Insights into the Status of Heavy Metal Resistant Rhizobacterial Communities in the Heavy Metal Contaminated Sites

Karthikeyan KirupaSree, Vijay Karuppiah, Sathiamoorthi Thangavelu, and Kavitha Thangavel

Abstract Anthropogenic activities viz. modern agricultural practices, industrialization and mining have long term detrimental effects on environment. All these factors lead to the increase in heavy metal concentration in both hydrosphere and lithosphere. The extreme use of chemical fertilizers in agricultural field possesses a major threat to human and animal health and also causes various environmental hazards. The utilization of the chemical fertilizers can be reduced through biological aspects. Rhizosphere bacterial community is majorly involved in the plant growth and colonized in root zones of the plants with enhanced symbiotic relationship with plant community. These bacteria support the plant growth at normal and stressed conditions. These naturally occurring bacteria will pave a way to minimize the use of chemical fertilizers and hence in reducing the risk hazards. Plant-soil-root ecosystem is an important interface between soil and plants and also plays a significant role in the biosorption of heavy metals from contaminated soils. The rhizobacteria dwelling in this soil are known to affect heavy metal mobility and availability to the growing plant through the release of chelating agents, acidification, phosphate solubilization and redox changes, therefore having tremendous potentials to enhance the bioremediation processes. Bioremoval strategies with appropriate heavy metal-adapted rhizobacteria have received considerable importance. This chapter aims to reveal the sources of heavy metals and its effects on various life forms with special emphasis on PGPR assisted mechanisms for bioremoval of heavy metals from heavy metal implicated sites in the environment.

Keywords Heavy metals · Chelating agents · Bioremoval · Plant growth promoting rhizobacteria · Chemical fertilizers

K. KirupaSree · V. Karuppiah · S. Thangavelu · K. Thangavel (✉)
Department of Microbiology, Science Campus, Alagappa University, Karaikudi, Tamil Nadu 630003, India
e-mail: kavithat@alagappauniversity.ac.in

M. Prashanthi et al. (eds.), *Bioremediation and Green Technologies*, Environmental Science and Engineering, https://doi.org/10.1007/978-3-030-64122-1_2

1 Introduction

Various industries such as cosmetics, textiles, tanneries emerge worldwide to fulfill the various requirements of enlarging population. These advancements have put an increasing burden on the environment by releasing large quantities of hazardous wastes, heavy metals, metalloids and chemicals that lead to serious problems in an ecosystem (Ayansina and Olubukola 2017). Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water (Tchounwou et al. 2012). The increase in heavy metal concentration in the environment at alarming rate may directly or indirectly affect plants, microbes, animals and human beings. Enormous amount of heavy metals are used in various fungicides and chemical fertilizers, wastewater irrigation and sewage sludge which in turn contaminates water resources as well as agricultural soils (Akcil et al. 2015). Copper conjugated pesticides are very expensive and formulated to have fungicidal and bactericidal actions (CIPAC 1992). Copper-(II) ion (Cu^{2+}) enters the fungal spores during germination and accumulates until a high concentration is achieved to kill the spores. However, antifungal activity is restricted to prevent the spore germination. Hence a prophylactic mode of fungicidal action is observed with copper based fungicides. Unfortunately, the deposition does happen on the crop before fungal spores begin to germinate indicating the essentiality of environment risk assessments. A similar mechanism is postulated for antibacterial action of copper based pesticides in day-to-day agriculture by US-EPA (United States Environmental Protection Agency) in the year 2000. Examples of copper based fungicides include GalbinAr, Efdalbakirox, Moltifen and Bromix. Waheed and Nahed (2017) have analyzed the presence of Arsenic (As), Cadmium (Cd) and Lead (Pb) as impurities and copper as conjugates in the above listed copper based fungicidal formulates before and after storage at 54 °C for 21 days.

Microbes play an important role in substance turnover of heavy metal contamination which will clean up the metal contaminated sites (Spain and Alm 2003). If the heavy metal is fewer in concentration it may act as active elements in plants and microorganisms. For example, Copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) are actually micronutrients which mean these heavy metals are needed at very low quantities for the normal growth of plants. Iron (Fe) is not generally considered as a heavy metal because it is essential for growth and metabolism of both plants and animals at its optimum level. However when the above listed heavy metals are presented at supra-optimum levels (i.e., above 0.1%) they are toxic to plants and rhizosphere microorganisms (Nies 1999).To circumvent the metal stress, bacteria progress through many types of mechanisms to overcome the uptake of heavy metal ions. The mechanisms include efflux of metal ions outside the cell, binding and accumulation of the metal ions inside the cell and reduction of heavy metal ions to the less toxic states (Nies 1999). Cations of the heavy metals bind to glutathione in gram negative bacteria, which would result in bisglutathionato complexes. Bisglutathionato complexes in turn react with the molecular oxygen forming the Oxidized bisglutathione (Kachur et al. 1998). Reduced forms of heavy metals, which are less toxic, are released from the biosglutothionato complex into the environment. More heavy metal is uptaken by Gram positive bacteria due to presence of glycoproteins. Less heavy metal uptake by Gram negative bacteria is observed due to phospholipids and Lipopolysaccharides (LPS) (Das et al. 2008).

Plant growth promoting rhizobacteria (PGPR) play a vital role in agriculture mainly in the host plant adaptation to metal contaminated area by activation of several physiological changes in the plant cell metabolism. This improves tolerance of growing plants towards high amount of heavy metal implications (Conrath et al. 2006).The plant growth promoting bacteria assist the phytoremediation process through mechanisms that support plant growth including nitrogen metabolisms, synthesis and secretion of phytohormones such as indole-3-acetic acid (IAA), Cytokinins, acetonin and 2,3 butanediol, and organic acids as well as defense molecules such as siderophores, I—aminocyclopropane—I–carboxylate deaminase (ACC) (Khan et al. 2009; Taghavi et al. 2009).

2 Various Methods for Removal of Heavy Metals

A variety of physical, chemical and biological methods were in use to remove metals from the environment which are as follows (Joo et al. 2010; Pagnanelli et al. 2010):

2.1 Physical Methods

Reverse osmosis, Membrane technology, Evaporation recovery, Filtration and Ion exchange.

2.2 Chemical Methods

Electrochemical treatment, chemical precipitation and oxidation/reduction reactions.

Though the above mentioned methods were not the initial choice as they are costly, ineffective, and labor-intensive or the treatment process lacks selectivity (Chen 2008; Tang 2008), the study carried out by Talos 2009, on bioremediation or biosorption—based remediation techniques concluded that the natural processes are found to be cost effective and in that line biological methods to remove heavy metals stands first and employs microorganisms such as bacteria, fungi and algae. The present chapter describes the types of heavy metals, chances of environmental release and its effect on earth and life forms. In addition it specially emphasizes the role of Bacteria and Rhizobacterial communities on heavy metal removal and detoxification and safe release into the environment.

