Chapter 18
Heavy Metals Pollution and Role of Soil PGPR: A Mitigation Approach

Smita Patil, Abullais Ansari, Ashwini Sarje, and Ashok Bankar

Abstract Heavy metal pollution is a serious threat to human health and the environment. It is severely augmented by several industrial activities. The main causes of metal pollution include several industrial processes such as metal forging, smelting, mining, fossil fuel burning, and the use of sewage sludge on agricultural sites. Toxic heavy metals discharged from these sources adversely affect the population of soil microorganisms and the physicochemical properties of the soil, reducing soil fertility and crop productivity. These heavy metals are not biodegradable and remain in the environment. Several conventional methods are used for removal or detoxification of heavy metals that have several drawbacks such as high cost, difficult to operate and toxic in nature. Therefore, bioremediation techniques have emerged as an alternative technique for remediation of heavy metals that have polluted soils. In metal-contaminated soil, the natural role of metal-tolerant plant growth-promoting rhizobacteria (PGPR) in maintaining soil fertility is fading with increasing use of pesticides. In addition to its role in detoxifying or removing toxic metals, rhizobacteria also promote plant growth via other mechanisms such as the production of growth promoting substances and siderophores. Phytoremediation is another new, low-cost in situ technology used to remove toxic pollutants from contaminated soil. The efficiency of phytoremediation can be enhanced by heavy-metal tolerant PGPR. In this book chapter, the significance of the PGPR for direct application to metal contaminated soil under a wide range of agro-ecological conditions has been discussed. The chapter also gives insight on re-establishment of metal contaminated soils and consequently, promotes crop productivity and their significance in phytoremediation. Thus, in the future bioremediation can be an effective technology for treatment of metal polluted environments.

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Introduction

Some heavy metals are essential for living organisms at low concentrations but can be harmful at high concentrations [1, 2]. Toxic heavy metals are those which are not essential to life and are often toxic at lower concentrations [3]. Heavy metals have several physicochemical properties such as ubiquity, toxicity, accumulation, non-biodegradability and persistence. Due to rapid urbanisation and several industrial activities a variety of toxic heavy metals are discharged into the soil environment [4, 5]. Heavy metals are constantly released into the environment through several human activities like mining, smelting, long-term use of mineral fertilizers, sewage sludge, pesticides, fuel and energy use, and wastewater [6, 7]. Most importantly, Cr, As, Cd, Ni, Cu, Pb, Co and Zn are commonly found in soil environment [8]. Heavy metal pollution has received special attention worldwide due to their negative impact on public health and the environment [6]. Heavy metals are accumulated in the human body through the food chain [2, 5, 9]. They have detrimental effects on various human body organs such as the digestive tract, kidneys, nervous system, skin, vascular muscles, and immune system. They can even cause congenital deficiencies and cancer [10]. The combined effects of several metals on humans can lead to complex stress regimes. Serious complications such as abdominal colitis, bloody diarrhoea, and renal failure due to high doses of heavy metals have been observed, but low dose exposure may be diagnosed as fatigue, anxiety, and neuropsychiatric disorders [11, 12]. Heavy metal soil pollution can reduce soil quality, soil fertility, microbial biodiversity, and plant productivity [13]. Accumulation of heavy metals in soil is a concern for the agricultural production sector, as increased uptake by plants can compromise food quality and quantity [14]. Management of heavy metal pollution is an important issue, as agricultural exports are sold internationally on the basis of environmental safety and sustainability [15].

Several methods have been used to remediate heavy metal-polluted soil and restore soil properties [6]. The suitable remediation techniques are selected based on the site characteristics, the nature of contaminants, the level of contamination, and the final use of the polluted soil. In general, physicochemical methods are widely used to remove heavy metals from polluted soil [6]. Traditional methods of heavy metal soil clean-up include extraction and immobilization of heavy metals, leading to excavation of land [16]. The conventional physicochemical techniques used to remove heavy metals are simple, quick, and effective. However, these techniques are costly, consume large amounts of energy, produce toxic by-products, and are not eco-friendly [17, 18]. In addition, these methods affect the physicochemical properties of the soil, affect the microbial biodiversity and can make the soil unsuitable for agriculture.

Therefore, to effectively manage heavy metal soil pollution, scientists have developed alternative biological approaches by using microorganisms [6, 17]. These microorganisms have some morphological, physiological, metabolic, and molecular characteristics to combat heavy metal toxicity. These properties can be used to remove heavy metals from polluted soil [17, 18]. Microbial remediation involves several microorganisms such as bacteria, microalgae, yeast and fungi to remove, transform, and detoxify heavy metals that remain in the environment [19–21]. Endogenous and exogenous microorganisms have several mechanisms to combat heavy metal toxicity. Microbial mechanisms such as extracellular or intracellular sequestration, metal chelating agent production, precipitation, enzymatic detoxification, and volatilization play important roles in bioremediation of heavy metal-polluted soils [20–24]. These biological approaches are chosen over physicochemical methods because they are simple, easy to implement, widely applicable, reliable, inexpensive, non-destructive, and eco-friendly [25]. Biological-based approaches are dependent on the type of microorganisms, the ability to resist metals, the degree of pollution, and the physicochemical properties of the soil. However, these limitations can be overcome by developing new microbial species that express specific genes of interest [6, 17, 26].

