake	Mir et al. (2018)
Cadmium	Affect growth, decrease chlorophyll content, inhibit growth, oxidative stress	Andresen and Küpper (2013), Shanying et al. (2017)
Zinc	Affect photosynthesis, inhibit growth rate, reduce chlorophyll content, germination rate and plant biomass	Dotaniya et al. (2018)

A wide variety of antioxidants that include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione-s-transferase (GST) may efficiently convert superoxide radicals into H_2O_2 and subsequently H_2O and O_2, whereas low molecular weight non-enzymatic antioxidants like proline, ascorbic acid, and glutathione may directly detoxify ROS. These two groups of antioxidants may professionally quench a wide range of toxic oxygen derivatives and prevent the cells from oxidative stress. Depending upon their localization at different compartments of the cell, their quenching mechanism also differs and acts in an organized manner. For example, SODs are a group of metalloenzymes that convert superoxide radical (SOR, $O_2^{\bullet-}$) into hydrogen peroxide, whereas CAT, guaiacol peroxidase (GPX), and a variety of general PODs are involved in breakdown of H_2O_2 (Gautam et al. 2018).

13.2.2 Cellular Homeostasis

Proline has been considered as one of the most important osmoticum found in the cellular system exposed to metal, water and saline stress, etc. Under different stresses including HM stress, proline accumulates in cytosol and helps in several ways: (1) maintain intracellular redox homeostasis potential, (2) protects enzymes, (3) sustains 3-D structure of proteins and vital organelles including cell membrane, and (4) reduces the risk of peroxidation of lipids and proteins (Aslam et al. 2017). In addition, proline prevents the disruption of membranes by forming clusters with H_2O molecules and stabilizes their structures (Slama et al. 2015). Under stress, proline provides variable benefits to plants such as (1) it chelates heavy metals in the cytoplasm (Sharma and Dietz 2006), (2) regulates the water potential which is often impaired by heavy metals (Kholodova et al. 2011), (3) maintains osmotic adjustment through cellular homeostasis and reduces metal uptake (Szabados and Savoure 2010). For instance, Hayat et al. (2013) showed that the exogenous application of proline alleviated the damaging effects of Cd in plants such as down regulation of water potential and thereby enhanced the growth and photosynthesis.

13.2.3 Role of Genes in Metal Uptake and Their Transportation

A number of genes are expressed differentially after the exposure to heavy metal stress which activates specific enzymes to overcome the negative impact of stress. For instance, genetically modified tobacco callus showed more resistance to methyl mercury (CH_3Hg^+) than the wild-type. MerB enzyme, a product of merB gene was found to dissociate CH_3Hg^+ to less toxic Hg^{2+} which accumulates as Hg-polyP complex in tobacco cells (Nagata et al. 2010). Mostly, the detoxification/sequestration process occurs in plant vacuoles and involves several transporters, namely ABC, CDF, HMA, and NRAMP (Singh et al. 2011). Moreover in another study, over expression of AtPCS1 and CePCS genes enhanced the remediation efficiency of tobacco plants under As and Cd stress by causing increase in phytochelatins level (Wojas et al. 2008). Besides, introduction of a gene encoding moth bean D1-pyrroline-5-carboxylate synthetase (P5CS) that initiates proline synthesis in green microalga *Chlamydomonas reinhardti* assists to increase HM tolerance. Accumulation of more than 80% higher proline level than the wild-type cells caused a genetically modified microalga grow more rapidly under higher Cd concentrations (Hassinen et al. 2009). The mechanism of heavy metal detoxification in hyperaccumulators that protects themselves by the overexpression of genes of reduced glutathione (GSH), cysteine, and o-acetylserine resulting in increase in the antioxidant activities (Anjum et al. 2014). Some genes are exclusively expressed in hyper accumulator phenotypes such as HMA4 gene under heavy metal stress. Similar to this, other studies have revealed that upstream regulation of salicylic acid, *NgSAT,* etc. results in increased serine acetyltransferase activity and higher GSH level and resulted in tolerance to Ni, Co, Zn, and to a small extent Cd (Freeman et al. 2004, 2005; Freeman and Salt 2007).

13.3 Metal Detoxification Approaches

13.3.1 Physico-Chemical Remediation of Heavy Metals

Taking into the account of the issues of metal toxicity, many techniques have been employed for the removal and/or recovery of heavy metals from polluted environments. Some established conventional procedures for heavy metal removal and/or recovery from solution include adsorption processes, chemical precipitation, electrochemical techniques, chemical oxidation or reduction reactions, ion exchange, evaporative recovery, reverse osmosis, and sludge filtration (Chen and Li 2010). However, these techniques have certain limitations as they are expensive, sometimes impractical, and not specific for metal-binding properties. Furthermore, high reagent requirement, generation of toxic waste, and unpredictable nature of metals are some of the disadvantages associated with these methods. Majority of these methods are ineffective when metal concentration in the solution is less than 100 mg/L (Ahluwalia and Goyal 2007). Separation by physical and chemical techniques is also challenging due to high solubility of heavy metal salts in solution. Thus, there is a need to develop inexpensive and suitable techniques which could be applicable under metal stressed conditions.

