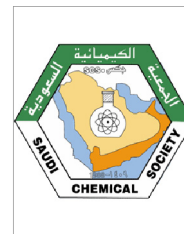




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## REVIEW

# Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: Paradigms and prospects

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## KEYWORDS

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**Abstract** Various industrial, agricultural and military operations have released huge amounts of toxic heavy metals into the environment with deleterious effects on soils, water and air. Under metal stress, soil microorganisms including plant growth promoting bacteria (PGPB) have developed many strategies to evade the toxicity generated by the various heavy metals. Such metal resistant PGPB, when used as bioinoculant or biofertilizers, significantly improved the growth of plants in heavy metal contaminated/stressed soils. Application of bacteria possessing metal detoxifying traits along with plant-beneficial properties is a cost effective and environmental friendly metal bioremediation approach. This review highlights the different mechanisms of metal resistance and plant growth promotion of metal resistant PGPB as well as the recent development in exploitation of these bacteria in bioremediation of heavy metals in different agroecosystems.

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## 1. Introduction

Heavy metals, having specific weight more than 5.0 g/cm<sup>3</sup>, are generally categorized in three classes: toxic metals (e.g. Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (e.g. Pd, Pt, Ag, Au, Ru, etc.) and radionuclides (e.g. U, Th, Ra, Am, etc.) (Nies, 1999; Bishop, 2002). Worldwide, smelting of metal-liferous surface finishing industry, fertilizer and pesticide industry, sewage sludge, energy and fuel production, mining, agriculture, leatherworking, metallurgy, combustion of fossil fuels, electroplating, faulty waste disposal, electrolysis, electro-osmosis, photography, electric appliance manufacturing, metal surface treatments, aerospace and atomic energy installation and military operations have directly or indirectly released huge amounts of toxic heavy metals into the environment with a subsequent hazardous impacts on both ecological and human health principally in developing countries (Wang and Chen, 2006; Kotrba et al., 2009; Ahemad and Malik, 2011). Heavy metal toxicity to various environmental niches is a great concern for environmentalists. Because these metals are difficult to be eliminated from the environment and unlike many other pollutants cannot be degraded chemically or biologically and are eventually indestructible and hence, their toxic effects last longer (Ahemad, 2012). Moreover, heavy metals display toxicity at low concentration (1.0–10 mg/L). Surprisingly, Hg and Cd metal ions show toxicity even at concentration of 0.001–0.1 mg/L. Furthermore, some metals (e.g. Hg) may transform from less toxic species into more toxic forms under some environmental conditions (Alkorta et al., 2004; Wang and Chen, 2006).

The metal concentration accumulated in soil is dependent upon the level of industrial discharge laden with metal species, the transportation of metals from the source to the disposing site and the retention of metals once these are reached (Alloway, 1995; Ahemad, 2012). Although some of the heavy metals are required by organisms at low concentration and are essential for different metabolic activities (Adriano, 2001). For instance, zinc is the component of a variety of metalloenzymes or it may act as cofactor for several enzymes (dehydrogenases, proteinases, peptidases, oxidase) (Hewitt, 1983). Moreover, it is also required for the metabolism of carbohydrates, proteins, phosphates, auxins, RNA and ribosome formation in plants (Shier, 1994). Likewise, copper at low concentration, contributes to several physiological processes, such as, photosynthesis, respiration, carbohydrate distribution, nitrogen synthesis, cell wall metabolism and seed production in plants (Kabata-Pendias and Pendias, 2001). However, the elevated concentration of such metals above threshold levels in soils negatively affects the composition of microbial communities including PGPB both quantitatively and qualitatively (Wani et al., 2008; Ahemad and Khan, 2012a) which in turn, leads to substantial changes in ecological dynamics of rhizosphere niche (Gray and Smith, 2005). In addition, the higher concentration of metals not only affects the growth and metabolism but also decreases the biomass of naturally occurring soil microbial communities of beneficial microorganisms around the roots (Giller et al., 1998; Pajuelo et al., 2008). As well, they also exert a negative impact on plant growth (Rajkumar et al., 2006; Wani and Khan, 2010). For example, cadmium halts the enzymatic activities, DNA-mediated transformation, symbiosis between microorganisms and plants and makes the plant

prone to fungal attack (Kabata-Pendias and Pendias, 2001; Wani et al., 2008). The remediation of metal-contaminated soils consequently becomes imperative, because such soils generally cover large areas that are rendered inappropriate for sustainable agriculture.