Significance of Heavy Metal Tolerance Mechanisms in PGPR

PGPR are soil bacteria that grow in the rhizosphere of plants and promote plant growth through several mechanisms. Plant roots interact with a number of different microorganisms, which affect the plant growth as well as soil conditions. Rhizosphere bacterial colonization is known to be beneficial to bacteria, but their presence may also be useful to plants. PGPR are found beneficial for several agricultural systems to enhance crop yield and quality [27, 28]. Heavy metal stress has been reduced by PGPR because they have various mechanisms to tolerate and allow the uptake of heavy metal ions inside cells. Such mechanisms include (1) metal transport through the plasma membrane (2) intracellular metal ion accumulation and sequestration (3) heavy metal precipitation (4) detoxification of heavy metals and (5) adsorption or desorption of metals as shown in Fig. 18.1 and metal tolerating PGPRs are listed in Table 18.1 [29–31].

The minimum inhibitory concentrations (MIC) of Cu, Cr, Ni, and Cd were 186.9 ± 29.60, 88.0 ± 12.36, 153.81 ± 34.38, and 130.54 ± 28.21 μg/mL for *P. aeruginosa*, respectively [32]. It was reported that 32 bacterial isolates were obtained from metal-contaminated soil samples. Among these bacterial isolates, *C. oceanosedimentum* showed high resistance to cadmium (18 mM) [34]. Similarly, *Stenotrophomonas rhizophila* was highly resistant to Cr (VI). This bacterial isolate completely reduced 50 mg/L Cr (VI) within 48 h [33]. It was found that 27 rhizobacterial isolates were tested against Cr (VI). NT 15, NT19, NT20, and NT27 isolates were found to exhibit high Cr (VI) resistance in the presence of Cr (VI) at concentrations of 100–200 mg/L without loss of PGPR trait [36]. Six strains of rhizobacteria were isolated from heavy metal-contaminated soil in abandoned mines. These strains used were multi-tolerant to heavy metals and had some plant growth-promoting properties [46]. The PGPR have been used as seed inoculants to intentionally metal-treated or modified soils or already contaminated soils. The obtained results have shown a significant reduction in metal toxicity [47]. The PGPR are known to protect plants from metal toxicity, as well as to improve soil fertility and promote plant productivity by providing essential nutrients and growth regulators [48–50].

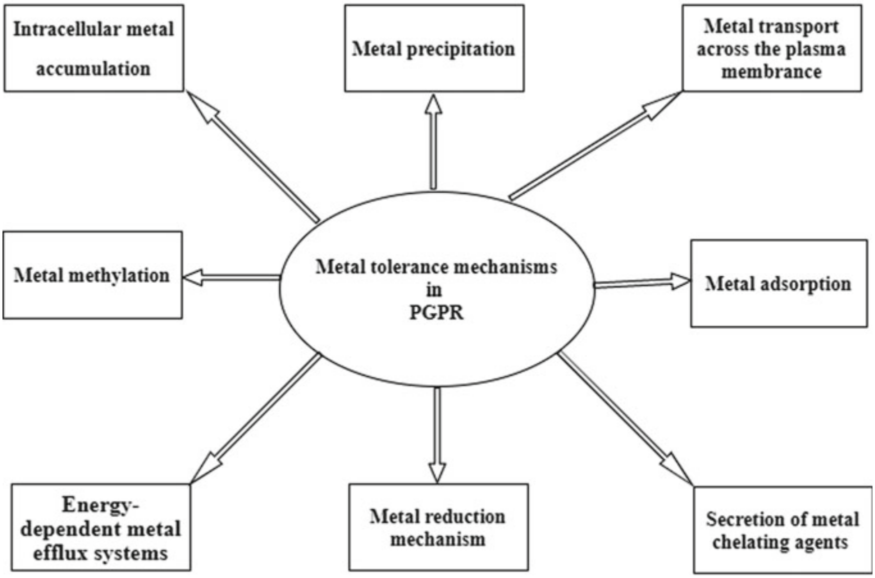

Fig. 18.1 Possible metal tolerance mechanisms in PGPR [29–31]

Table 18.1 List of heavy metal tolerating PGPR

PGPR	Metal tolerated	Reference
P. aeruginosa	Cu, Cr, Ni, and Cd	[32]
Stenotrophomonas rhizophila	Cr (VI)	[33]
C. oceanosedimentum	Cd	[34]
P. aeruginosa and *B. gladioli*	Cd	[35]
Pseudomonas sp	Cr (VI)	[36]
Bacillus spp	Cr	[37]
B. subtilis SJ101	Ni	[38]
B. licheniformis, *M. luteus*, and *P. fluorescens*	As	[39]
Pseudomonas Sp, *Bacillus Sp*, *Cupriavidus Sp*, and *Acinetobacter Sp*	Pb, Cd, and Cu	[40, 41]
P. fluorescens	Cd and Pb	[42]
Rhizobium sp. RP5	Zn and Ni	[43]
Rhizobacterium sp. D14	As	[44]
Sinorhizobium sp. Pb002	Pb	[45]