3 Effects of Heavy Metal Accumulation on the Earth

Environment is polluted by various ways and heavy metals are the main source for pollution which affects many biological systems in the world as heavy metals does not undergo biodegradation. According to the Biological functions, these metals has been classified as: (i) toxic metalloids and metals with undefined biological functions, (ii) fundamental metals with known biological functions, (iii) non-essential and nontoxic metal with no biological functions (Pepper et al. 2015). Heavy metals ions are chemical moieties which would influence its negative effects through diverse mechanisms such as protein damage, DNA damage and oxidative damage through the production of reactive oxygen species etc., (Assal et al. 2017). Most of the heavy metals are toxic to the environment in that Pb, Co, Cd stands in the first line and are differentiated from other pollutants; those are not biodegraded however these heavy metals can be accumulated in living organisms resulting in various diseases and disorders even at lower concentrations (Pehlivan et al. 2009). Soil is the backbone of agriculture and the worst effects are caused due to the accumulation of heavy metals in plants. Higher organisms that consume heavy metal accumulated plants face numerous health issues. This may also have a negative crash on balance of soil micro flora, plant growth and in the ground cover at the level of ground water purity (Roy et al. 2005). The toxicity of heavy metal against the plant and plant associated microbes are described in the table as well as the most of the PGPR which are involved in the phytoremediation has shown in the Table 1.

3.1 Arsenic

Arsenic is moderately distributed in natural waters which are related with geological sources. Likewise in various locations of anthropogenic inputs, such as the use of pesticides, insecticides as well as the combustion of fossil fuels are the enormously important additional sources. Oxidation states III and V of arsenic occurs in natural waters, in the form of arsenic acid (H_3AsO_5) and its salts, arsenous acid (H_3AsO_3) and its salts, respectively (Sawyer et al. 2003). Arsenic contamination in groundwater may create major problem during irrigations. For example, it accumulates in plant tissues including grains and contaminates food chain (Verma et al. 2016). In recent, study has been carried out to inspect the molecular mechanisms and physiology of arsenic accumulation, toxicity, detoxification and tolerance in various plants (Kumar et al. 2015) some of the plant growth promoting rhizobacteria which is resistant to the arsenic is shown in the Table 1.

Table 1 Toxicity of heavy metals towards plants and plant-associated microbes under heavy metal stress

Metal	Source	Effects on plants	Effects on microbes	Host plant	PGPR involved in phytoremediation	References
Antimony	Soil erosion, volcanic eruption, coal combustion, mining, smelting	Decrease synthesis of some metabolites, growth inhibition, inhibit chlorophyll synthesis	Inhibit enzyme activities, reduced growth rate	Not yet reported	Not yet reported	Blais et al. (2008) and An et al. (2009)
Beryllium	Coal and oil combustion, volcanic dust	Inhibits seed germination	Chromosomal aberration, mutation	Not yet reported	Not yet reported	Finnegan et al. (2012) and Bissen et al. (2003)
Arsenic	Atmospheric deposition, mining, pesticides, rock sedimentation, smelting	Damage cell membrane, inhibition of growth, inhibits roots extension and proliferation, interferes with critical metabolic processes, loss of fertility, yield and fruit production, oxidative stress, physiological disorders	Deactivation of enzymes	*Brassica nigra, Brassica juncea*	*Microbacterium sp. CE3R2, Curtobacterium sp. NM1R1, Staphylococcus arlettae*	Román-Ponce et al. (2017), Srivastava et al. (2013)
Chromium	Dyeing, electroplating, paints production, steel fabrication, tanning, textile	Chlorosis, delayed, senescence, wilting, biochemical lesions, reduced biosynthesis germination, stunted growth, oxidative stress	Elongation of lag phase, growth inhibition, inhibition of oxygen uptake	*Cicer arietinum*	*Mesorhizobium* sp. RC3	Wani and Khan (2010)

(continued)

Table 1 (continued)

Metal	Source	Effects on plants	Effects on microbes	Host plant	PGPR involved in phytoremediation	References
Cadmium	Fertilizer, mining, pesticide, plastic, refining, welding	Chlorosis, decrease in plant nutrient content, growth inhibition, reduced seed germination	Damage nucleic acid, denature protein, inhibit cell division and transcription, inhibits carbon and nitrogen mineralization	*Brassica Juncea, Brassica napus*	*Variovox paradoxus*, *Rhodococcus* sp., *Xanthomonas* sp. RJ3, *Azomonas* sp. RJ4, *Pseudomonas* sp. RJ10, *Bacillus* sp. RJ31	Belimov et al. (2005), Sheng and Xia (2006)
Copper	Copper polishing, mining, paint, plating, printing operations	Chlorosis, oxidative stress, retard growth	Disrupt cellular function, inhibit enzyme activities	*Brassica Juncea, Cicer arietinum, Brassica nigra*	*Bacillus* sp. PSB10, *Achromobacter xylosoxidans* Strain Ax10, *Bacillus* sp. PSB10, *Microbacterium* sp. CE3R2, *Curtobacterium* sp. NM1R1	Ma et al. (2009), Wani and Khan (2010), Román-Ponce et al. (2017)
Mercury	Batteries, coal combustion, geothermal activities, mining, paint industries, paper industry, volcanic eruption, weathering of rocks	Affects antioxidative system, affects photosynthesis, enhance lipid peroxidation, induced genotoxic effect, inhibit plant growth, yield, nutrient uptake and homeostasis, oxidative stress	Decrease population size, denature protein, disrupt cell membrane, inhibits enzyme function	*Triticumaestivum*	*Enterobacterludwigii*, *Klebsiella pneumoniae*	Gontia-Mishra et al. (2016)

(continued)

Table 1 (continued)