Soil is a complex ecosystem where different microorganisms play important roles in maintaining the soil fertility and plant productivity through the interactions with both biological and physico-chemical components (Ahemad et al., 2009; Ilieva and Vasileva, 2014; Kosev and Vasileva, 2014). Under metal stress, soil microorganisms including PGPB have developed many strategies to evade the toxicity generated by the various heavy metals. These mechanisms include the expulsion of metal species outside the microbial cell surface, bioaccumulation the metal ions inside the cell actively or passively, biotransformation of toxic metals to less toxic forms and metal adsorption on the cell wall (Ahemad, 2012). Therefore, bacterial strains isolated from polluted environments were shown to be tolerant to higher concentrations of metals than those isolated from unpolluted areas (Rajkumar et al., 2010). Through these metal stress evading mechanisms, PGPB, when used as bioinoculant or biofertilizers, substantially improved the growth of plants implanted in heavy metal contaminated/stressed soils by lowering the metal toxicity (Madhaiyan et al., 2007; Wani and Khan, 2010). In addition, there are other mechanisms of plant growth promotion by PGPB e.g. they protect colonizing plants from the pathogens attack directly by inhibiting/killing pathogens through the production of antibiotics, HCN and phenazines, etc. (Saravanakumara et al., 2007; Cazorla et al., 2007). As well, PGPB also facilitate the plant growth through N<sub>2</sub> fixation (Jha and Kumar, 2007), solubilization of insoluble phosphorus (Ahemad and Khan, 2012c), production of siderophores (Tian et al., 2009; Jahanian et al., 2012), production of phytohormones (Tank and Saraf, 2010; Ahemad and Khan, 2012a,b,c,d,e,f), lowering of ethylene concentration (Rodrigues et al., 2008; Tank and Saraf, 2010), production of antibiotics and antifungal metabolites and induced systemic resistance (Glick, 2012). In this way, PGPB are known to boost the soil fertility in turn, the plant yield by supplying essential nutrients and growth regulators (Ahemad and Khan, 2012e) and alleviating the ethylene-mediated stress by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase and improving plant stress tolerance to drought, salinity, and metal and pesticide toxicity (Khan, 2005; Ahemad and Khan, 2012c; Glick, 2012). Exploitation of PGPB possessing metal detoxifying traits as well as multiple plant beneficial properties is a promising, cost competitive and environment friendly metal bioremediating tool.

## 2. Mechanisms of plant growth promotion by PGPB

PGPB mediated plant growth promotion occurs by the alteration of the whole microbial community in rhizosphere niche through the production of various substances (Table 1). Generally, PGPB promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (Glick, 2012; Ahemad and Kibret, 2014).

**Table 1** Growth promoting substances released by selected plant growth promoting bacteria (PGPB).

PGPB	Plant growth promoting traits	References
<i>Pseudomonas putida</i>	IAA, siderophores, HCN, ammonia, EPS phosphate solubilization	Ahemad and Khan (2012a,c, 2011c)
<i>Pseudomonas aeruginosa</i>	IAA, siderophores, HCN, ammonia, EPS, phosphate solubilization	Ahemad and Khan (2012e, 2011a,k, 2010d)
<i>Rhizobium</i> sp. (pea)	IAA, siderophores, HCN, ammonia, EPS	Ahemad and Khan (2012b, 2011i, 2010c, 2009b)
<i>Mesorhizobium</i> sp.	IAA, siderophores, HCN, ammonia, EPS	Ahemad and Khan (2012d, 2010e,h, 2009a)
<i>Bradyrhizobium</i> sp.	IAA, siderophores, HCN, ammonia, EPS	Ahemad and Khan (2012f, 2011d,h,l)
<i>Klebsiella</i> sp.	IAA, siderophores, HCN, ammonia, EPS, phosphate solubilization	Ahemad and Khan, (2011b,f,g)
<i>Pseudomonas</i> sp. A3R3	IAA, siderophores	Ma et al. (2011a)
<i>Rhizobium</i> sp. (lentil)	IAA, siderophores, HCN, ammonia, EPS	Ahemad and Khan, (2011e,j, 2010f,g)
<i>Psychrobacter</i> sp. SRS8	Heavy metal mobilization	Ma et al. (2011b)
<i>Enterobacter asburiae</i>	IAA, siderophores, HCN, ammonia, exo- polysaccharides, phosphate solubilization	Ahemad and Khan, (2010a,b)
<i>Bradyrhizobium</i> sp. 750, <i>Pseudomonas</i> sp., <i>Ochrobactrum</i> <i>cytisi</i>	Heavy metal mobilization	Dary et al. (2010)
<i>Bacillus</i> species PSB10	IAA, siderophores, HCN, ammonia	Wani and Khan (2010)
<i>Proteus vulgaris</i>	Siderophores	Rani et al. (2009)
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Ralstonia</i> <i>metallidurans</i>	Siderophores	Braud et al. (2009)
<i>Pseudomonas</i> sp.	Phosphate solubilization, IAA, siderophore, HCN, biocontrol potentials	Tank and Saraf (2009)
<i>Azospirillum amazonense</i>	IAA, nitrogenase activity	Rodrigues et al. (2008)
<i>Pseudomonas</i> sp.	ACC deaminase, IAA, siderophore	Poonguzhali et al. (2008)
<i>Serratia marcescens</i>	IAA, siderophore, HCN	Selvakumar et al. (2008)
<i>Pseudomonas fluorescens</i>	ACC deaminase, phosphate solubilization	Shaharoon et al. (2008)
<i>Acinetobacter</i> sp., <i>Pseudomonas</i> sp.	ACC deaminase, IAA, antifungal activity, N <sub>2</sub> - fixation, phosphate solubilization	Indiragandhi et al. (2008)
<i>Enterobacter</i> sp.	ACC deaminase, IAA, siderophore, phosphate solubilization	Kumar et al. (2008)
<i>Burkholderia</i>	ACC deaminase, IAA, siderophore, heavy metal solubilization, phosphate solubilization	Jiang et al. (2008)
<i>Pseudomonas jessenii</i>	ACC deaminase, IAA, siderophore, heavy metal solubilization, phosphate solubilization	Rajkumar and Freitas (2008)
<i>Pseudomonas aeruginosa</i>	ACC deaminase, IAA, siderophore, phosphate solubilization	Ganesan (2008)

ACC: 1-aminocyclopropane-1-carboxylate; EPS: exopolysaccharides; IAA: indole acetic acid.