Heavy metals adhere to extracellular polymeric substances (EPSs) that are naturally secreted by several bacterial cells, such as proteins, nucleic acids, fatty acids, polysaccharides, and humic substances. These EPSs have a very high binding affinity for heavy metals such as lead, cadmium and copper. Bacteria such as *Staphylococcus aureus*, *Micrococcus luteus*, and *Azotobacter* spp. have been reported for production of exopolymer that show high metal binding affinity [51]. Plant growth is promoted by reducing the stress induced by the ethylene-mediated effects on plants by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme [52–54]. Some microbes have the ability to produce low molecular weight siderophores as iron-chelating agents for immobilization of iron. Siderophores also have a binding affinity for other toxic heavy metals. Therefore, siderophores have the ability to minimize the bioavailability of heavy metals and reduce their metal toxicity. Bacterial metabolites are capable of crystallizing or precipitating heavy metals to reduce cellular uptake of heavy metals [55, 56].

The advantages of such microorganisms, with their multiple properties of metal resistance or reduction and the ability to promote plant growth through various mechanisms in metal-contaminated soil, are the most suitable options for bioremediation studies. PGPR can impose various indirect impacts on plants such as plant pathogen inhibition activity by competing for nutrients and space [57, 58]. In addition to the direct and indirect positive effects on biomass production, plant-associated bacteria can also contribute to increased metal availability and uptake, and reduced phytotoxicity of metals [59]. In recent years, PGPR has been shown to be effective in enhancing phytoremediation of petroleum and other pollutants [60, 61]. PGPR interacts with toxic heavy metals in soil, reducing their bioavailability. Energy-dependent metal efflux systems such as ATPases and chemiosmotic ion or proton pumps have been reported for the uptake of Cr and Cd metallothionein by bacterial cells [55]. The mechanism of cytosolic metal sequestration has been previously reported. In this mechanism, metallothionein, a low-molecular weight, bacterial cells to detoxify heavy metals such as Cd, Cu, Hg, and Ag secrete cysteine rich metal binding protein. Methylation of heavy metals by bacterial cells has been reported as an alternative mechanism of bacteria [56, 62]. The metal reduction mechanism has been studied in several bacteria. For example, detoxification of chromium involves the reduction of Cr (VI) to Cr (III) reported previously [63].

PGPR has the ability to produce various metal chelating agents, such as siderophores and organic acids, in the soil environment. They can acidify the microenvironment and induce the changes in redox potential [64, 65]. Due to these inherent mechanisms, the rhizosphere bacterium, which promotes plant growth, is a potential candidate for soil metal remediation. PGPR can also contribute to the reduction of phytotoxicity of metals via biosorption and bioaccumulation mechanisms. Bacterial cells have a very high surface-area-to-volume ratio and may adsorb more heavy metals than inorganic soil components either by a metabolism-independent passive or by a metabolism-dependent active process [66, 67]. Many authors suggest that the bacterial biosorption or bioaccumulation mechanism, along with other plant growth-promoting properties, including ACC deaminase and plant hormone production, is involved in promoting plant growth in metal-contaminated soils [38, 68]. The genes encoding heavy metal resistance of microorganisms need to be identified. Several molecular techniques have been used to identify metal resistance genes in microorganisms [69]. DNA microarray technique has been adopted as a powerful tool for identifying gene regulation under stress heavy metals [70]. The mass spectrometry-based proteomic techniques have been used to investigate the patterns of proteins expression due to intracellular metal accumulation [71]. Whole-genome sequencing method has been shown to help identify genes that play an important role in enhancing metal accumulation process [72]. Similarly, transcriptomics analysis techniques have been used to identify genes responsible for effective metal accumulation processes [73]. In addition, bioinformatics and mathematical modelling have been used to analyse the microbial metal resistance capability [74]. Therefore, advanced techniques have the potential to improve the metal bioaccumulation processes in the future.

Rhizoremediation of Heavy Metal-Polluted Soil

Rhizoremediation is the remediation of polluted soil by rhizobacteria observed in the rhizosphere of plants. The symbiosis of microorganisms and plants in the plant rhizosphere found to be useful as an effective restoration technique. This is a relatively novel approach and may provide a practical remedy [75, 76]. PGPR, which promote plant growth, are soil bacteria that grow in the rhizosphere of plants and promote plant growth through various mechanisms. Plant roots interact with a number of different microorganisms, which affect plant growth as well as soil conditions. Rhizosphere bacterial colonization is known to be beneficial to bacteria, but their presence may also be beneficial to plants [27, 28, 77]. Some PGPR strains have been applied to plants that grow in poor soils that are heavily contaminated with heavy metals. Under these conditions, uninoculated plants and plants inoculated with the LMR250 strain did not grow, while the other five bacterial inoculants restored plant growth. The best performing strain, *Pseudarthrobacter oxydans* LMR291, has been reported as an excellent biofertilizer or biostimulant that promotes plant growth in contaminated soil [46].

In addition, a pot assay was performed to determine if the *Curtobacterium oceanosedimentum* strain could promote Chili growth under cadmium stress. Bacterial colonization significantly increased root and shoot lengths by up to 58% and 60%, respectively, compared to controls. After inoculation with the cadmium-resistant strain, the plants gained both fresh and dry weight. In both the control and inoculated plants, cadmium accumulates more in the roots than in shoots, indicating that Chili stabilizes Cd levels. In addition to improving plant properties, Cd-resistant strains have also been shown to increase the amount of total plant chlorophyll, total phenol, proline, and ascorbic acid. The PGPR inoculants protect the plants from adverse effects of cadmium [34]. Inoculations of *P. aeruginosa* and *B. gladioli* showed improvements in root length, shoot length, and photosynthetic pigments.