13.3.2 Biological Approaches to Combat Heavy Metal Stress

Bioremediation is an environment friendly innovative technique for the removal and recovery of heavy metal from the polluted areas. It involves living organisms (e.g. algae, bacteria, fungi, or plants) and/or their associated activities that reduce and/or recover heavy metal pollutants into less hazardous forms. It has been employed for the removal of heavy metals from contaminated wastewaters and soils. This method is considered a viable and appealing alternative to physical and chemical techniques since it involves the use of inexpensive microorganisms and provides long-term environmental benefits (Emenike et al. 2018). These organisms help to detoxify hazardous components in the environment. The bioremediation process can function naturally or can be improved through the addition of electron acceptors, nutrients, or other factors.

13.3.2.1 Microbial Resistance Strategies Towards Heavy Metals

The rhizospheric microbes play important role in HM detoxification in contaminated soils. According to Pires et al. (2017) the predominant bacterial populations in HM contaminated sites belong to Firmicutes, Proteobacteria, and Actinobacteria and the most common genera are *Bacillus*, *Pseudomonas*, and *Arthrobacter*. In order to survive under metal stress conditions, bacteria have developed certain strategies to regulate the intracellular levels of HMs. Microbial resistance to heavy metals occurs via acquisition of specific resistance systems such as efflux and uptake and extracellular precipitation. In a study, Karthik et al. (2017) isolated rhizobacterial strain AR6 from the rhizosphere of *Phaseolus vulgaris* which showed high Cr (VI) tolerance and multifarious plant growth promoting traits. Also, it was observed that the detoxification of toxic Cr (VI) occurred directly by enzymatic reduction to less toxic Cr (III) by chromate reductase or indirectly by making complexes with metabolites (Karthik et al. 2017). Different chromate reductases (e.g. ChrR, YieF, NemA, and LpDH) have been identified that are located either in cytoplasm or membrane bound in a bacterial cell (Huang et al. 2016). Similarly, other PGPR with improved metal remediation efficiency have been found to facilitate the growth of plants under adverse toxic conditions (Gopalakrishnan et al. 2018). Apart from traditional PGPR, both symbiotic and free living rhizobia have also been found capable of detoxifying HM and consequently upgrading the quality of contaminated soils (Checcucci et al. 2017; Rangel et al. 2017). Summarily, the metal removal mechanism can be grouped into five categories (1) extracellular precipitation, (2) intracellular accumulation, (3) oxidation and reduction reactions, (4) methylation and demethylation, and (5) extracellular binding and complexation (Ojuederie and Babalola 2017) as presented in Table 13.3.

13.3.2.2 Metal Extrusion Strategies

Mostly the resistance mechanisms in bacteria known till date are encoded on plasmids and transposons and consequently have high probability of gene transfer or spontaneous mutation that help bacteria to acquire resistance against heavy metals. For example, in gram-negative bacteria (e.g. *Ralstonia eutropha*), a czc system is found responsible for the resistance to Cd, Zn, and Co. The czc-genes

Table 13.3 Strategies adopted by metal-tolerant bacteria to overcome metal stress

Mechanism	Organism	Description and effectiveness	References
Bioaccumulation and biosorption	*Sinorhizobium* sp.	Improved growth and nodulation in *M. sativa* under cd, cu, Pb, and Zn stress	Zribi et al. (2012)
Bioaccumulation	*Delftia* sp. B9	Intracellular dissolution of cd Reduce cd accumulation in rice grain	Liu et al. (2018)
Biotransformation and bioaccumulation	*Micrococcus* KUMAs 15	Arsenite oxidation and accumulation	Paul et al. (2018)
Biosorption	*Bacillus* sp. MC3B-22 and *Microbacterium* MC3B-10	EPS mediated sorption of Cd^{2+}	Camacho-Chab et al. (2018)
Bioreduction	*Bacillus subtilis* MA13	Reduction of Cr(VI) via Cr reductases enhanced growth and photosynthetic pigments of soybean	Wani et al. (2018)

encode the cation-proton antiporter (CzcABC) that exports Cd, Zn, and Co. Similarly, ncc system found in *Alcaligenes xylosoxidans* displayed resistance to Ni, Cd, and Co. On the contrary, Cd resistance mechanism in *Staphylococcus*, *Bacillus,* or *Listeria* operates through Cd-efflux ATPase. Two most well studied Cu resistance systems (cop) are observed in *P.syringae* pv. tomato and pco in *E.coli*. The cop genes encode for different Cu-binding proteins, which sequester Cu in the periplasm or in the outer membrane. However, the pco system acts through an ion-dependent Cu antiporter (Kunito et al. 1997). Naturally occurring PGPR also show resistance to zinc which is mostly through efflux system, for example, a P-type ATPase efflux system transports Zn ions across the cytoplasmic membrane via ATP hydrolysis (Beard et al. 1997), while RND-driven transporter system moves Zn across the cell wall of gram-negative bacteria through a proton gradient (Nies 1999). Likewise, Ni resistance is inducible and depends on energy-dependent efflux system driven by chemiosmotic proton-antiporter system (Taghavi et al. 2001).

13.3.2.3 Biotransformation

Numerous microorganisms have ability to reduce/transform a wide variety of multivalent metals that pose major threat to the environment. Though, various PGPR strains possessing metal reducing ability have been identified (Mallick et al. 2018), reduction of chromium only by PGPR will be discussed in the following section.