Metal	Source	Effects on plants	Effects on microbes	Host plant	PGPR involved in phytoremediation	References
Lead	Coal combustion, electroplating, manufacturing of batteries, mining, paint, pigments	Affects photosynthesis and growth, chlorosis, inhibit enzyme activities and seed germination, oxidative stress	Denatures nucleic acid and protein, inhibits enzymes activities and transcription	*Brassica Juncea,*	*Azotobacter chroococcum* HKN-5, *Bacillus megaterium* HKP-1, *Bacillus mucillaginosus* HKK	Wu et al. (2006)
Nickel	Electroplating, non-ferrous metal, paints, por	Decrease chlorophyll content, inhibit	Enzyme activities and growth, reduced	*Oryzasativa, Brassica juncea*	*Bacillus licheniformis* NCCP-59, *Bacillus subtilis* SJ-101	Jamil et al. (2014), Zaidi et al. (2006)
Thallium	Cement production, combustion of fossil fuels, metal smelting, oil refining	Inhibits enzyme activities, reduced growth	Damages DNA, inhibits enzyme activities and growth	Not yet reported	Not yet reported	Blais et al. (2008) and Babula et al. (2008)
Selenium	Coal combustion, mining	Alteration of protein properties, reduction of plant biomass	Inhibits growth rate	Not yet reported	Not yet reported	Dixit et al. (2015) and Germ et al. (2007)
Zinc	Brass manufacturing, mining, oil refinery, plumbing	Affects photosynthesis, inhibits growth rate, reduced chlorophyll content, germination rate and plant biomass	Death, decrease in biomass, inhibits growth	*Brassica juncea*	*Bacillus megaterium* HKP-1, *Bacillus mucillaginosus* HKK	Wu et al. (2006)

3.2 Lead

Lead can affect the various life forms through contamination from old lead plumbing pipes, dusts, fuels from various industrial sites and also in the old orchard sites in production where arsenate and lead is mostly used (Tangahu et al. 2011). Long term exposure to lead is found to be intensely toxic to both animals as well as plants which is non-biodegradable and has several harmful effects on organic systems including soil properties i.e., pH, organic carbon, amorphous iron, aluminium oxides (FEAL), and cation exchange capacity. (Bradham et al. 2006; Pehlivan et al. 2009). Several bacteria, such as *Arthrobacter* spp., *Bacillus megaterium, Pseudomonas marginalis, Citrobacter freundii, Staphylococcus aureus*, and *E. coli* have been found to be resistant to lead (Das et al. 2016).

3.3 Cadmium

Cadmium is highly soluble in water therefore it is easily up taken by plants which results in phytotoxicity followed by entry through the pathways into the food chain causing serious harmful effects to human beings (Buchet et al. 1990). This heavy metal has been classified into carcinogenic to humans by The International Agency for Research on Cancer (IARC 1993). Still at low concentrations, cadmium alters some enzyme activities including those enzymes involved in Calvin cycle, CO_2 fixation, Carbohydrate and Phosphorus metabolisms (Gill and Tuteja 2011). This may result in the underdeveloped growth, alterations in chloroplast ultrastructure, Chlorosis, leaf epinasty, inhibition of photosynthesis and pollen germination, alterations in nitrogen (N) and sulphur (S) metabolism and disruption of the antioxidant machinery (Gill and Tuteja 2011). In a report by Roane et al. (2001), *Pseudomonas* strain H1 and *Bacillus* strain H9 showed an intracellular mechanism of cadmium sequestration (36%) for reducing cadmium toxicity. These strains showed the production of exopolymers (EPS) which accumulated cadmium and reduced soluble cadmium levels by 22% and 11%, respectively.

3.4 Chromium

Seventh most rich metal on earth is chromium and exists in two stable states in the environment: they are trivalent Cr^{3+} and hexavalent Cr^{6+}. Source of Chromium contamination is due to the use of Cr in many industries such as leather tanning, metal plating, and other metallurgical procedures. The insufficient disposal of their wastes may give rise to concentrations above the natural values (Wuana 2011). Chromium inhibits sulphate membrane transport and causes oxidative damage in bacteria. Against chromium toxicity, microbes involve in two mechanisms. The first and foremost is chromate efflux from the cells, and another one is the reduction of toxic form Cr^{6+} into less toxic form Cr^{3+}. The efflux protein of chromate is encoded by chrAgene, which has homologs in eubacteria, archaea, and also in some eukaryotes (Das et al. 2016). Nies et al. (1998) defined about two chromate efflux pumps with six transmembrane segments but Diaz-Magana et al. (2009) described that in *E. coli*, these two pumps are not separate ones, however together form a heterodimer of 12 segments.

3.5 Copper

Copper is third oldest abundantly used metal having wide applications in wires, architecture, motors and medicines. Consuming more amount of copper in the body causes Copperiedus, leading to the production of Reactive Oxygen Species (ROS), which have the potential to damage proteins, DNA and lipids. Two oxidation states Cu(I) and Cu(II) involve in copper cycles and dislocate iron (Fe) from available Fe–S clusters in dehydratases and other iron sulphur proteins (Macomber and Imlay 2009). Plasmid pRJ1004-mediated copper resistance in bacteria was first reported by Tetaz and Luke (1983). Later on Bender and Cooksey (1986) identified native pPT23D plasmids in *Pseudomonas syringae* helping tomato from copper toxicity. However, the removal of cytoplasmic copper is involved by ATPase-driven copper efflux system. Periplasmic copper handling, metallochaperones, multicopper oxidases and Resistance-Nodulation-Division (RND) systems are involved in this process (Bondarczuk and Piotrowska-Seget 2013).

3.6 Mercury

Compared to other toxic metal pollutants, mercury is one of the most toxic elements in the earth which has severe health concerns. It is strong in sediments, soils, water and atmosphere. Through anthropogenic activities mercury enters into the environment. Mercury resistant bacteria have two operons. One of the operons is narrow-spectrum mer operon and another one is broad spectrum mer operon (Matsui et al. 2016). Consensus sequence GMTCAAC is present in the mercury binding site. MerP scavenges inorganic mercury ions and transports them to the MerT protein (Hamlett et al. 1992). merC expression in *Arabidopsis thaliana* and *Nicotiana tabacum* has led to their doubling ability to accumulate mercury (Sasaki et al. 2006).

3.7 Nickel

Nickel is most abundantly disturbed in the environment which exists as five stable isotopes ^{58}Ni, ^{60}Ni, ^{61}Ni, ^{62}Ni, ^{64}Ni. Nickel has vital role in the biochemistry of microbes and plants. Most of the enzyme contains nickel active site such as ureases, hydrogenases, superoxide dismutases, and glyoxalase enzyme contains in the form of Ni–Fe clusters or use nickel as a co-factor. Nickel is highly toxic to the humans and animals because it's potential to cross the placenta and affect the developing fetus. Nickel resistance in bacteria is generally mediated by metal efflux pumps, this resistance mechanism is well studied in *Cupriavidus metallidurans* CH34 formerly called *Ralstonia metallidurans* by Grass et al. (2000). It has been reported that in the presence of CnrCBA transenvelope efflux pump encoded by the cnrYHXCBAT gene system, Ni is expelled out of the cell (Maillard et al. 2015). Through cnrY and cnrC the cnr promoter is initiated while the nickel enters into the periplasm, transcription occurs. The CnrCBA encodes a highly efficient pump which is activated only in micromolar concentrations of nickel. These gene products form an efflux pump to efflux excess nickel outside the cell. Certain standard levels of heavy metals allowed to be present in mg per Kg of soil varies with countries as below in Table 2.