Levels of protein-bound and non-protein bound thiols were also increased in Cd-treated seedlings. Therefore, microorganisms have growth promoting properties that allow them to reduce the metal toxicity in plants [35].

The PGPR NT27 isolate was a strain of the genus *Pseudomonas*. In the presence of Cr (VI), the shoot and root dry weights of *M. sativa* was increased by 97.6 and 95.4%, respectively, compared to uninoculated control plants. Chlorophyll content has also increased significantly, and the stress markers, hydrogen peroxide, malondialdehyde, and proline have decreased. Thus, chromium-tolerant *Pseudomonas* sp had a positive effect on shoots and roots of *M. sativa* plants by reducing chromium toxicity [36]. Six Cr-tolerant PGPR strains were isolated and identified as *Bacillus* spp. The consortium of Cr-tolerant strains was used for the inoculation in combination with Biochar. The highest increase in shoot and root length was (22–23.4%) and the highest increase in chlorophyll and SOD was (28–40%). Similarly, proline and sugar levels improved to 20.5% and 9.6%, respectively. A significant reduction in Cr uptake was recorded in the dry biomass of wheat plants, with Cr concentrations of 0.28 ± 1.01 mg/kg compared to controls. Therefore, according to the results, PGPR and biochar are an important tools for protecting plants from chromium toxicity and can be used as inoculum for better crop production [37]. Nearly 180 Cr (VI) resistant PGPRs were isolated, and after screening, 10 efficient bacteria that could function under Cr (VI) stress conditions were selected. Wheat seeds (*Triticum aestivum* L.) were inoculated with selected bacterial isolates and sown in Cr (VI) contaminated (20 mg/kg) pots. The results showed that Cr (VI) contaminated soil significantly suppressed plant growth and development. However, inoculation significantly improved plant growth parameters compared to uninoculated plants. In inoculated pots, soil Cr (VI) levels were reduced by up to 62%. Cr (VI) levels were up to 36% lower in roots and up to 60% lower in shoots than uninoculated plants grown in contaminated pots [78].

The effects of PGPR, which stimulates plant growth under stress, are considered an effective strategy. It has been studied that plant grown in heavy metals polluted areas in the presence of PGPR were able to accumulate significant amounts of heavy metals in some plant parts than plants grown in soils without microbial flora [79]. The IAA-producing strain *B. subtilis* SJ101 promoted the growth of *Brassica juncea* in Ni-contaminated soil [38]. Similarly, Zn, Cu, Ni, and Co tolerant IAA producing strains were found to promote rapid root growth of *B. juncea* in soil contaminated with Cd [53]. Pinter et al. [39] found that siderophore production, phosphate solubilization, and nitrogen fixation activity of As-resistant *B. licheniformis*, *M. luteus*, and *P. fluorescens* increase the biomass of grapevine in the presence of high As concentrations. Environmental adaptability of Cd, Pb, and Cu resistant bacterial strains obtained from rhizospheric soil of *Boehmeria nivea* growing around mine refineries [80]. Scientists revealed rhizosphere bacteria of the genera *Pseudomonas*, *Bacillus*, *Cupriavidus*, and *Acinetobacter* are resistant to Pb, Cd, and Cu. A wide range of plant growth promoting properties of rhizobia including nitrogen fixation, solubilization of insoluble minerals such as phosphate, phytohormones and siderophores production, ACC deaminase synthesis, and volatile compounds such as acetoin and 2, 3-butanediol. Thus, rhizobia are found to be good candidates for detoxification of heavy metals [40, 41].

Of the 58 PGPR isolates, 8 bacterial strains were screened for multiple heavy metal tolerance, salt tolerance, indole-3-acetic acid, phosphate solubilization, and siderophore production, and finally the WW-40 strain was selected as a potent PGPR. Applying this strain under greenhouse conditions, the highest 52% of seed germination, 1078% of vigour index, 68.57% of shoot length, 71% root length, 44.44% of shoot fresh weight, 50% of root fresh weight, 52.38% of shoot biomass, and 66.66% of root biomass increased significantly compared to heavy metal treatment maize seedlings. Chlorophyll content increased by 68.75% in the consortium with Zn compared to the Zn inoculated pot. Similarly, the carotenoid content of Zn consortium pot increased by 57.89% and the xanthophylls content of the Zn consortium pot increased by 65.62% compared to other metal treatment pots. Therefore, the heavy metal resistant isolates that stand out in this study may be PGPR strains for both bioremediation and crop growth promotion [81]. The use of PGPR supports plant growth in contaminated soil, and urea-degrading bacteria can immobilize heavy metals by carbonate precipitation process. Therefore, dual treatment with such bacteria may be useful for plant growth and bioremediation in polluted soil. Pot experiments were carried out to grow radish plants in soil contaminated with Cd and Pb treated with PGPR *P. fluorescens*, and the results were compared with dual inoculation of *P. fluorescens* in combination with ureolytic *S. epidermidis* HJ2. The removal rate of Cd and Pb from the soil was 17% with PGPR alone, and more than 83% was reported with combined treatment [42]. Table 18.2 shows the importance of PGPR in phytoremediation of heavy metal contaminated soil.