### 3. Speciation versus bioavailability of heavy metal in soils

Bacterial traits such as, the releasing of chelating substances, acidification of the microenvironment and influencing changes in redox potential affect heavy metals bioavailability in soils (Lasat, 2002). Despite of the fact that microbial physiology exposed to high concentration of heavy metals is negatively affected, microbes essentially require various heavy metals as essential micronutrients for normal growth and development (Ahemad, 2012). Among metals, some are essential for most

redox reactions and are fundamental to normal cellular functions (Table 2). The interaction of bacteria with metal species, whether for basic metabolic requirements or to protect from their toxic effects, depends upon the metal speciation, i.e., bioavailable forms (Table 3).

Bacteria directly affect metal bioavailability by changing heavy metal speciation in the rhizosphere. In addition, they protect the plants from the phytotoxicity of excessive metals by changing the speciation from bioavailable to the non-bioavailable forms in soils (Jing et al., 2007). Generally,

**Table 2** Heavy metals and their significance in bacteria.

Heavy metals	Implications
Molybdate	Most important metal; part of molybdoenzymes, regulate nitrogenase synthesis in <i>Klebsiella</i>
Iron	Fe <sup>3+</sup> essentially is required by all bacteria while Fe <sup>2+</sup> is important for anaerobic bacteria Due to low solubility, Fe <sup>3+</sup> is not toxic to aerobic bacteria Microbial uptake siderophore-mediated
Manganese	Low toxicity, Mn(II) is used as an electron acceptor (in anaerobic respiration), a cofactor for some free radical detoxifying enzymes and in the photosynthetic photosystem II
Cobalt	Biologically important, part of cofactor B <sub>12</sub> Found mainly in the Co <sup>2+</sup> (medium toxicity) form, Co <sup>3+</sup> is only stable in complex compounds Resistance due to transenvelope efflux or owing to resistance to either nickel or zinc
Nickel	Toxicity similar to cobalt, required for a few enzymatic reactions Occurs as Ni <sup>2+</sup> (common form) and Ni <sup>3+</sup> Resistance is through sequestration and/or transport
Copper	Component of superoxide dismutase and cytochrome c oxidase Toxicity is due to interaction with free radicals Resistance by efflux system and compartmentalization
Zinc	Component of various cellular enzymes, DNA-binding proteins (zinc fingers) Lower toxicity compared to other metals Resistance by P type ATPase efflux and RND-driven transporter systems
Chromium	Cr(VI) is mainly derived anthropogenically and more toxic than Cr(III) due to greater solubility and generation of free radicals Resistance attributed to Cr(VI) reduction and efflux mechanism
Vanadium	Highly toxic, ATPase inhibitor Occurs in the form of V(V) or trivalent oxyanion vanadate Part of vanadate-dependent nitrogenase; used as an electron acceptor in anaerobic respiration
Arsenic	Structural similarity of arsenate to PO <sub>4</sub> <sup>3-</sup> makes it toxic for phosphorus metabolism No biological function except as an electron acceptor in anaerobic respiration Resistance through the action of <i>ars</i> operon-encoded proteins
Lead	Limited toxicity due to its low solubility Resistance due to efflux mechanism
Cadmium	More toxic than zinc Resistance based on cadmium efflux/metallothioneins
Silver	Mainly occurs as Ag <sup>+</sup> Toxicity because of forming a tight complex with sulfur
Mercury	Most toxic metal, no beneficial function Strong affinity of Hg <sup>2+</sup> to thiol groups Resistance through <i>mer</i> operon encoded proteins (MerT: uptake protein and MerA: mercuric reductase)

Based on the information from Vangronsveld and Clijsters (1994), Cooksey (1994), Nies and Silver (1995), Giller et al. (1998), Nies (1999), Kehres and Maguire (2003), Rubio and Ludden (2008), Hynninen (2010) and Ahemad (2012).

the low bioavailability of metals in soils decreases their uptake by organisms (Whiting et al., 2001; Braud et al., 2006). The bioavailability is influenced by various edaphic and ecological factors, such as (i) soil properties including soil pH, cation exchange capacity (CEC), organic matter content, the content of clay minerals and hydrous metal oxides, buffering capacity, redox potential, water content, and temperature, (ii) metal chemical properties, (iii) soil biological properties including exudation by plant roots and microbial activities in soil, and (iv) climate (Roane and Pepper, 2000; Fischerová et al., 2006). In addition, bioavailability of heavy metals increases under oxidizing/aerobic conditions/low pH owing to their presence in ionic forms. In contrast, under reducing/anaerobic conditions/high pH, their availability decreases because of the existence of insoluble metal species as sulfide, phosphates or carbonates (Lena and Rao, 1997).