4 Bioremediation of Heavy Metals by Microorganisms

Bioremediation is the process of removing heavy metals through biological aspects which also involves microorganisms. These microbes decrease the heavy metal ion toxicity by immobilizing, uptake, mobilizing and transformation of heavy metals (Hassan et al. 2017). Numerous symbiotic PGPR resides in plant roots and also as free-living bacteria in the rhizosphere soil that positively alters plant growth and increases the productivity by the production of growth regulators, through supplying and facilitating nutrient uptake from soil (Nadeem et al. 2014). Most of the studies

Table 2 Permissible level of heavy metals

Heavy metal (mg/kg)	Austria standard	The European Union standard	Indian standard
Al	–	–	–
As	50	–	–
Cd	5	3	3–6
Co	50	–	–
Cr	100	150	–
Cu	100	140	135–270
Ni	100	75	75–150
Pb	100	300	250–500
Zn	100	300	–

have reported that PGPR act as potential elicitors for heavy metal tolerance as well as abiotic stress tolerance (Dary et al. 2010; Tiwari et al. 2016, 2017). These PGPR bind with the bioavailablemetals by forming complexes with siderophores such as Desferroxamines, Dihydroxybenzoic acids and Rhizoferrins (Dimpka et al. 2016), particular metabolites like heme in *Bacillus japonicum* by ferrochelatases, metallothionein cation-binding proteins (Chandrangsu 2017) and bacterial heavy metal transporters such as Pb(II)/Cd(II)/Zn(II)-transporting ATPase in *E. coli* (Rajkumar et al. 2010; Ahemad 2012). The agriculturally important microorganisms evolved various mechanisms to overcome heavy metal stress which includes (a) transport of metals across cytoplasmic membrane; (b) biosorption and bioaccumulation to the cell walls; (c) metal entrapment in the extracellular capsules; (d) heavy metals precipitation; and (e) metal detoxification via oxidation–reduction reactions (Zubair et al. 2016). The harmful effects of heavy metals are reduced through various microbes of Heavy-metal-tolerant PGPR including *Bacillus, Pseudomonas, Streptomyces* and *Methylobacterium* which has the potentials to improve the growth and yield of the crops (Sessitsch et al. 2013).

Plant growth promoting bacteria are involved in the biosorption of heavy metals in which siderophores and IAA are accountable for metal uptake with which indirect defence mechanisms are activated (Spaepen and Vanderleyden 2011). Siderophores reduce the abiotic stresses forced on plants by making stable complexes with toxic heavy metals of environmental concern such as Cd, Cu, Cr, Pb and Zn (Rajkumar et al. 2010).

4.1 Role of Microbes in Detoxification of Heavy Metal

In order to survive at high concentration of heavy metals, bacteria need to develop different mechanisms to confer resistances to these heavy metals. There is no general mechanism for resistance in bacteria towards all heavy metal ions. Though it is well known that both living and dead cells are capable of metal accumulation but there are differences in the mechanism involved.

There are four possible known mechanisms postulated in bacterial heavy metal resistances. They are as follows:

- The first mechanism is by keeping the toxic ion out of the cell by altering a membrane transport system involved in initial cellular accumulation.
- The second mechanism is the intracellular or extracellular sequestration by specific metal-ion binding components (analogous to the phytochelatins in plants and the metallothioneins of eukaryotes, but generally binding occurs at the level of the cell wall in bacteria). Extracellular accumulation/precipitation may be facilitated by using viable microorganisms. However, cell surface sorption or complexation can occur within alive or dead microorganisms, while intracellular accumulation requires microbial activity (Macek et al. 2011).

- The third mechanism includes plasmid mediated bacterial metal ion resistance, which involves highly specific anion or cation efflux systems encoded by resistance genes associated with plasmids.
- The fourth and widely known mechanism involves detoxification of the toxic anion or cation by enzymatically converting it from a more toxic to a less toxic form. This mechanism does essentially occur in detoxification of inorganic and organomercurials.

Some other major mechanisms of microbial metal transformations between soluble and insoluble metal species include chemolithotrophic leaching, chemoorganotrophic leaching, rock and mineral bioweathering and biodeterioration, biocorrosion, redox mobilization, methylation, complexation (For example, complex formation with microbial products such as metallothionein like proteins and extracellular polymers (EPS) in case of soluble metal species whereas for insoluble metal species the process includes biosorption, accumulation, biomineral formation, redox immobilization, metal sorption to biogenic minerals and formation of metalloid nanoparticlesare well notable processes (Tunali 2006)). The key factors in controlling these mechanisms include:

- The nature of the biomass i.e. living or non-living;
- The type of biological ligands available for heavy metal sequestration;
- The chemical, stereochemical and coordination characteristics of the targeted metals and metalloid species (Remoudaki et al. 1999).
- The hysic-chemical characteristics of the metal solution such as pH, and presence of competing co-ions (Esposito 2002).

4.2 Interaction Between the Heavy Metals and Microbes

Numerous mechanisms have been executed by bacteria to detoxify and resist heavy metals by which the metal ions bind to the cell surface and incorporate electrostatic interactions, covalent binding, Van der Waals forces, redox interactions and extracellular precipitation or by means of all these as combined processes (Blanco et al. 2000). In general the response of bacteria may fall into two categories: (1) mechanisms dependent on activation by specific metals, and (2) general mechanisms, which convey resistance but do not depend on metal stress for their activation. Uptake of heavy metals and detoxification through Heavy metal tolerant—plant growth promoting microbes is largely responsible for the Bioaccumulation. There are two methods involved in bioaccumulation of Heavy metals: one is passive uptake which is also known as biosorption, a metabolism independent accumulation of heavy metals by inactive non-living biomass or living cells/biological materials. Another one is active uptake that occurs only in alive cells, it requires energy and metabolism for the exchange of metals (Gutierrez-Corona et al. 2016). One or a blend of different processes involved in the biosorption includes coordination, complexation, chelation, ion exchange, entrapment and micro precipitation (Pokethitiyook and Poolpak 2016).

Biosorption of heavy metals from the contaminated site by means of microbes are associated within the cell wall and functional groups like –SH, –OH, and –COOH and other biomolecules have more affinity for heavy metals. Metal-binding peptides and chelators involved in metal binding such as metallothioneins (MT) and glutathione—derived peptides (PC). These PC and MT are secreted by rhizosphere fungi, bacteria and also by plants in response to heavy metals stress (Miransari 2011).