Table 18.2 PGPR-assisted phytoremediation of heavy metal contaminated soil

PGPR	Plant/s	Heavy metal/s	Impact on plant	Reference
B. licheniformis, *M. luteus*, and *P. fluorescens*	Grapevine	Pb, Cd, and Cu	Increased the biomass of grapevine	[80]
B. subtilis SJ101	*B. juncea*	Ni	Promoted the growth of plant	[38]
Pseudomonas Sp	*M. sativa*	Cr (VI)	Increased shoot and root length, chlorophyll content enhanced	[36]
Bacillus Sp with biochar	Wheat plant	Cr	Increased shoot and root length, chlorophyll content enhanced	[37]
C. oceanosedimentum	Chili	Cd	Significantly increased root and shoot lengths	[34]

(continued)

Table 18.2 (continued)

PGPR	Plant/s	Heavy metal/s	Impact on plant	Reference
B. licheniformis, *M. luteus*, *P. fluorescens*	*Vitis vinifera*	As	*M. luteus* increased plant biomass, protein content, and POX activity *B. licheniformis* increased plant biomass and APX *P. fluorescens* augmented POX activity	[39]
Bacillus megaterium	*B. campestris* and *B. rapa*	Cd	Inoculation increased biomass, soluble proteins, and vitamin C content	[82]
B. safensis and *P. fluorescens*	*Helianthus annuus*	Zn and Pb	Inoculation reduced Zn and Pb uptake by plant tissues	[83]
Klebsiella oxytoca	*H. annuus*	Co, Pb, and Zn	Inoculation enhanced plant growth	[84]
Klebsiella sp.	*Vigna radiata*	Cd, Cu, and Pb	Inoculation promoted plant growth under HM stress	[85]
Kocuria flava and *B. vietnamensis*	*Oryza sativa*	As	Inoculation promoted plant growth (shoot and root length and weight)	[86]

Possible Rhizobacterial Strategies for Heavy Metals Bioremediation

Rhizobacterial Biosorption of Heavy Metals

Biosorption is a new biological technique that has been employed for the last 20 years. It is an inexpensive approach to remove heavy metals from polluted environments [87]. Biosorption is based on the ionic interactions between the extracellular surface of living cells or dead biomass with metal ions. Therefore, most of the pollutants adhere on the cell surfaces instead of being oxidised by aerobic or anaerobic metabolism. Biosorption is considered as an effective technique for removal of various heavy metals from aqueous solutions [88, 89]. Researchers have shown that charged functional groups act as nucleation sites for the biosorption of various metal-containing precipitates. There are three mechanisms reported by which heavy metals can be adsorbed from contaminated environment: (1) Adsorption on the bacterial cell surfaces (2) Additional surface complexation and precipitation of actinides and (3) Precipitation of actinides with bacterial cell lysates [90]. In microorganisms, heavy metals are accumulated through adsorption or absorption processes reported previously [91–93]. Adsorption is the main mechanism of heavy metal accumulation observed in several microorganisms. Adsorption is an energy-independent process that occurs in both living and non-living bacterial cells. However, absorption is an energy-dependent process that occurs in living bacterial cells [94]. Bacterial cell walls have some specific functional groups such as carboxyl, amine, phosphonate, and hydroxyl groups [95]. These functional groups are involved in metal binding on the cell surfaces [96]. Anionic carboxyl and phosphate groups contribute to overall negative charge on microbial cell walls. Almost all heavy metals are positively charged and easily interact with cell walls. Therefore, metal ions bind or accumulate inside the cell via cell membrane [97]. Thus, the success of the metal adsorption process depends on the diverse structure of the bacterial cell wall. Gram-positive bacterial cell wall consists of a thick layer of peptidoglycan, which has high adsorption capacity [98, 99]. Gram-positive bacteria have the ability to remove heavy metal cations due to their electronegative charges due to the presence of teichoic and teichuronic acids in the cell wall. Thus, metal binding mechanism depends on the chemical nature of cell biomass and ionic strength of metal ions [100, 101] (Fig. 18.2).

Uptake of Cd (II) by biomass of *Sphingomonas paucimobilis* has been reported earlier. The ability of living cells to remove Cd (II) was found to be significantly higher than that of dead cells [104]. Another study also reported that live cells of *Enterobacter cloacae* TU cells were superior in removing Cd (II) compared to dead cells [105]. Huang et al. [106] studied those dead cells have been shown to have higher Cd (II) biosorption capacity than live cells [106]. It has also been shown that live and dead biomass of *P. plecoglossicida* have approximately the same Cd (II) biosorption capacity [107].