Among different forms of chromium, the hexavalent chromium is the more toxic and carcinogenic owing to its high solubility in water, rapid permeability through biological membranes, and subsequent interaction with intracellular proteins and nucleic acids (Kamaludeen et al. 2003). Among various forms of Cr, Cr (III) does not migrate freely in natural systems because it tends to precipitate as Cr (III) minerals or is removed by adsorption. Hence, reduction of toxic Cr (VI) to Cr (III) is a useful approach to remediate Cr (VI) affected environments (Thatoi and Pradhan 2017). In this regard, numerous chromium resistant PGPR like *Pseudomonas* sp., *Bacillus, Stenotrophomonas*, *Serratia*, *Arthrobacter,* and rhizobia have been identified and applied in Cr (VI) contaminated soils (Baldiris et al. 2018; Dong et al. 2018). Detoxification of chromium by microbes may occur directly or indirectly and can be affected by pH, incubation period, chromate concentration, and types of microbes (aerobic/anaerobic) involved (Narayani and Shetty 2013). In the direct mode, the microbes absorb chromium and then enzymatically (chromium reductases) reduce it (Mala et al. 2015). While in the indirect mode, metabolic products (reductants or oxidants) of the microbes in soil, such as H_2S, chemically reduce chromium by redox reactions. Jin et al. (2017) observed that the removal of Cr (VI) by *Acinetobacter* strain WB-1 was due to surface immobilization along with intracellular and extracellular reduction. Enzymatic reduction of Cr (VI) to Cr (III) usually is accomplished by chromium reductases and occurs both anaerobically (Masaki et al. 2015) as well aerobically (He et al. 2015) and sometimes involves chemical reactions associated with compounds such as nucleotides, sugars, amino acids, vitamins, organic acids or glutathione.

13.3.2.4 Bioaccumulation

Bioaccumulation strategy involves the uptake of metal ions by an organism either directly following exposure to a contaminated medium or indirectly by consumption of contaminants. Intracellular accumulation of toxic metals is an energy-dependent transport system which depends on (1) intrinsic properties, (2) physiological and genetic adaptation, (3) metal speciation, availability, and toxicity (Sinha et al. 2013). Once taken up, toxic metals pass through biological membranes via carrier mediated transport, endocytosis, ion pumps, ion channels, complex permeation, and lipid permeation (Adriano 2017). Permeabilization of cell membranes to toxic elements can lead to further exposure of intracellular metal-binding sites resulting in enhanced passive accumulation. Several methods have been used for detecting the accumulation and localization of metals inside the bacterial cells. For example, using TEM, Podder and Majumder (2018) found that the growth and bioaccumulation of arsenic ions by *Corynebacterium glutamicum* MTCC 2745 varied with pH, inoculum size, contact time, temperature, and concentrations of peptone and As. In another study, AAS analysis of the culture products from *B. amyloliquefaciens* treated with Cr (VI) for 45 h showed the distribution of Cr(III) in pellet and culture supernatant in the range of 37.4 ± 1.7 and 62.6 ± 3.4 mg L^{-1}, respectively (Das et al. 2014). In SEM images, the Cr (VI) treated bacterial pellets looked rough, coagulated, and porous, whereas the untreated pellets appeared smooth, regular, and non-porous. Also, TEM–EDX study of the bacterial precipitates under Cr (VI) treatment had nanometric range of intracellular Cr (III). Bioaccumulation process has several advantages like it is a metabolically active process of living organisms that works through adsorption, intracellular accumulation, and bioprecipitation. However, bioaccumulation also has limitation because it is applied on live cells only. The living cells however have the potential of recombination or mutant formation which can change morphological and physiological features of the strain. Besides, high concentration of applied or already present metals significantly damage the surface of living cells and may lead to partial loss in cell-binding abilities and consequently release of accumulated metals back into solution (Kadukova and Vircikova 2005).

13.3.2.5 Biosorption

Biosorption of heavy metals by certain living or dead microbial biomass is a very effective solution to remediate even very dilute aqueous solutions (Dadrasnia et al. 2015). Biosorption consists of several mechanisms, such as ion exchange, adsorption, chelation, and diffusion through cell walls and membranes, which differs and depends on the species used, the origin and processing of biomass, and the chemistry of solution. Biosorption in fact is a non-enzymatic process wherein pollutants are adsorbed onto the cell surface (Sulaymon et al. 2012). The uptake of metal could be active or passive (Vijayaraghavan and Yun 2008) and both may occur independently or simultaneously. Of these, passive process is relatively nonspecific (Volesky 2007) and does not involve cellular metabolism. Here, metal binds to poly ionic cell walls through ion exchange. This process is not affected by physical conditions such as pH and ionic strength. It is a reversible and fairly rapid process requiring only 5–10 min. For complete biosorption of heavy metals. The active process is slow and depends on cellular metabolism and therefore is influenced by uncouplers, metabolic inhibitors, and temperature. In the active process, the metal complexes with specific proteins like metallothioneins which is found in vacuole. For example, biosorption capacity of live and dead cells of a novel *Bacillus* strain for chromium showed that both live and dead biomass followed the monolayer biosorption on the active surface sites. Scanning electron microscopy and FTIR indicated significant influence on the morphological features of the dead cells during biosorption of chromium. Approximately 92% and 70% desorption efficiencies were achieved using dead and live cells, respectively (Dadrasnia et al. 2015). However, whatever may be the mode of metal uptake, the adsorption occurs due to the nonspecific binding of ionic species to cell surface associated or extracellular polysaccharides and proteins (Cristani et al. 2012) of different bacterial cell organization, bacterial cell walls, and envelopes (Puyen et al. 2012). Among bacteria, the cell walls of gram-positive bacteria in general bind larger quantities of toxic metals than the envelopes of the gram-negative bacteria (Silver and Phung 1996). FTIR studies have revealed that various functional moieties such as hydroxyl, amino, carboxylate, phosphoryl, etc. were present on the surface of bacterial cell which participated in metal binding and hence assisted biosorption process (Patil and Unnikrishnan 2017).