4.3 Mechanisms Behind the Utility of Heavy Metals by Microorganisms

Heavy metal ions are trapped by the cellular structure of microorganisms and consequently attached to the binding sites of the cell wall (Malik 2004). This method is known as passive uptake or biosorption, and is exclusively independent of the metabolic cycles. The amount of metals biosorbed depends on the kinetic equilibrium and composition of the metals at the cellular surface. The mechanism involves several process including electrostatic interaction, ion exchange and surface complexation. Absorption of heavy metal is carried out by fragments of cells and tissues, or living cells or by dead biomass as passive uptake by surface complexation on the cell wall and other outer layers (Fomina et al. 2014). In cellular metabolic cycles, these heavy metal ions may pass across the cell membrane and the process is known as bioaccumulation or active uptake.

4.4 Bioaccumulation or Active Uptake of Heavy Metals in Bacteria

In gram positive bacteria peptidoglycan layer contains alanine, glutamic acid, meso-diaminopimelic acid, polymers of glycerol and teichoic acid whereas in gram negative bacteria, cell wall contains enzymes, lipoproteins, glycoproteins, lipopolysaccharides and phospholipids which concerned as the active site for metal binding (Lesmana et al. 2009; Fomina et al. 2014; Gupta et al. 2015). The process involved in the bioaccumulation of heavy metals in the living cell is dependent on a variety of physical, chemical and biological mechanisms. The intercellular and extracellular process plays a partial and ill-defined role in biosorption as well (Fomina et al. 2014). The microbes which accumulate heavy metals have the tolerance capacity to one or more metals at higher concentrations, and it has the ability to change the toxic forms to harmless forms thereby reducing the toxic levels of the metals in the environment (Fig. 1). Simultaneously these organisms do retain the heavy metals contained in the cells (Mosa et al. 2016).

Microorganisms hide many kinds of metal-binding metabolites and produce extracellular polymeric substances like polysaccharides and associated components such

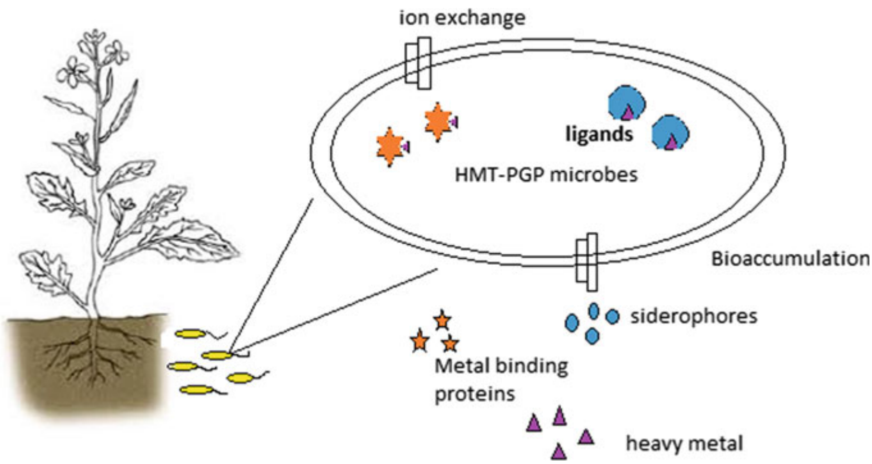

Fig. 1 Bioaccumulation/active uptake of heavy metals by plant growth promoting rhizosphere bacteria

as capsules, slimes and sheaths, and biofilms for with heavy metals (Fomina et al. 2014).

4.5 Biosorption or Passive Uptake in Bacteria

Among microorganisms, bacteria constitute of being the most abundant, versatile, most diverse creature on this planet earth (Norberg et al. 1984; Abbas et al. 2014). They are fundamentally classified on the basis of their morphology as rod, cocci or spirillum (Wang et al. 2009; Abbas et al. 2014). A bacterium has relatively simple morphology consisting of cell wall, cell membrane, capsule, slime layer and internal structures, such as ribosomes, mesosomes etc. Slime layer contains functional groups like carboxyl, amino, phosphate or sulfate for metal chelation (Abbas et al. 2014). Cell wall in general, is dependable for surface binding sites and the binding strength for different metal ions depend on different binding mechanisms. There exist many intracellular and extracellular events in bacteria involving microbial bioremediation of toxic pollutants from the environment. In response to the presence of toxic metals in the environment, resistant bacteria synthesize many intracellular and extracellular enzymes to remove/degrade the toxic form of metals to non-toxic/less toxic forms (Fig. 2). Various bacterial species belonging to the genus such as *Bacillus, Pseudomonas, Escherichia* exhibit biosorption property because of their small size and ability to grow in different environmental conditions (Vasudevan et al. 2001; Vijayaraghavan et al. 2008; Kinoshita et al. 2013).

Gram positive bacteria are comprised of thick peptidoglycan layer connected by amino acid bridges, also known to contain polyalcohols and teichoic acids. On the

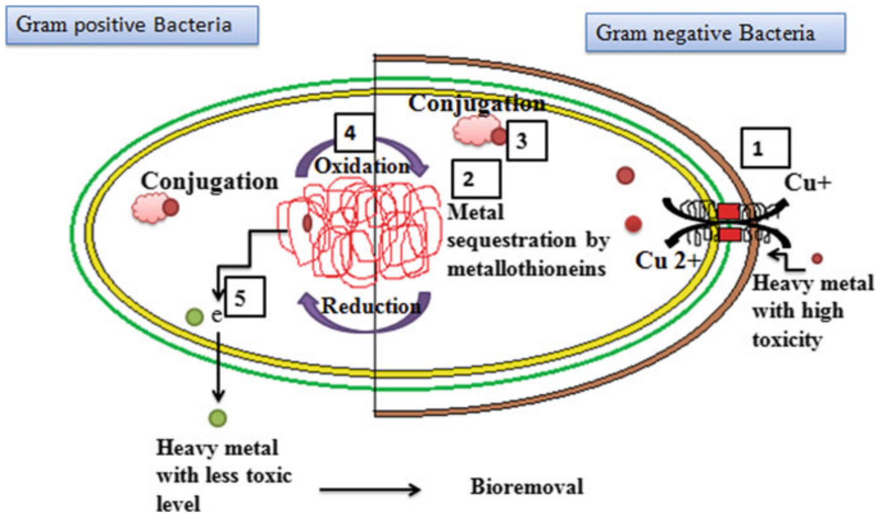

Fig. 2 Bioremoval of heavy metals from the environment by resistant bacteria through passive uptake in response to the presence of toxic metals in the environment. Here, resistant bacteria synthesize many intracellular and extracellular enzymes to remove/detoxify the toxic form of heavy metals to non-toxic/less toxic forms. These processes include (1) Entry of heavy metal with high toxicity (2) Metal sequestration by metallothioneins, (3) Conjugate formation with organic compounds/precipitation, (4) Oxidation–reduction of metals, (5) Metal efflux by metal transporters followed by bioremoval through microbial products such as biosurfactants or EPS