However, being biosorbent, little research has been carried out on live and dead cells of PGPR. The use of live or dead biomass to remove heavy metals continues

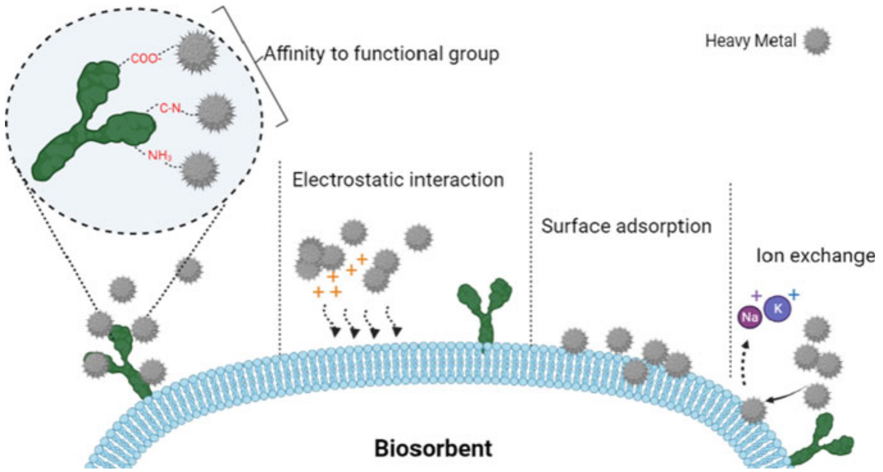

Fig. 18.2 Biosorption of heavy metals on bacterial cell surface [90, 102, 103]

to be debated. Therefore, living and non-living biomass of *C. necator* GX_5, *Sphingomonas* sp. GX_15, and *Curtobacterium* sp. GX_31 have been used as biosorbents to compare their Cd (II) adsorption capacities [108]. Dead cells showed higher adsorption capacity than the live cells of *Curtobacterium* sp. GX_31. However, in the case of *C. necator* GX_5 and *Sphingomonas* sp. GX_15, the loading capacity of non-living biomass was stronger when compared with living biomass at 20 mg/L of Cd (II). After autoclaving, slight changes in the spectrum were observed, and FTIR analysis showed that more functional groups of the dead biosorbents were involved in Cd (II) binding. FTIR study also revealed that functional groups such as hydroxyl, amino, amide, and carboxyl groups played a vital role in complexation with Cd (II). Thus, it was concluded that dead cells are more effective biosorbents for Cd (II) remediation [108]. In another study, 10 different PGPRs were isolated, and identified as *Arthrobacter globiformis*, *B. megaterium*, *B. cereus*, *B. pumilus*, *S. lentus*, *E. asburiae*, *S. paucimobilis*, *Pantoea* spp., *Rhizobium rhizogenes*, and *R. radiobacter*. These isolates were tested for their arsenic biosorption capability. It was observed that all rhizobacteria showed arsenic biosorption capability. However, *S. paucimobilis* showed the highest biosorption capacity for arsenic (146.4 ± 23.4 mg/g dry cell weight) [109].

Therefore, PGPR not only promotes plant growth, but are also promising biosorbents for removing heavy metals from the environment. However, there is still some debate about the biosorption and bioaccumulation processes, and their role in cadmium adsorption. Therefore, cadmium biosorption and bioaccumulation study was carried out by using three different Cd (II)-resistant PGPR such as *C. necator* GX_5, *Sphingomonas* sp. GX_15, and *Curtobacterium* sp. GX_31. The study found that the highest Cd (II) removal efficiency values for GX_5, GX_15, and GX_31 were 25.05%, 53.88%, and 86.06%, respectively at 20 mg/L of Cd (II) [110]. Recently, several microorganisms are genetically modified to improve the metal sorption capacity [111, 112]. Bacteria such as *S. xylosus* and *S. carnosus* are transgenic strains that express two different polyhistidyl peptides (His3-Glu-His3 and His6) reported earlier [113]. Similarly, *E. coli* and *P. putida* strains have been employed for phosphate biosorption through phosphate-binding protein [114]. *E. coli* was genetically modified to express the Ni21 transport system and at the same time overexpress pea MT as a carboxyl-terminal fusion with glutathione S-transferase (GSTMT). This change improved the Ni21-accumulating capacity of *E. coli* [115].

Bioaccumulation of Heavy Metals by Rhizobacteria

Uptake of heavy metals by microorganisms occurs in two main stages: (i) metabolism-independent; and (ii) metabolism-dependent [90]. In the first stage, metal binding takes place on the cell surface via various mechanisms such as adsorption, precipitation, complexation, ion-exchange, and crystallization [116]. In the second stage, the metal uptake in microorganisms occurs through bioaccumulation process. Heavy metal ions are adsorbed on the cell surface and slowly enter the cytoplasm of the cell. Therefore, the metal species remain immobilized within the cell cytoplasm of the cell. This process is also known as metal sequestration [69]. This process is slow and dependent on several factors such as metabolic energy, temperature and metabolic inhibitors [90].

Bioaccumulation process in which microorganisms use importer complexes to take up heavy metals into the intracellular space via translocation pathways through the lipid bilayer. Once heavy metals enter cells, they can be sequestrated by several proteins and peptide ligands [69]. Bacteria synthesize metal-binding proteins such as metallothionein (MT) after exposure to high concentrations of metals to enhance their metal-binding capacity [117]. Therefore, MTs have metal-binding capacity and are encoded by genes expressed in a diverse group of rhizobacteria to facilitate the accumulation of heavy metals [118]. Recombinant expression of inner membrane importers from three major transporter classes: (i) channels, (ii) secondary carriers, and (iii) primary active transporters are studied well to enhance heavy metal bioaccumulation by increasing cytoplasmic uptake from the periplasmic membrane [119] as shown in Fig. 18.3.