13.4 Role of Bioactive Molecules Secreted by PGPR in HM Removal

Many PGPR colonizing plants have been found to play significant roles in mobilization or immobilization of heavy metals and consequently reducing the availability and/or toxicity of metals to plants (Table 13.4). However, this metal accumulating ability and plant colonizing potential of rhizospheric bacteria together could be of practical importance in alleviating metal toxicity when bioinoculated plants are grown in metal-contaminated soils (Kidd et al. 2017). Fatnassi et al. (2015) reported that co-inoculation of plants, treated with 1 mM Cu and 2 mM Cu (1) increased the dry weight as compared with Cu-treated and uninoculated plants, (2) decreased Cu uptake in the roots, (3) increased copper tolerance status of *Vicia faba* compared to uninoculated plants exposed to Cu stress.

In a similar study, Mohamed and Almaroai (2017) found that the phosphate solubilizer *Bacillus* sp., *Azotobacter* sp., and *Pseudomonas* sp. produced a substantial amount of IAA both in the absence and presence of heavy metals. Besides these merits, they significantly decreased the uptake of heavy metals in corn plants grown under metal stress. Similarly, Subrahmanyam et al. (2018) reported that green gram, inoculated by *Enterobacter* sp. C1D had significantly better length and dry biomass of shoot and root and chlorophyll content when grown in the presence of Cr (VI). Confocal laser scanning microscopy (CLSM) of the roots showed heavy bacterial loads on root surface specifically at the root tip and the point of root hair/lateral root formation. Moreover, the elevated IAA levels and ACC deaminase activity enabled *Enterobacter* sp. C1D to enhance green gram production in Cr (VI)-amended soils.

13.5 Role of EPS as Biosorbents in Heavy Metal Removal

Extracellular polysaccharides, the complex biomolecules are consisted of proteins, polysaccharides, uronic acid, humic-like substances, nucleic acid, lipids, and glycoproteins and surround the bacterial cells (Sheng et al. 2010). Metal sorption by EPS is believed to be an important self-protection strategy of microbial cells against toxic substances (Bhunia et al. 2018). The ionic nature of metal, its size, and charge density regulate its interaction with negatively charged EPS (Gupta and Diwan 2017). Since EPS are usually the first barrier of microbial cells that directly contact and interact with metals, they are of vital importance not because they protect the interior microbial cells but also play important role in remediation of metal-contaminated environments (Ayangbenro and Babalola 2018). Recently, numerous PGPR found to secrete EPS are involved in metal removal, for example, EPS of *B. subtilis* and *P. putida* strains significantly enhanced their Cu (II) adsorption capacity (Fang et al. 2013). The complex and diverse EPS properties make it usually difficult to understand the adsorption behavior of microbial biomass. In recent times, FTIR has frequently been used to obtain structural information on metal-binding

Table 13.4 Role of metal-tolerant PGPR secreting bioactive molecules in phytoremediation process

Bacteria	Bioactive molecules	Role of PGPR	References
Pseudomonas and rhizobium sullae	IAA, siderophores, P solubilization	Increased growth of Sulla coronaria under cd stress, enhanced antioxidant response and cd accumulation in roots	Chiboub et al. (2018)
Bacillus	IAA, Ni, ACC deaminase	Increased plant growth and facilitated Ni accumulation	Akhtar et al. (2018)
Mesorhizobium ciceri	N_2 fixation	Enhanced growth of chickpea and Cr concentration in roots favoring phytostabilization	Velez et al. (2017)
Gordonia alkanivorans, Cupriavidus necator, and Sporosarcina luteola	EPS, IAA, NH3, N2 fixation	Increased phytoextraction of As and Hg in B. juncea and L. albus	Franchi et al. (2017)
Pseudomonas sp.	Auxin, siderophore, P solubilization, ACC deaminase	Enhanced plant growth and increased nodule biomass Influenced phytostabilization	Soussou et al. (2017)
Bacillus sp.	Zn solubilization	Zn mobilization and accumulation, yield enhancement in soybean and wheat	Khande et al. (2017)
Enterobacter	Siderophore, IAA	Increased Fe uptake, immobilized Cd^{2+} in rhizosphere, influenced phytostabilization of Cd^{2+}	Chen et al. (2017)
Burkholderia cepacia	IAA, Siderophore	Enhanced growth of Brassica rapa, Zn uptake	Kang et al. (2017)
Bradyrhizobium liaoningensis	–	Increased Ni and Fe uptake in Pongamia pinnata from V Fe magnetite mine failing site	Yu et al. (2017)
Mesorhizobium	IAA, Siderophore, ACC, TCP solubilization	Enhanced multi element tolerance in Leucaena leucocephala	Rangel et al. (2017)
Variovorax paradoxus		Increased biomass of plant and Ni uptake in roots and shoots	Durand et al. (2016)
Brevibacterium casei	NH3, ACC, IAA, HCN	Increased biomass, enhanced cd, Zn, cu accumulation in shoots	Plociniczak et al. (2016)
Bacillus sp.	Zn solubilization, P and K solubilization, biocontrol	Enhanced Zn translocation	Shakeel et al. (2015)
P. aeruginosa	Siderophore	As accumulation in all plant	Jeong et al. (2014)

complex associated with various functional groups of EPS. Hence, the chemical state and the IR absorption spectrum of EPS functional groups change sensitively upon binding with metals. The FTIR spectral band in the region between 4000 and