#### 4. Mechanisms to overcome heavy metal stress in PGPB

It is well known that heavy metal cations are essentially required as trace elements to carry out the various biochemical

reactions in microbial cell metabolism (Ahemad, 2012). However, heavy metal ions form unspecific complexes in the microbial cells at concentrations above threshold levels thereby toxic effects of these metals are manifested. For example, heavy metals like, Hg<sup>2+</sup>, Cd<sup>2+</sup> and Ag<sup>+</sup> form highly toxic complexes which adversely affect the physiological functions of bacteria cells (Nies, 1999). Metal concentration exceeding the biological requirement inhibits the bacterial growth or bacteria respond to the elevated levels of metals by various resistance mechanisms (Ahemad and Malik, 2011). For instance, an *in vitro* assessment of the sensitivity of plant growth promoting *Rhizobium*, *Bradyrhizobium* and *Pseudomonas* to Cu<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup>, Mo<sup>2+</sup> and Fe<sup>2+</sup> by Bíró et al. (1995) revealed that *Rhizobium leguminosarum* strains were most sensitive to Cu<sup>2+</sup>, Zn<sup>2+</sup> and Co<sup>2+</sup> while *Bradyrhizobium*, *Pseudomonas* isolates, however, tolerated the highest (10 µg/ml) dose of these metals. This study also showed that sulfate forms of Cu<sup>2+</sup> and Zn<sup>2+</sup> were more deleterious than the chloride counterparts. Generally, long term exposure of heavy metals to microorganisms enforces a selection pressure which facilitates the proliferation of microbes, tolerant/resistant to metal stress. This



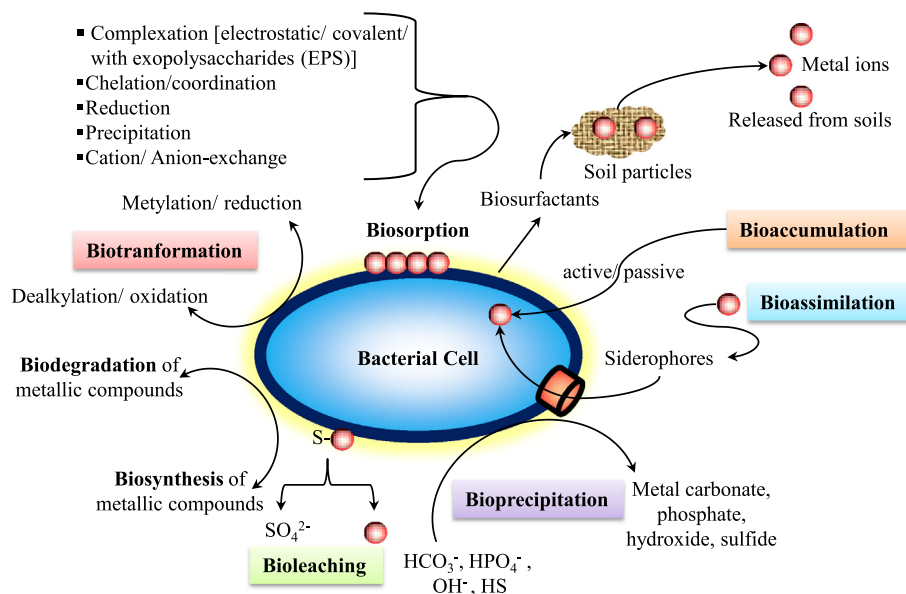
**Table 3** Speciation and chemistry of some heavy metals in soils.