Microorganisms employed for metal bioaccumulation must be metal tolerant to one or more metal contaminants at high concentrations. They also should have the metal biotransformational potential to convert toxic heavy metals into non-toxic

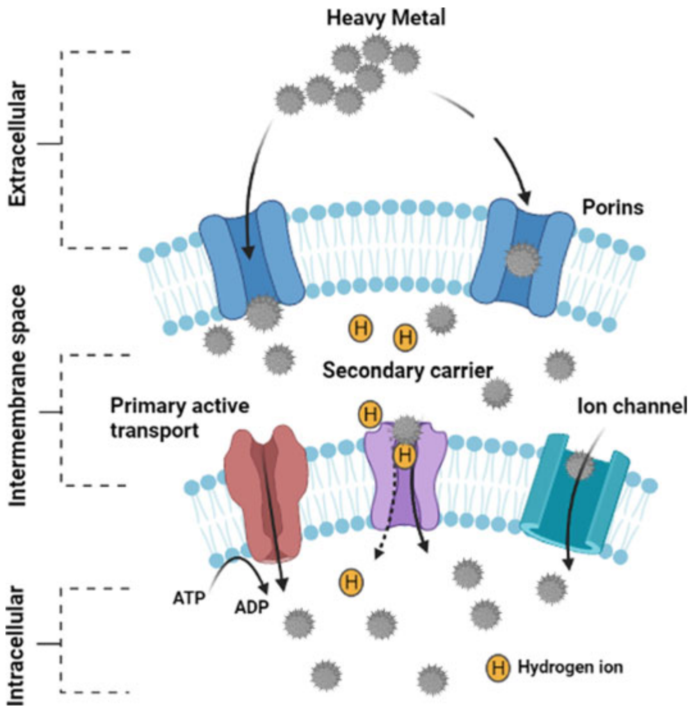

Fig. 18.3 Bioaccumulation of heavy metals by bacterial cell [90, 119]

forms [120, 121]. Thus, PGPRs not only promote plant growth but also found to be promising agents for heavy metal remediation. Li et al. [110] isolated three cadmium-resistant PGPR namely *Cupriavidus necator* GX_5, *Sphingomonas* sp. GX_15, and *Curtobacterium* sp. GX_31 and used for bioaccumulation study under different Cd (II) concentrations. The study revealed that bioaccumulation was dominant in *C. necator* GX_5 and metal uptake was about 50.66–60.38%. The bioaccumulation study was also evidenced by different techniques such as SEM–EDX, TEM and FTIR spectroscopy. Further bioaccumulation study showed that heavy metals (cadmium and zinc) were mostly adhered on the cell wall instead of accumulating inside the cells [122]. In case of rhizobacteria, heavy metals in soluble and complex form are accumulated by live bacterial cells [123]. Studies on bioaccumulation of heavy metals by PGPR are very less reported and thus there is scope to carry out research in future.

Rhizobacterial Exopolysaccharides (EPS) for Heavy Metal Remediation

EPS is a complex mixture of high molecular weight biopolymer metabolites produced by several microorganisms that protects against harsh environmental conditions. Rhizobacterial EPS has high metal binding capability which composed of polysaccharides, proteins, uronic acid, humic substances, lipids nucleic acid, and glycoproteins. Alginate (EPS) obtained from *Azotobacter* shows a strong metal binding capability. This property of EPS helps in remediation of toxic heavy metals by creating a microenvironment of essential metal ions to maintain the health of soil ecosystem and promotes plant growth [124–127]. EPS can assist in biofilm formation that protect cells in adverse conditions and helping plants by absorbing more water and nutrients [128]. Biofilms have been employed in bioremediation processes because of their inherent ability to thrive in harsh environments. Bacterial biofilms are highly dense biomass embedded in EPS used for metal remediation via biosorption and bioaccumulation processes [129]. EPS of bacterial biofilm have high metal binding affinity. EPS form organometal complexes via electrostatic forces of attraction [129]. Thus, heavy metals are immobilised by bacterial biofilms via EPS and cell membrane components due to their high affinity towards heavy metals [130]. The ionic charges on the EPS of biofilm are due to several functional groups such as carboxyl, amino, phenol, phosphate, and sulfhydryl groups. These functional groups are responsible net negative charges on the EPS surface that assist the formation of organometallic complexes with heavy metals [129, 130]. Three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy was used to study the interaction of EPS of biofilm and Hg (II). In this study, EPS of biofilm is a class of organic ligands that are important for complexing with Hg (II) and have profound effects on chemical forms, mobility, bioavailability, and ecotoxicity of heavy metals in the aquatic environment [130]. Thus, EPS could be an effective biosorbent for heavy metals. EPS obtained from rhizobacteria exhibited strong heavy metal binding capacity, removing precipitated metal sulfides and oxides, leading to formation of EPS-metal complexes and thus, promoting remediation of heavy metals [131]. Carboxyl and phosphate groups of EPS produced by *P. putida* have been reported for adsorption of Cd^{2+} [132]. EPS of *A. chroococcum* strain XU1 exhibited biosorption capacity about 33.5 and 38.9 mg/g for lead and mercury, respectively [126].