400 cm^{-1}usually shows the major characteristic bands of various bonds present in functional groups of EPS. For example, the Cu^{2+} complexation by carboxyl functional group of EPS can be clearly reflected by the change in peak intensity of FTIR spectral band at both 1400 cm^{-1} (associated with the stretching vibration of COO^{2-} bond from carboxylic group and deformation vibration of -OH from alcohols and phenol groups) and 1080 cm^{-1} (attributed to the stretching vibration of -OH group) (Mohite et al. 2018). An interesting feature called *rescuing* was highlighted wherein EPS secreting (metal-tolerant) strains displayed a protective effect towards non-EPS secreting sensitive strain in co-culture experiments (Nocelli et al. 2016). Similarly, the non-symbiotic N_2 fixers such as *A. chroococcum* secreted EPS and formed complexes with Pb and Hg ions in pH dependent manner. At lower pH (between 4 and 5), the maximum adsorption was 40–47% of initial metal ion concentration in solution (Rasulov et al. 2013).

13.6 Role of Metallothioneins and Phytochelatins

Metallothioneins (MTs) are cysteine-rich, heavy metal-binding protein molecules synthesized due to mRNA translation (Guo et al. 2013) and play a crucial role in uptake, detoxification, and accumulation of metal. MTs are gene-encoded polypeptides. They show greater affinity for metals such as Cd, Cu, Zn, and As by cellular sequestration, homeostasis of intracellular metal ions as well as modification of metal transport. In addition to detoxification of heavy metals, plant MTs also play an important role in cell growth and proliferation, repair of plasma membrane, repair of damaged DNA, scavenge ROS, and maintenance of redox level (Emamverdian et al. 2015). Naik et al. (2012) explored the role of bacterial MTs in Pb-resistant bacterial isolates: *Salmonella choleraesuis* strain 4A, *Proteus penneri* strain GM10, *Bacillus subtilis* strain GM02, *Pseudomonas aeruginosa* strain 4EA, *Proteus penneri* strain GM03, and *Providencia rettgeri* strain GM04 which were quarantined from soil, polluted with car battery waste from Goa, India. All the isolates except *P. aeruginosa* strain 4EA had plasmids. Both bacterial MTs and intracellular bioaccumulation of Pb in *S.choleraesuis* strain 4A and *P.penneri* strain GM10 were responsible for the Pb resistance. Similarly Li et al. (2015) found that expression of PcPCS1gene from bean pear (*Pyrus calleryana* Dcne.) was induced after Cd and Cu treatments and *E.coli* with over-expressed PcPCS1 had enhanced tolerance to Cd, Cu, Na, and Hg. *E.coli* cells transformed with pPcPCS1 survived in solid M9 medium containing 2 mM Cd^{2+},4 mM Cu^{2+}, 4.5%(w/v)Na^{+}, or200 μMHg^{2+}. Moreover, the growth curve showed that1.5 mM Cd^{2+}, 2.5 mMCu^{2+}, 3.5% (w/v)Na^{+}, and100 μMHg^{2+}had no effect on growth of *E.coli* cells transformed with pPcPCS1. Also, the content of PCs and accumulation of Cd, Cu, Na, and Hg ions were enhanced in the recombinant *E.coli* strain Rosetta™(DE3).

Phytochelatins (PCs) are short-chain thiol-rich repetitions of peptides synthesized from sulfur-rich glutathione by the enzyme phytochelatin synthase which defend plants against environmental stresses such as salinity, drought, herbicide, and heavy metals (Emamverdian et al. 2015). They are used as biomarkers for an early detection of heavy metal stress in plants (Saba et al. 2013). Metallothioneins have much affinity for a wide range of metals such as Cu, Zn, Cd, and As by cellular sequestration, homeostasis of intracellular metal ions as well as adjustment of metal transport. Apart from detoxification of heavy metals, plant MTs also play a role in maintenance of the redox level, repair of plasma membrane, cell proliferation, and its growth, repair of damaged DNA, and scavenge ROS (Emamverdian et al. 2015). Glutathione (GSH), a precursor of phytochelatin, has been reported to play a vital role in metal detoxification (Hasanuzzaman et al. 2017) and in protecting plant cells from other environmental stresses including intrinsic oxidative stress reactions.