whole, Gram positive bacterial cell wall comprised of 90% peptidoglycan. Some teichoic acids are linked to lipids of lipid bilayer forming lipoteichoic acid. These lipoteichoic acids again forma part of cytoplasmic membrane. They constitute linkage of peptidoglycan to cytoplasmic membrane. This results in cross linking of peptidoglycan forming a grid like structure. These teichoic acids are responsible for negative charge on cell wall due to presence of phosphodiester bonds between teichoic acid monomers (Abbas et al. 2014). Hence, affinity towards the heavy metals is more favoured in cell wall of gram positive bacteria as these heavy metals are cationic in nature. On the other hand, Gram negative bacterial cell wall contains an additional outer membrane composed of phospholipids and lipopolysaccharides. Gram negative cell wall contains 10–20% peptidoglycan. The negative charge on the Gram negative bacteria is due to lipopolysaccharides, teichoic acids, teichuronic acids. Extracellular polysaccharides also exhibit the properties of metal binding. Bacterial cell wall encountering the metal ion is the first component of biosorption. The metal ions get attached to the functional groups (amine, carboxyl, hydroxyl, phosphate, sulfate, and amine) present on the cell wall (Abdi 2015). The general metal uptake process involves binding of metal ions to reactive groups present on bacterial cell wall followed by internalization of metal ions inside the cell (Abbas et al. 2014). More metal uptake is carried out by Gram positive bacteria due to presence of glycoproteins which facilitates the internalization. However, fewer metal uptakes by Gram negative bacteria are observed due to presence of phospholipids and LPS which favours the phenomenon of adsorption (Gourdon et al. 1990; Das et al. 2008). Microorganisms have advanced mechanisms to protect themselves from the toxic doses of heavy metals such as adsorption, oxidation/reduction, or methylation. These mechanisms can be adopted with some manipulation in treatment strategies for the removal of metals from polluted environments (Hashim et al. 2011).

5 Applications and Future Prospects

Detection of novel genes and proteins associated with the ability of eco-friendly clean-up will be a great benefit to achieve enhanced bioremediation. To identify new genes which may be expressed in the presence of a particular heavy metal pollutant, gene expression studies employing microarray technology and whole genome sequencing assays shall be worthwhile. Microbes possess many unique characteristic features such as biofilm formation, biosurfactant productions, secondary metabolites synthesis, and many more to withstand the adverse conditions in the environment. These properties of metal resistant bacteria may be harnessed for their enhanced utilization in bioremediation. Recently, multispecies biofilm communities have been explored for their metal tolerance and bio-mineralization properties (Golby et al. 2014). Though bacteria develop metal-resistant phenotypes and genotypes as a mode of adaptation in the contaminated environments, the gene pool of these resistant bacteria can be a choice of research thirst in near future to have a proper insight into the molecular genetics approaches for an enhanced heavy metal bioremediation so as to save the environment.

6 Conclusion

This chapter revealed both the active (Bioaccumulation) and passive (Biosorption) mechanisms of bioremoval of toxic heavy metals by Heavy metal tolerant-plant growth promoting rhizobacteria. These communities are widely involved in the removal of heavy metals from the polluted environment. At this juncture, Bacterial siderophores gain importance because of their capability to interact with the heavy metals like Fe, Ni, Cd, Cu, and Zn. In which the metal uptake is concerned through two special proteins. They are metallo-proteins or metal-binding proteins and peptides. The use of PGPR for plant growth improvement is presently receiving substantial worldwide concentration and the latest successive PGPR researches have exhibit luminous prospects for bioremediation of polluted soil environments. In addition, the rapid development of molecular biological methods is bringing valuable advantages to identify and enhance the rhizobacterial traits involved in heavy metal bioremoval from the areas under heavy metal stress.

Acknowledgements Authors gratefully acknowledging the RUSA—Phase 2.0 grant sanctioned vide Letter No. F. 24-51/2014-U, Policy (TN Multi-Gen), Department of Education, Govt. of India, dated 09.10.2018.

References

Abbas SH, Ismail IM, Mostafa TM, Sulaymon AH (2014) Biosorption of heavy metals: a review. J Chem Sci Technol 3(4):74–102

Abdi O, Kazemi M (2015) A review study of biosorption of heavy metals and comparison between different biosorbents. J Mater Environ Sci 6(5):1386–1399

Ahemad M (2012). Implications of bacterial resistance against heavy metals in bioremediation: a review. J Inst Integr Omics Appl Biotechnol (IIOAB) 3(3)

Akcil A, Erust C, Ozdemiroglu S, Fonti V, Beolchini F (2015) A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J Cleaner Prod 86:24–36

An YJ, Kim M (2009) Effect of antimony on the microbial growth and the activities of soil enzymes. Chemosphere 74(5):654–659

Belimov, AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.). Soil Biol Biochem 37(2):241–250

Bender CL, Cooksey DA (1986) Indigenous plasmids in Pseudomonas syringae pv. tomato: conjugative transfer and role in copper resistance. J Bacteriol 165(2):534–541

Bissen M, Frimmel FH (2003) Arsenic—a review. Part I: occurrence, toxicity, speciation, mobility. Acta Hydrochim Hydrobiol 31(1):9–18

Blais JF, Djedidi Z, Cheikh RB, Tyagi RD, Mercier G (2008) Metals precipitation from effluents. Pract Periodical Hazard Toxic Radioactive Waste Manage 12(3):35–149

Blanco A, Sampedro MA, Sanz B, Llama MJ, Serra JL (2000). Immobilization of non-viable cyanobacteria and their use for heavy metal adsorption from water. Environ Biotechnol Cleaner Bioprocess 135–151

Bondarczuk K, Piotrowska-Seget Z (2013) Molecular basis of active copper resistance mechanisms in gram-negative bacteria. Cell Biol Toxicol 29:397–405

Bradham KD, Dayton EA, Basta NT, Schroder J, Payton M, Lanno RP (2006) Effect of soil properties on lead bioavailability and toxicity to earthworms. Environ Toxicol Chem 25:769–775

Buchet JP, Lauwerys R, Roels H, Bernard A, Bruaux P, Claeys F, Lijnen P (1990) Renal effects of cadmium body burden of the general population. Lancet 336(8717):699–702