It has been also reported that biofilm-grown cells have showed high resistance to heavy metals. Further study revealed that *Pseudomonas* biofilms was developed in presence of lead and zinc. However, there was no direct evidence provided by authors to prove the metal resistance potential of biofilms [133]. The nitrogen-fixing species *Sinorhizobium meliloti* has the ability to synthesize two different symbiosis-promoting EPSs: (1) succinoglycan and (2) galactoglucan. These EPSs have been studied to play important roles in plant development and protection from environmental stress. Researchers evaluated the role of EPS in bacterial resistance to heavy metals and metalloids, which are known to affect various biological processes. A recent study showed that EPS is essential for protecting bacteria from the toxicity of Hg (II) and As (III) stress. Biofilm formation has also been observed in the presence of heavy metals. Therefore, it was finally concluded that bacterial strain, which produces EPS have higher metal resistance ability compared to non-EPS bacterial strain [134]. PGPR such as *Pseudomonas* sp. H13 and *Brevundomonas* sp. H16 were reported for their ability to form biofilm and adsorbing heavy metals including Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. It has been observed that C–OH and P=O groups related to polysaccharides showed a significant role in heavy metal adsorption and immobilization [135]. A biofilm forming cadmium tolerant PGPR, *Aeromonas* sp enhanced the root length and shoot height of augmented plant by 21.4 and 17.36%, respectively, as compared to the non-augmented plants. It was also noticed that bioaugmentation of *Aeromonas* sp. in the rhizosphere of *Vetiveria zizanioides* increased cadmium uptake by 67.7% in the soil treated with 15 mg/kg of Cd [136].

Rhizobacterial Biosurfactant Mediated Heavy Metal Remediation

Biosurfactant-mediated metal remediation from metal-polluted soils is considered a promising environmental green technology [137]. Biosurfactants are surface-active molecules that reduce the surface tension between liquid and liquid or liquid and solid [138]. Several microorganisms such as bacteria, yeast, and fungi have been reported to be capable of producing biosurfactants. These biosurfactants are commonly used for remediation of heavy metals such as cadmium, lead and zinc [139]. Several bacterial isolates within the genus *Pseudomonas*, *Bacillus*, *Micrococcus*, *Arthrobacter*, and *Rahnella* have been reported as potent producers of biosurfactants [140]. Endophytic *Rahnella* sp. JN6 significantly enhanced the phytoremediation efficacy in cadmium, lead and zinc contaminated soil [141]. Rhizobacteria produce biosurfactants that not only contribute to metal bioavailability but also promote plant growth. Biosurfactants are composed of polysaccharides, proteins, lipoproteins, lipopolysaccharides, or complex mixtures. Many species of *Acinetobacter* have produced high-molecular weight emulsifiers [77, 138]. However, rhamnolipids are the major class of biosurfactants produced by *P. aeruginosa* and other several microorganisms [139].

A potential of biosurfactant producing the endophytic *Pseudomonas* sp. Lk9 was tested for cadmium uptake and growth promotion of *Solanum nigrum* L. Researcher has found that *Solanum nigrum* L inoculated by *Pseudomonas* sp. Lk9 increases the cadmium availability, increases shoot dry biomass by 14% and total Cd accumulated in the shoot by 46.6% mg/kg [142]. Similarly, *Miscanthus sinensis* inoculation with the biosurfactant-producing multimetal-tolerant endophytic *P. koreensis* AGB-1 improved plant biomass by 54% and also increased metals content in roots and shoots [143]. Further study has been performed on the metal speciation by biosurfactant-producing *B. subtilis*, *P. aeruginosa*, and *P. fluorescence*. This study showed that *P. aeruginosa* strain has high metal exchangeable fraction concentrations compared to other strains [144].

Conclusion

Restoring soil contaminated with toxic metals is a major challenge. Several physico-chemical methods are available for treating metal-contaminated soil. These methods have several disadvantages. Therefore, searching an alternative method is of high priority. A biological approach that fascinates many scientists because it has many advantages over traditional methods. Microbial remediation of heavy metal-polluted environment has emerged as an efficient green technology. There are several reports available on bioremediation of heavy metal-polluted soil by PGPR.

It has been investigated that PGPR is a promising agent for remediation of heavy metal-contaminated soils. There are various strategies like biosorption, bioaccumulation, EPS-assisted, bioleaching, biosurfactant-assisted, and biofilm-based techniques that have been used for restoration purposes. In the future, further research is needed to improve the bioremediation process with PGPR. Heavy metal tolerance in PGPR needs to be understood in detail, and genes responsible for metal tolerance need to be thoroughly studied in the future. Since the bioaccumulation of heavy metals by PGPR has not been sufficiently studied, it is very important to carry out the research work in detail. In order to develop efficient green technology in the future, it is necessary to study the interaction between PGPR and heavy metals at the molecular level. PGPR-metal interactions need to be study at molecular level in order to develop efficient green technology in future. Further genetic modification in PGPR is of high importance to improve efficacy of bioremediation process. Another genetic manipulation in PGPR is very important for improving the efficiency of the bioremediation process.

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