Heavy metals Speciation and chemistry	
Lead	Pb occurs in 0 and +2 oxidation states. Pb(II) is the more common and reactive form of Pb. Low solubility compounds are formed by complexation with inorganic ( $\text{Cl}^-$ , $\text{CO}_3^{2-}$ , $\text{SO}_4^{2-}$ , $\text{PO}_4^{3-}$ ) and organic ligands (humic and fulvic acids, EDTA, amino acids). The primary processes influencing the fate of Pb in soil include adsorption, ion exchange, precipitation and complexation with sorbed organic matter
Chromium	Cr occurs in 0, +6 and +3 oxidation states. Cr(VI) is the dominant and toxic form of Cr at shallow aquifers. Major Cr(VI) species include chromate ( $\text{CrO}_4^{2-}$ ) and dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) (especially $\text{Ba}^{2+}$ , $\text{Pb}^{2+}$ and $\text{Ag}^+$ ). Cr(III) is the dominant form of Cr at low pH (<4). Cr(VI) can be reduced to Cr(III) by soil organic matter, $\text{S}^{2-}$ and $\text{Fe}^{2+}$ ions under anaerobic conditions. The leachability of Cr(VI) increases as soil pH increases
Zinc	Zn occurs in 0 and +2 oxidation states. It forms complexes with anions, amino acids and organic acids. At high pH, Zn is bioavailable. Zn hydrolyzes at pH 7.0–7.5, forming $\text{Zn}(\text{OH})_2$ . It readily precipitates under reducing conditions and may coprecipitate with hydrous oxides of Fe or manganese
Cadmium	Cd occurs in 0 and +2 oxidation states. Hydroxide [ $\text{Cd}(\text{OH})_2$ ] and carbonate ( $\text{CdCO}_3$ ) dominate at high pH whereas $\text{Cd}^{2+}$ and aqueous sulfate species dominate at lower pH (<8). It precipitates in the presence of phosphate, arsenate, chromate, sulfide, etc. Shows mobility at pH range 4.5–5.5
Arsenic	As occurs in -3, 0, +3, +5 oxidation states. In aerobic environments, As(V) is dominant, usually in the form of arsenate ( $\text{AsO}_4^{3-}$ ). It behaves as chelate and can coprecipitates with or adsorbs into Fe oxyhydroxides under acidic conditions. Under reducing conditions, As(III) dominates, existing as arsenite ( $\text{AsO}_3^{3-}$ ) which is water soluble and can be adsorbed/coprecipitated with metal sulfides
Iron	Fe occurs in 0, +2, +3 and +6 oxidation states. Organometallic compounds contain oxidation states of +1, 0, -1 and -2. Fe(IV) is a common intermediate in many biochemical oxidation reactions. Many mixed valence compounds contain both Fe(II) and Fe(III) centers, e.g. magnetite and prussian blue
Mercury	Hg occurs in 0, +1 and +2 oxidation states. It may occur in alkylated form (methyl/ethyl mercury) depending upon the pH of the system. $\text{Hg}^{2+}$ and $\text{Hg}_2^{2+}$ are more stable under oxidizing conditions. Sorption to soils, sediments and humic materials is pH-dependent and increases with pH
Copper	Cu occurs in 0, +1 and +2 oxidation states. The cupric ion ( $\text{Cu}^{2+}$ ) is the most toxic species of Cu, e.g., $\text{Cu}(\text{OH})^+$ and $\text{Cu}_2(\text{OH})_2^{2+}$ . In aerobic alkaline systems, $\text{CuCO}_3$ is the dominant soluble species. In anaerobic environments $\text{CuS}(\text{s})$ will form in presence of sulfur. Cu forms strong solution complexes with humic acids

Adapted from Hashim et al. (2011).

adaptive mechanism of metal resistance has been explored by assaying habitats exposed to anthropogenic or natural metal contamination over an extended period of time (Hutchinson and Symington, 1997), or by experimentally adding heavy metals to samples, and assaying changes over periods up to a few years (Diaz-Ravina and Baath, 1996). Hence, metal entry within the bacterial cell is first prerequisite to manifest the metal toxicity. Generally, bacterial cells uptake the heavy metal cations of the similar size, structure and valency with the same mechanism (Nies, 1999). Bacteria generally possess two types of uptake system for heavy-metal ions: one is fast and unspecific and driven by the chemiosmotic gradient across the cytoplasmic membrane and another type is slower, exhibits high substrate specificity, and is coupled with ATP hydrolysis (Nies and Silver, 1995). Bacteria including PGPB have devised several resistance mechanisms, by which they can immobilize, mobilize or transform metals, thus reducing their toxicity to tolerate heavy metal ion uptake (Ahemad, 2014a). The major mechanisms are physical sequestration, exclusion, complexation and detoxification etc. (Fig. 1). In fact, binding of heavy metals to extracellular materials can immobilize the metal and further, prevent its intake into bacterial cell. For instance, many metals bind the anionic functional groups (e.g. sulfhydryl, carboxyl, hydroxyl, sulfonate, amine and amide groups) present on cell surfaces. Likewise, bacterial extracellular polymers, such as polysaccharides, proteins and humic substances, also competently bind heavy metals (biosorption) (Ahemad and Kibret, 2013). These substances thus detoxify metals merely by complex formation or by forming an effective barrier surrounding the cell (Rajkumar et al., 2010). Moreover,

siderophores secreted by a range of PGPB can also diminish metal bioavailability and in turn, its toxicity by binding metal ions that have chemistry akin to that of iron (Gilis et al., 1998; Dimkpa et al., 2008; Rajkumar et al., 2010). Sometimes, crystallization and precipitation of heavy metals takes place because of bacteria-mediate reactions or due to the production of specific metabolites (Diels et al., 2003; Rajkumar et al., 2010). Furthermore, numerous bacteria exhibit efflux transporters (e.g. ATPase pumps or chemiosmotic ion/proton pumps) with high substrate affinity by which they expel high concentration of toxic metals outside the cell (Haferburg and Kothe, 2007; Ahemad, 2012). For instance, plasmid encoded and energy dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are also reported for arsenic, chromium and cadmium resistance in other bacteria (Roane and Pepper, 2000). Moreover, several bacteria have developed a cytosolic sequestration mechanism for protection from heavy metal toxicity. In this process, metal ions might also become compartmentalized or converted into more innocuous forms after entering inside the bacterial cell. This process of detoxification mechanism in bacteria facilitates metal accumulation in high concentration (Haferburg and Kothe, 2007; Ahemad, 2012). For this, a marvelous example is the synthesis of low-molecular mass cysteine-rich metal-binding proteins, metallothioneins which have high affinities for cadmium, copper, silver and mercury, etc. The production of these novel metal detoxifying proteins is induced by the presence of metals. In addition, certain bacteria utilize methylation as an alternative for metal resistance or detoxification mechanism. It involves the transfer of methyl groups to metals and



**Figure 1** Depiction of various types of bacterial interaction with heavy metals in metal polluted soils [modified from Tsezos (2009)].