Chandrangsu P, Rensing C, Helmann JD (2017) Metal homeostasis and resistance in bacteria. Nat Rev Microbiol 15(6):338

Chen G, Zeng G, Tang L, Du C, Jiang X, Huang G, Liu H, Shen G (2008) Cadmium removal from simulated wastewater to biomass byproduct of Lentinusedodes. Bioresour Technol 99(15):7034–7040

Conrath U, Beckers GJ, Flors V, García-Agustín P, Jakab G, Mauch F, Pugin A (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19(10):1062–1071

Dary M, Chamber-Pérez MA, Palomares AJ, Pajuelo E (2010) "In situ" phytostabilisation of heavy metal polluted soils using Lupinus luteus inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177(1–3):323–330

Das N, Vimala R, Karthika P (2008) Biosorption of heavy metals—an overview. Indian J Biotechnol 2008(7):159–169

Das S, Dash HR, Chakraborty J (2016) Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants. Appl Microbiol Biotechnol 100(7):2967–2984

Diaz-Magana A, Aguilar-Barajas E, Moreno-Sánchez R, Ramírez- Díaz MI, Riveros-Rosas H, Vargas E, Cervantes C (2009) Short-chain chromate ion transporter proteins from Bacillus subtilis confer chromate resistance in *Escherichia coli*. J Bacteriol 191:5441–5445

Dimkpa C (2016) Microbial siderophores: production, detection and application in agriculture and environment. Endocytobiosis Cell Res 27(2):7–16

Dixit R, Malaviya D, Pandiyan K, Singh U, Sahu A, Shukla R, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7(2):2189–2212

Dubey S, Shri M, Misra P, Lakhwani D, Bag SK, Asif MH (2014) Heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice root. Funct Integr Genomics 14:401–417

El Assal FM, Abdel-Meguid ZA (2017) Impact of Heavy Metal Pollution on Procambarus clarkii (Crustacea: Decapoda), from Egypt. Int J Waste Resour 7(3):270

Esposito A, Pagnanelli F, Vegliò F (2002) pH-related equilibria models for biosorption in single metal systems. Chem Eng Sci 57(3):307–313

Finnegan P, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. Front Physiol 3:182

Fomina M, Gadd GM (2014) Biosorption: current perspectives on concept, definition and application. Bioresour Technol 160:3–14

Germ M, Kreft I, Stibilj V, Urbanc-Berčič O (2007) Combined effects of selenium and drought on photosynthesis and mitochondrial respiration in potato. Plant Physiol Biochem 45(2):162–167

Gill SS, Tuteja N (2011) Cadmium stress tolerance in crop plants: probing the role of sulfur. Plant Signal Behav 6:215–222

Golby S, Ceri H, Marques LLR, Turner RJ (2014) Mixed-species biofilms cultured from an oil sand tailings pond canbiomineralize metals. Microb Ecol 68:70–80

Gontia-Mishra I, Sapre S, Sharma A, Tiwari S (2016) Alleviation of mercury toxicity in wheat by the interaction of mercury-tolerant plant growth promoting rhizobacteria. J Plant Growth Regul 35:1000–1012

Gourdon R, Bhende S, Rus E, Sofer SS (1990) Comparison of cadmium biosorption by gram-positive and gram-negative bacteria from activated sludge. Biotech Lett 12(11):839–842

Grass G, Grobe C, Nies DH (2000) Regulation of the cnr cobalt and nickel resistance determinant from Ralstonia sp. strain CH34. J Bacteriol 182:1390–1398

Gupta VK, Nayak A, Agarwal S (2015) Bioadsorbents for remediation of heavy metals: current status and their future prospects. Environ Eng Res 20(1):1–18

Gutierrez-Corona JF, Romo-Rodriguez P, Santos-Escobar F, Espino-Saldana AE, Hernandez-Escoto H (2016) Microbial interactions with chromium: basic biological processes and applications in environmental biotechnology. World J Microbiol Biotechnol 32:191

Hamlett NV, Landale EC, Davis BH, Summers AO (1992) Roles of the Tn21 merT, merP, and merC gene products in mercury resistance and mercury binding. J Bacteriol 174(20):6377–6385

Hashim MA, Mukhopadhyay S, Sahu JN, Sengupta B (2011) Remediation technologies for heavy metal contaminated groundwater. J Environ Manag 92(10): 2355–2388

Hassan TU, Bano A, Naz I (2017) Alleviation of heavy metals toxicity by the application of plant growth promoting rhizobacteria and effects on wheat grown in saline sodic field. Int J Phytorem 19(6):522–529

Jamil M, Zeb S, Anees M, Roohi A, Ahmed I, ur Rehman S, Rha ES (2014) Role of Bacillus licheniformis in phytoremediation of nickel contaminated soil cultivated with rice. Int J Phytorem 16(6):554–571

Joo JH, Hassan SH, Oh SE (2010) Comparative study of biosorption of Zn2+ by Pseudomonas aeruginosa and Bacillus cereus. Int Biodeterior Biodegradation 64(8):734–741

Kachur AV, Koch CJ, Biaglow JE (1998) Mechanism of copper-catalyzed oxidation of glutathione. Free Radical Res 28(3):259–269

Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7(1):1–19

Kinoshita H, Sohma Y, Ohtake F, Ishida M, Kawai Y, Kitazawa H, Kimura K (2013) Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. Res Microbiol 164(7):701–709

Kumar S, Dubey RS, Tripathi RD, Chakrabarty D, Trivedi PK (2015) Omics and biotechnology of arsenic stress and detoxification in plants: current updates and prospective. Environ Int 74:221–230

Lesmana SO, Febriana N, Soetaredjo FE, Sunarso J, Ismadji S (2009) Studies on potential applications of biomass for the separation of heavy metals from water and wastewater. Biochem Eng J 44:19–41

Ma Y, Rajkumar M, Freitas H (2009) Inoculation of plant growth promoting bacterium Achromobacter xylosoxidans strain Ax10 for the improvement of copper phytoextraction by Brassica juncea. J Environ Manag 90(2):831–837

Macek T, Mackova M (2011) Potential of biosorption technology. In: Microbial biosorption of metals. Springer, Dordrecht, pp 7–17

Macomber L, Imlay JA (2009) The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proc Natl Acad Sci 106(20):8344–8349

Maillard AP, Künnemann S, Grobe C, Volbeda A, Schleuder G, Petit-Härtlein I, Covès J (2015) Response of CnrX from Cupriavidus metallidurans CH34 to nickel binding. Metallomics 7(4):622–631