13.6.1 Phytoremediation Strategies

Some of the strategies mediated by plants often termed as phytoremediation include:

1. Phytoextraction (phytoaccumulation): Uptake of the contaminant by plant roots from the environment and its translocation into harvestable plant biomass.
2. Rhizofiltration: Use of plant roots to absorb or adsorb contaminants present in solution form in the surrounding of root zone.
3. Phytostabilization: Reduction of mobility and bioavailability of pollutants in environment either by physical or chemical effects.
4. Phytodegradation (phytotransformation): Phytodegradation is the use of plants and microorganisms to uptake, metabolize, and degrade the contaminant. In this approach, plant roots are used in association with microorganisms to detoxify soil contaminated with organic compounds. It is also known as phytotransformation. Some plants are able to decontaminate soil, sludge, sediment, and ground and surface water by producing various enzymes. This approach involves organic compounds, including herbicides, insecticides, chlorinated solvents, and inorganic contaminants (Pivetz 2001).
5. Rhizodegradation: The breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of root zone is called rhizodegradation. This process uses microorganisms to consume and digest organic substances for nutrition and energy. Natural substances released by the plant roots, sugars, alcohols, and acids contain organic carbon that provides food for soil microorganisms and establishes a dense root mass that takes up large quantities of water. This process is used for the removal of organic substance (contaminants) in soil medium (Moreno et al. 2008).
6. Phytovolatilization: Phytovolatilization is the use of green plants to extract volatile contaminants, such as Hg and Se, from the polluted soils and to ascend them into the air from their foliage (Karami and Shamsuddin 2010).

Phytoremediation as a technique is inexpensive since it involves plants which can be grown and monitored easily (Saraswat and Rai 2012). Moreover, the recovery and re-use of valuable products of this method are easy, because it uses natural biological

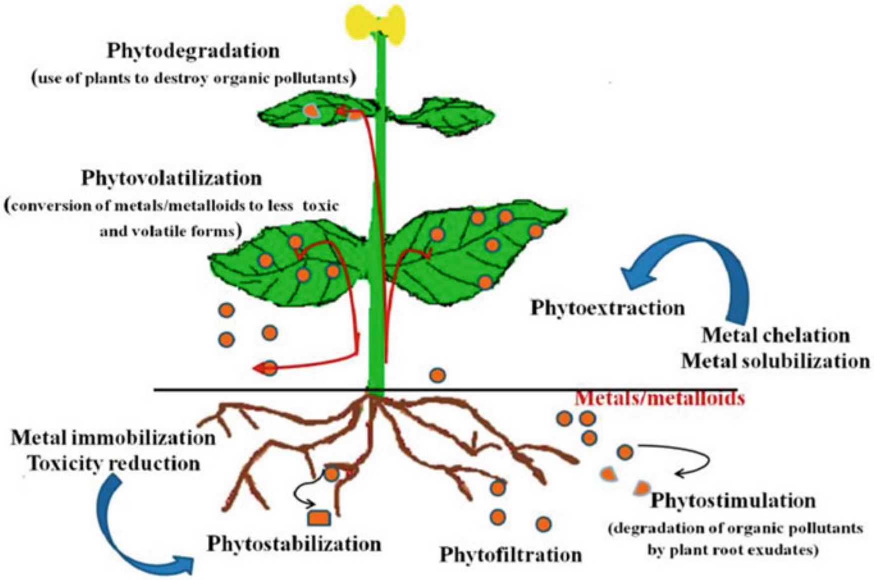

Fig. 13.1 Strategies employed for HM removal using plants. Adapted from Ojuederie and Babalola (2017)

materials. Additionally, plants can be modified for any target characteristics, and the original state of the environment could be restored.

It is relatively a recent technology and perceived as cost-effective, efficient, novel, eco-friendly, and solar-driven technology with good public acceptance. Phytoremediation is an area of active current research (Fig. 13.1). New efficient metal hyperaccumulators are being explored for their application in phytoremediation and phytomining.

Plant species for phytoremediation are selected based on their root depth, the nature of the contaminants and the soil, and regional climate. The root depth directly impacts the depth of soil that can be remediated. It varies greatly among different types of plants, and can also vary significantly for one species depending on local conditions such soil structure, depth of a hard pan, soil fertility, cropping pressure, contaminant concentration, or other conditions. The cleaning depths are approximately <3 feet for grasses, <10 feet for shrubs, and <20 feet for deep rooting trees. The nature of on-site contaminants is a principal factor in the selection of a plant for phytoremediation (Sharma and Reddy 2004). Grasses are the most commonly evaluated plants used for phytoremediation (Shu et al. 2002). They are preferably used for phytoremediation because as compared to trees and shrubs, herbaceous plants, especially grasses, rapid growth, large amount of biomass, strong resistance, effective stabilization to soils, and ability to remediate different types of soils (Elekes 2014). They are pioneers and usually adapted to adverse conditions such as low soil nutrient content, stress environment, and shallow soils (Malik et al. 2010). The large surface area of fibrous roots of grasses and their intensive penetration of soil reduces leaching, runoff, and erosion via stabilization of soil and offers advantages for phytoremediation. Molecular tools are being used to better understand the mechanisms of metal uptake, translocation, sequestration, and tolerance in plants.