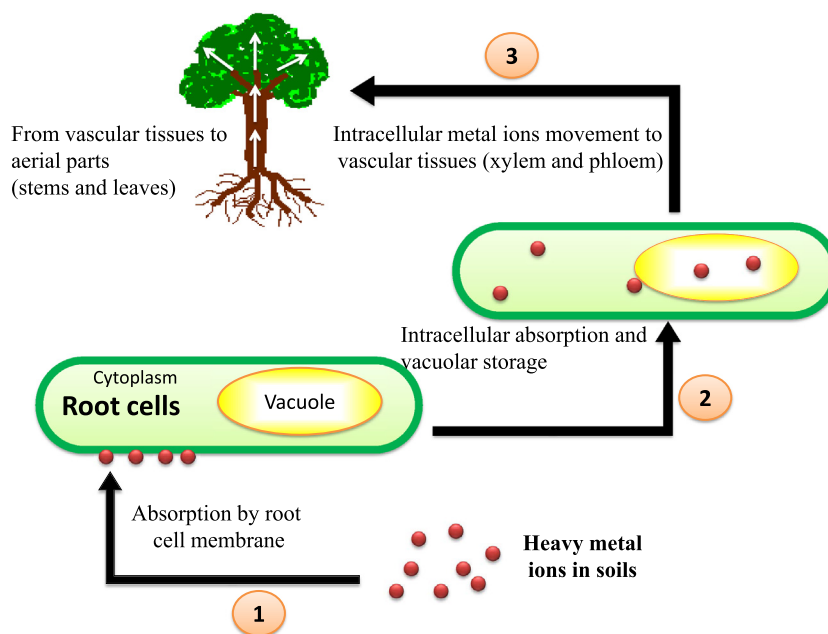
metalloids. However, limitation of application of this methylation related metal detoxification is that only some metals can be methylated (Ranjard et al., 2003; Rajkumar et al., 2010).

In addition, microorganisms can eliminate several heavy metals from the metal polluted soils by reducing them to a lower redox state (Lovley, 1995; Jing et al., 2007). Bacterial species that catalyze such reducing reactions are referred to as dissimilatory metal-reducing bacteria, exploit metals as terminal electron acceptors in anaerobic respiration; even though, most of them use  $\text{Fe}^{3+}$  and  $\text{S}^0$  as terminal electron acceptors (Lovley et al., 1997; Jing et al., 2007). For example,

the anaerobic or aerobic reduction of Cr(VI) to Cr(III) by an array of bacterial isolates is an effective means of chromium detoxification (Lovley, 1995; Wang and Shen, 1995; Jing et al., 2007). Moreover, metal-chelating agents, siderophores secreted by different bacteria too have an important role in the acquisition of several heavy metals (Rajkumar et al., 2010).

### 5. PGPB as bioremediating agents

Elevated levels of heavy metals in soils not only decrease soil microbial activity but also decrease crop production by accumulating in plant organs (Ahemad, 2012). These metals ions