Malik A (2004) Metal bioremediation through growing cells. Environ Int 30(2):261–278

Matsui K, Yoshinami S, Narita M, Chien MF, Phung LT, Silver S, Endo G (2016) Mercury resistance transposons in Bacilli strains from different geographical regions. FEMS Microbiol Lett 363(5)

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29(6):645–653

Mosa A, El-Ghamry A, Trüby P, Omar M, Gao B, Elnaggar A, Li Y (2016) Chemo-mechanical modification of cottonwood for Pb2+ removal from aqueous solutions: sorption mechanisms and potential application as biofilter in drip-irrigation. Chemosphere 161:1–9

Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol Adv 32:429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Nies DH, Koch S, Wachi S, Peitzsch N, Saier MH (1998) CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfateantiporters. J Bacteriol 180:5799–5802

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51(6):730–750

Norberg AB, Persson H (1984) Accumulation of heavy-metal ions by Zoogloea ramigera. Biotechnol Bioeng 26(3):239–246

Pagnanelli F, Viggi CC, Toro L (2010) Isolation and quantification of cadmium removal mechanisms in batch reactors inoculated by sulphate reducing bacteria: biosorption versus bioprecipitation. Bioresour Technol 101(9):2981–2987

Pehlivan E, Özkan AM, Dinç S, Parlayici Ş (2009) Adsorption of Cu^{2+} and Pb^{2+} ion on dolomite powder. J Hazard Mater 167(1–3):1044–1049

Pepper IL, Gerba CP, Gentry TJ (2015) Environmental Microbiology, 3rd edn. Academic, San Diego USA

Pokethitiyook P, Poolpak T (2016) Biosorption of heavy metal from aqueous solutions. In: Phytoremediation, pp. 113–141. Springer, Cham

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28(3):142–149

Remoudaki E, Tsezos M, Hatzikioseyian A, Karakoussis V (1999) Mechanism of palladium biosorption by microbial biomass. The effects of metal ionic speciation and solution co-ions. Process Metall 9:449–462

Román-Ponce B, Reza-vázquez DM, Gutiérrez-paredes S, María de jesús DE, Maldonado-hernández J, Bahena-osorio Y (2017) Plant growth-promoting traits in rhizobacteria of heavy

metal-resistant plants and their effects on *Brassica nigra* seed germination. Pedosphere 27:511–526. https://doi.org/10.1016/S1002-0160(17)60347-3
Roy S, Labelle S, Mehta P, Mihoc A, Fortin N, Masson C, Olsen C (2005) Phytoremediation of heavy metal and PAH-contaminated brownfield sites. Plant Soil 272(1):277–290
Sasaki Y, Hayakawa T, Inoue C, Miyazaki A, Silver S, Kusano T (2006) Generation of mercury-hyperaccumulating plants through transgenic expression of the bacterial mercury membrane transport protein MerC. Transgenic Res 15(5):615
Sawyer RH, Washington LD, Salvatore BA, Glenn TC, Knapp LW (2003) Origin of archosaurian integumentary appendages: the bristles of the wild Turkey beard express feather-type β keratins. J Exp Zool Part B: Mol Dev Evol 297(1):27–34
Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K et al (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60:182–194. https://doi.org/10.1016/j.soilbio.2013.01.012
Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harbor Perspect Biol 3(4). Retrieved February 7, 2015 from https://doi.org/10.1101/cshperspect.a001438
Spain A, Alm E (2003) Implication of microbial heavy metal tolerance in the enivornment. Rev Undergraduate Res 2:1–6
Srivastava S, Verma PC, Chaudhary V, Singh N, Abhilash PC, Kumar KV (2013) Inoculation of arsenic-resistant Staphylococcus arlettae on growth and arsenic uptake in Brassica juncea (L.) Czern. Var. R-46. J Hazard Mater 262:1039–1047. https://doi.org/10.1016/j.jhazmat.2012.08.019
Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, van der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 75(3):748–757
Talos K, Pager C, Tonk S, Majdik C, Kocsis B, Kilar F, Pernyeszi T (2009) Cadmium biosorption on native Saccharomyces cerevisiae cells in aqueous suspension. Acta Univ Sapientiae Agric Environ 1:20–30
Tang L, Zeng GM, Shen GL, Li YP, Zhang Y, Huang DL (2008) Rapid detection of picloram in agricultural field samples using a disposable immunomembrane-based electrochemical sensor. Environ Sci Technol 42(4):1207–1212
Tangahu BV, Abdullah S, Rozaimah S, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int J Chem Eng. https://doi.org/10.1155/2011/939161
Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. Molecular, clinical and environmental toxicology. Springer, Basel, pp 133–164
Tetaz TJ, Luke RK (1983) Plasmid-controlled resistance to copper in Escherichia coli. J Bacteriol 154(3):1263–1268
Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) Pseudomonas putida attunes morphophysiological, biochemical and molecular responses in Cicerarietinum L. during drought stress and recovery. Plant Physiol Biochem 99:108–117. https://doi.org/10.1016/j.plaphy.2015.11.001
Tiwari S, Prasad V, Chauhan PS, Lata C (2017) Bacillus amyloliquefaciens confers tolerance to various abiotic stresses and modulates plant response to phytohormones through osmoprotection and gene expression regulation in rice. Front Plant Sci 8:1510. https://doi.org/10.3389/fpls.2017.01510
Tunali S, Çabuk A, Akar T (2006) Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. Chem Eng J 115:203–211
Vasudevan P, Padmavathy V, Tewari N, Dhingra SC (2001) Biosorption of heavy metal ions. J Sci Ind Res 2001(60):112–120
Verma PK, Verma S, Pande V, Mallick S, Tripathi RD, Dhankher OP (2016) Overexpression of rice glutaredoxin OsGrx_C7 and OsGrx_C2. 1 reduces intracellular arsenic accumulation and increases tolerance in Arabidopsis thaliana. Front Plant Sci 7:740
Vijayaraghavan K, Yun KS (2008) Bacterial Biosorbents and Biosorption. Biotechnol Adv 26(3):266–291. https://doi.org/10.1016/j.biotechadv.2008.02.002

Wang JL, Chen C (2009) Biosorbents for heavy metals removal and their future—a review. Biotechnol Adv 2009(27):195–226
Wu SC, Cheung KC, Luo YM, Wong MH (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by Brassica juncea. Environ Pollut 140(1):124–135
Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol 2011
Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of Bacillus subtilis strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea. Chemosphere 64(6):991–997
Zubair M, Shakir M, Ali Q, Rani N, Fatima N, Farooq S, Nasir IA (2016) Rhizobacteria and phytoremediation of heavy metals. Environ Technol Rev 5(1):112–119