13.6.2 PGPR: Assisted Phytoremediation

Degraded soils, following the harmful effects of emissions from non-ferrous metal smelters, are usually arid from organic material and devoid of proper microflora. Therefore, the poor condition of the soil environment makes it impossible to carry out an effective biological remediation of the degraded area. For this purpose, some organic substances, such as sewage sludge, are used to support the process of phytoremediation, because they are a source of biogenic elements and soil microorganisms (Kacprzak et al. 2014). Among the disadvantages, phytoremediation is a lengthy process and is affected greatly by the changing environmental conditions. Thus, the ability of plants to remove/sequester metals in contaminated sites can be improved by applying PGPR with various phytoremediation methods. When single or mixture of inoculants are applied along with phytoremediation process, PGPR affect the mobility and availability of metals to plants by releasing numerous chelating substances, acidification, phosphate solubilization, and redox changes (Zoomi et al. 2017). Chen et al. (2018a) reported that successful in situ phytoremediation depends on beneficial interactions between roots and microbes and plant cultivation. Soil amendment increases microbial diversity and restructures microbial communities. Rhizo-compartmentalization through selection of a specific core root microbiome by the metal-tolerant plant *H. cannabinus* with Enterobacteriaceae, Pseudomonadaceae, and Comamonadaceae included a large number of metal-tolerant and plant growth-promoting bacteria. The root-associated microbial community formed niche-assembled patterns and predominantly had *Proteobacteria*, *Actinobacteria,* and *Chloroflexi* under metal-contaminated conditions.

Złoch et al. (2017) evaluated the role of three metallotolerant siderophore-producing *Streptomyces* sp. B1–B3 strains in the phytoremediation of heavy metals using *S. dasyclados*. The bacterial inoculation significantly stimulated biomass and reduced oxidative stress. Moreover, the bacteria affected the speciation of heavy metals and finally their mobility, thereby enhancing the uptake and bioaccumulation of Zn, Cd, and Pb in the biomass. The best capacity for phytoextraction was noted for strain B1, which had the highest siderophore secretion ability. Five metal resistant PGPR (*Ralstonia eutropha* 1C2, *Chryseobacterium humi* ECP37, *Pseudomonas fluorescens* S3X, *Rhizobium radiobacter* EC1B, and *Pseudomonas reactans* EDP28) were investigated by Moreira et al. (2016) for their in vitro growth promoting traits and for their ability to induce growth of maize seedlings exposed to Zn and Cd. They showed that some bacteria only enhanced PGP traits when exposed to metals. The bacterial strains ECP37 and EDP28 were most efficient in improving seedling growth with increasing metal concentrations, followed by S3X. When inoculated in maize grown in mine soil, these strains also outperformed the others by increasing shoot biomass and elongation, metal accumulation, and by decreasing it in roots. The most evident effect of doubling the inoculum size was the increase in Cd accumulation, which was of 17% and 31% in roots and shoots, respectively. Other effects included a slight reduction in shoots' biomass (13%) and a general decrease in P content.

Sinorhizobium meliloti CCNWSX0020 was used to assess its effect on *Medicago sativa* seedlings under Cu stress (Chen et al. 2018b). This rhizobium inoculation alleviated Cu-induced growth inhibition, regulating antioxidant enzyme activities and increased nitrogen concentration in *M. sativa* seedlings. Moreover, the total amount of Cu uptake in inoculated plants was significantly increased compared with non-inoculated plants, and the increase in the roots was much higher than that in the shoots, thus decreasing the transfer coefficient and promoting Cu phytostabilization.

Bianucci et al. (2018) in a recent investigation observed no changes in growth variables (shoot and root dry weight) of soybean plants inoculated with *Bradyrhizobium* sp. grown in As (V) contaminated soils. Regarding As uptake by plants, metalloid accumulation followed the same distribution pattern among strains. Furthermore, at 6 μM As (V), *Bradyrhizobium* inoculated soybean revealed a significantly lower translocation factor (TF) in comparison to other inoculated strains and promoted As phytostabilization. At the highest As (V) concentration, only *B. diazoefficiens* USDA110 was able to nodulate the legume; however, a significant decrease in the number and dry weight of nodules and N content was observed. Similarly, Mallick et al. (2018) reported that two As-resistant halophilic bacterial strains *Kocuria flava* AB402 and *Bacillus vietnamensis* AB403 from mangrove rhizosphere of Sundarban could tolerate 35 mM and 20 mM of arsenite, respectively. Also, As had a variable impact on EPS synthesis, biofilm formation, and root association ability of both the bacterial strains. When used as inoculum they promoted the growth of rice seedlings by decreasing As uptake and accumulation in plants. Gupta et al. (2018) isolated Cr resistant plant growth promoting *Pseudomonas* sp. (strain CPSB21) from the tannery effluent contaminated agricultural soils and evaluated the plant growth promoting activities, oxidative stress tolerance, and Cr^{6+} bioremediation potential. Assessment of different plant growth promoting traits, such as P solubilization, IAA production, siderophores, ammonia, and HCN production, revealed that the strain CPSB21 could serve as an efficient plant growth promoter under laboratory conditions. Further, the plant growth, pigment content, N and P uptake, and Fe accumulation were reduced when sunflower and tomato were grown in Cr (VI) amended soils. However, inoculation of strain CPSB21 alleviated the Cr^{6+} toxicity and enhanced the plant growth parameters and nutrient uptake. Apart from these, Cr toxicity had varied response on oxidative stress tolerance at graded Cr^{6+}concentration on both plants, and increase in SOD and CAT activity and reduction in MDA were observed following inoculation of strain CPSB21. Additionally, inoculation of CPSB21 enhanced the uptake of Cr^{6+} in sunflower, while no substantial increment was observed in inoculated tomato plants.