**Figure 2** Graphical presentation of the movement of heavy metals in plants [modified from Jing et al. (2007)].

**Table 4** Plant growth promoting bacteria (PGPB) applied in heavy metal detoxification.

PGPB	Plant	Heavy metals	Conditions	Role of PGPB	References
<i>Pseudomonas</i> sp. A3R3	<i>Alyssum serpyllifolium</i> , <i>Brassica juncea</i>	Ni	Pots	Increased significantly the biomass ( <i>B. juncea</i> ) and Ni content ( <i>A. serpyllifolium</i> ) in plants grown in Ni-stressed soil	Ma et al. (2011a)
<i>Psychrobacter</i> sp. SRS8	<i>Ricinus communis</i> , <i>Helianthus annuus</i>	Ni	Pots	Stimulated plant growth and Ni accumulation in both plant species with increased plant biomass, chlorophyll, and protein content	Ma et al. (2011b)
<i>Bacillus</i> species PSB10	Chickpea ( <i>Cicer arietinum</i> )	Cr	Pots	Significantly improved growth, nodulation, chlorophyll, leghaemoglobin, seed yield and grain protein; reduced the uptake of chromium in roots, shoots and grains	Wani and Khan (2010)
<i>Bradyrhizobium</i> sp. 750, <i>Pseudomonas</i> sp., <i>Ochrobactrum cytisi</i>	<i>Lupinus luteus</i>	Cu, Cd, Pb	Fields	Increased both biomass, nitrogen content, accumulation of metals (improved phytostabilization potential)	Dary et al. (2010)
<i>Pseudomonas</i> sp. SRI2, <i>Psychrobacter</i> sp. SRS8, <i>Bacillus</i> sp. SN9	<i>Brassica juncea</i> , <i>Brassica oxyrrhina</i>	Ni	Pots	Increased the biomass of the test plants and enhanced Ni accumulation in plant tissues	Ma et al. (2009a)
<i>Psychrobacter</i> sp. SRA1, <i>Bacillus cereus</i> SRA10	<i>Brassica juncea</i> , <i>Brassica oxyrrhina</i>	Ni	Pots	Enhanced the metal accumulation in plant tissues by facilitating the release of Ni from the non-soluble phases in the soil	Ma et al. (2009b)
<i>Achromobacter xylosoxidans</i> strain Ax10	<i>Brassica juncea</i>	Cu	Pots	Significantly improved Cu uptake by plants and increased the root length, shoot length, fresh weight and dry weight of plants	Ma et al. (2009c)
<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Ralstonia metallidurans</i> <i>Pseudomonas</i> sp.	Maize Chickpea	Cr, Pb Ni	Pots Pots	Promoted plant growth, facilitated soil metal mobilization, enhanced Cr and Pb uptake Enhanced fresh and dry weight of plants even at 2 mM nickel concentration	Braud et al. (2009) Tank and Saraf (2009)
<i>Bacillus weihenstephanensis</i> strain SM3	<i>Helianthus annuus</i>	Ni, Cu, Zn	Pots	Increased plant biomass and the accumulation of Cu and Zn in the root and shoot systems, also augmented the concentrations of water soluble Ni, Cu and Zn in soil with their metal mobilizing potential	Rajkumar et al. (2008)
<i>Bacillus edaphicus</i>	Indian mustard ( <i>Brassica juncea</i> )	Pb	Pots	Stimulated plant growth, facilitated soil Pb mobilization, enhanced Pb accumulation	Sheng et al. (2008)
<i>Pseudomonas aeruginosa</i> strain MKRh3	Black gram	Cd	Pots	Plants showed lessened cadmium accumulation, extensive rooting, and enhanced plant growth	Ganesan (2008)
<i>Mesorhizobium</i> sp. RC3	Chickpea ( <i>Cicer arietinum</i> )	Cr (VI)	Pots	Increased the dry matter accumulation, number of nodules, seed yield and grain protein by 71%, 86%, 36% and 16%, respectively, compared to noninoculated plants. Nitrogen in roots and shoots increased by 46% and 40%, respectively, at 136 mg Cr/kg	Wani et al. (2008)
<i>Pseudomonas putida</i> KNP9	Mung bean	Pb, Cd	Greenhouse	Stimulated the plant growth, reduced Pb and Cd uptake	Tripathi et al. (2005)
<i>Pseudomonas aeruginosa</i>	Indian mustard and pumpkin	Cd	Pots	Stimulated plant growth, reduced Cd uptake	Sinha and Mukherjee (2008)
<i>Bradyrhizobium</i> sp. (vigna) RM8	Greengram ( <i>Vigna radiate</i> )	Ni	Pots	Enhanced the nodule numbers by 82%, leghaemoglobin by 120%, seed yield by 34%, grain protein by 13%, root N by 41% and shoot N by 37% at 290 mg Ni/kg soil	Wani et al. (2007a)

(continued on next page)

**Table 4** (continued)

PGPB	Plant	Heavy metals	Conditions	Role of PGPB	References
<i>Rhizobium</i> sp. RP5	Pea ( <i>Pisum sativum</i> )	Ni	Pots	Enhanced the dry matter, nodule numbers, root N, shoot N, leghaemoglobin, seed yield, and grain protein by 19%, 23%, 26%, 47%, 112%, 26%, and 8%, respectively, at 290 mg Ni/kg	Wani et al. (2007b)
<i>Methylobacterium oryzae</i> , <i>Berkholderia</i> sp.	<i>Lycopersicon esculentum</i>	Ni, Cd	Gnotobiotic conditions, pots	–	Madhaiyan et al. (2007)
<i>Azotobacter chroococcum</i> HKN-5, <i>Bacillus megaterium</i> HKP-1, <i>Bacillus mucillaginosus</i> HKK-1	<i>Brassica juncea</i>	Pb, Zn	Greenhouse	Protected plant from metal toxicity, stimulated plant growth	Wu et al. (2006)
<i>Bacillus subtilis</i> SJ-101	<i>Brassica juncea</i>	Ni	Growth chamber	Facilitated Ni accumulation	Zaidi et al. (2006)
<i>Sinorhizobium</i> sp. Pb002	<i>Brassica juncea</i>	Pb	Microcosms	Increased the efficiency of lead phytoextraction by <i>B. juncea</i> plants	Di Gregorio et al. (2006)
<i>Xanthomonas</i> sp. RJ3, <i>Azomonas</i> sp. RJ4, <i>Pseudomonas</i> sp. RJ10, <i>Bacillus</i> sp. RJ31	<i>Brassica napus</i>	Cd	Pots	Stimulated plant growth and increased cadmium accumulation	Sheng and Xia (2006)
<i>Pseudomonas</i> sp, <i>Bacillus</i> sp.	Mustard	Cr (VI)	Pots	Stimulated plant growth and decreased Cr (VI) content	Rajkumar et al. (2006)
<i>Ochrobactrum</i> , <i>Bacillus cereus</i>	Mungbean	Cr (VI)	Pots	Lowers the toxicity of chromium to seedlings by reducing Cr (VI) to Cr (III)	Faisal and Hasnain (2006)
<i>Brevibacillus</i>	<i>Trifolium repens</i>	Zn	Pots	Enhanced plant growth and nutrition of plants and decreased zinc concentration in plant tissues	Vivas et al. (2006)
<i>Variovox paradoxus</i> , <i>Rhodococcus</i> sp, <i>Flavobacterium</i>	<i>Brassica juncea</i>	Cd	<i>In vitro</i>	Stimulating root elongation	Belimov et al. (2005)
Bacterial strains A3 and S32	<i>Brassica juncea</i>	Cr	Pots	Promoted the plant growth under chromium stress	Rajkumar et al. (2005)
<i>Pseudomas fluorescens</i>	Soybean	Hg	Greenhouse	Increased plant growth	Gupta et al. (2005)
<i>Ochrobactrum intermedium</i>	Sunflower	Cr (VI)	Pots	Increased plant growth and decreased Cr(VI) uptake	Faisal and Hasnain (2005)
<i>Pseudomonas fluorescens</i> Avm, <i>Rhizobium leguminosarum</i> bv <i>phaseoli</i> CPMex46	Alfalfa	Cu	Growth chamber	Improved Cu and Fe translocation from root to shoot	Carrillo-Castaneda et al. (2003)
<i>Pseudomonas</i> sp.	Soybean, mungbean, wheat	Ni, Cd, Cr	Pots	Promotes growth of plants	Gupta et al. (2002)
<i>Brevundimonas</i> Kro13	–	Cd	Culture media	Sequestered cadmium directly from solution	Robinson et al. (2001)
<i>Kluyvera ascorbata</i> SUD165	Indian mustard, canola, tomato	Ni, Pb, Zn	Growth chamber	Both strains decreased some plant growth inhibition by heavy metals, No increase of metal uptake with either strain over non-inoculated plants	Burd et al. (2000)