In order to select the PGPR for phytoremediation of heavy metal contamination, Roman-Ponce et al. (2017) isolated bacterial strains from the rhizosphere of two endemic plants, *Prosopis laevigata* and *Sphaeralcea angustifolia,* grown in a heavy metal-contaminated zone in Mexico. These rhizobacterial strains were characterized for the growth at different pH and salinity, extracellular enzyme production, solubilization of phosphate, heavy metal resistance, and plant growth-promoting (PGP) traits, including production of siderophores and IAA. Overall, the rhizobacteria showed multiple PGP traits. These rhizobacteria were also resistant to high levels of heavy metals including As as a metalloid (up to 480 mmol L^{-1} As(V), 24 mmol L^{-1} Pb(II), 21 mmol L^{-1} Cu(II), and 4.5 mmol L^{-1} Zn(II)). Inoculation of *Brassica nigra* seeds with *Microbacterium* sp. CE3R2, *Microbacterium* sp. NE1R5, *Curtobacterium* sp. NM1R1, and *Microbacterium* sp. NM3E9 facilitated the root development and significantly improved the seed germination and root growth in the presence of 2.2 mmol L^{-1}Zn (II).

Additionally, siderophores released by PGPR including legume nodulating rhizobia into the rhizosphere serve as an Fe source for plants (Ivanov et al. 2012) and therefore help to fulfil the Fe deficiency of plants in Fe limiting soils. Considering this, it is generally suggested to use PGPR in soils deficient in Fe. To substantiate this, metal resistant PGPR such as *P. putida* strains and *P. fluorescens* strains (showing IAA, siderophores and ACC deaminase production) were reported to show significant improvement in growth attributes of inoculated canola and barley plants even when grown with various concentrations of $CdCl_2$ and $PbNO_3^{2-}$. Furthermore, the translocation factor indicated that inoculated canola and barley had abilities of Cd and Pb phytoextraction in soil contaminated with respective metal. Conclusively, the enhancement in the inoculated canola and barley plants occurred due to the protection against the inhibitory effects of Cd and Pb by PGPR in addition to their ability to provide IAA, siderophore, and ACCD to the developing plants (Yancheshmeh et al. 2011). Nayak et al. (2018) used *B. cereus* (T1B3) strain able to produce various bioactive compounds such as ACC deaminase, indole acetic acid, and siderophores, nitrogen fixation, and P solubilization. Removal capacity (mg L^{-1}) of T1B3 strain was 82% for Cr^{+6} (100), 92% for Fe (100), 67% for Mn (50), 36% for Zn (50), 31% for Cd (30), 25% for Cu (30), and 43% for Ni (50) during the active growth cycle in HM-amended soil.

Ma et al. (2015) isolated a PGPB strain SC2b from the rhizosphere of *Sedum plumbizincicola* grown in Pb/zinc (Zn) mine soils and characterized as *Bacillus* sp. using partial 16S ribosomal DNA sequencing analysis. Strain SC2b exhibited high levels of resistance to Cd (300 mg/L), Zn (730 mg/L), and Pb (1400 mg/L). Besides possessing various PGP features such as secretion of ACC deaminase, utilization of 1-aminocyclopropane-1-carboxylate, solubilization of P, and production of IAA and siderophore, the strain mobilized high concentration of heavy metals from the soils and exhibited different biosorption capacity toward the tested metal ions. Under pot trial, this metal resistant PGPB SC2b elevated the shoot and root biomass and leaf chlorophyll content of *S. Plumbizincicola* besides enhanced Cd and

Zn uptake through metal mobilization or plant-microbial mediated changes in chemical or biological soil properties.

A metal resistant bacterium *Enterobacter ludwigii* isolated from the rhizosphere of Kair "*Capparis decidua*" by Singh et al. (2018) was screened for its phytoextraction ability under gradient metal stress conditions. Among the PGP traits, isolate showed the production of ACC deaminase, produced IAA, and solubilized the inorganic P. The isolate was resistant to Zn, Ni, Cu, and Cd. Furthermore, inoculation of the test isolate significantly increased various growth parameters of wheat plants and also improved the photosynthetic pigments. In addition, inoculation resulted in significant increase in the Zn content in wheat plants under metal stress. Bacterial application significantly increased various compatible solutes such as proline content, total soluble sugar and decreased the malondialdehyde (MDA) content as compared to control, illustrating its protective effect under metal induced oxidative stress in wheat plants.

13.7 Conclusion

The toxic effect of heavy metals may result from the accumulation of HMs over time in vital parts of humans, plants as well as microbes. The direct and indirect mode of deleterious effect of HMs in plants results due to several reasons such as reduction in dry biomass, alteration in chlorophyll content and at molecular level due to over generation of ROS, damage to essential macromolecules, and thus constrain crop productivity. Undoubtedly the PGPR-assisted HM phytoremediation used in native or engineered forms is reported to have greater remediation potential but their impact on ecosystems needs to be elucidated before commercialization. Various steps of regulatory networks via plant-associated microbes including the synergistic action of plants and microbes and their mechanism for metal mobilization, transformation, and detoxification should be investigated for unravelling the dynamics of plant–microbe–metal interactions in the soils. Further monitoring and managing microbial heavy metal remediation require the characterization of the fate and behavior of the compounds of interest in the environment.

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