are excessively absorbed by roots and translocated to different plant organs and tissues (Fig. 2). Further, a number of mercapto ligands present in enzymes and proteins of plant cells have affinity for heavy metals and chelate them. Due to this interaction, proteins generally, lose their functional traits. Modification of structure of several essential proteins by metallic stress, thus results in chlorosis, growth impairment, browning of roots, and inactivation of photosystems in plants (Shaw et al., 2004; Gorhe and Paszkowski, 2006). Moreover, metals also generate oxidative stress by the production of free radicals (Seth et al., 2008) in turn; they adversely affect biochemical and physiological processes by impairing photosynthetic and respiratory reactions which subsequently bring about the overall decline in plant growth and development (Vangronsveld and Clijsters, 1994). As explained above, microorganisms including PGPB which are continuously exposed to heavy metal stress have adapting mechanisms to the metal contaminants (Munoz et al., 2006). Bacteria respond to these molecules by diverse biological processes like, transportation across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation–reduction (Singh et al., 2010). The bacterial response to a specific heavy metal is of great significance in exploiting them in the remediation of metal contaminated sites (Hemambika et al., 2011). Although PGPB has been used largely as growth promoting agents in agronomic practices, substantial emphasis is being placed on them in order to exploit their metal detoxifying potential in phytoremediation (phytoextraction and phytostabilization) of metal contaminated soils using as bioinoculants (Ahemad, 2014b). For example, Abou-Shanab et al. (2003) reported that inoculation of *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens*, and *Microbacterium arabinogalactanolyticum* to *Alyssum murale* plants appreciably increased Ni uptake by plants when compared to the un-inoculated control on account of decline in soil pH. Similarly, Carrillo-Castaneda et al. (2003) reported the potential of plant growth promoting *Pseudomonas fluorescens* Avm, *R. leguminosarum* CPMex46, *Azospirillum lipoferum* UAP40 and UAP154 in protecting alfalfa *Medicago sativa* seeds from the copper toxicity. This stimulatory effect was attributed to expedite the iron translocation by bacteria from roots to shoots in the seedlings. In other study, Dimkpa et al. (2009) found that the hydroxamate siderophores increased the iron uptake by plants despite of the presence of heavy metals (such as Al, Cu, Mn, Ni and U). Moreover, siderophores secreted by these PGPB strains reduced the free radical formation by binding the heavy metals around the roots, in this manner, protecting microbially secreted auxins from oxidative damage and consequently, enabling them to promote the plant growth. Correspondingly, the inoculation with the lead and cadmium resistant *Pseudomonas putida* KNP9 significantly increased *Phaseolus vulgaris* growth protecting them from lead and cadmium toxicity compared to controls (Tripathi et al., 2005). Inoculation with other PGPB like, *Pseudomonas* sp. Ps29C and *Bacillus megaterium* Bm4C isolated from nickel contaminated soils significantly reduced the toxicity of nickel in *Brassica juncea* and augmented the plant growth significantly. In this study, it was suggested that plant growth-promoting traits such as, the production of phytohormones, siderophores and l-aminocyclopropane-l-carboxylic acid deaminase was responsible for the increase in the plant growth (Rajkumar and Freitas, 2008). Consistent

with the similar findings, Barzanti et al. (2007) observed that bacteria facilitated plant growth under Ni stress. Taken as a whole, these studies evidently pointed out the potential of inoculation of PGPB to increase plant biomass under heavy metal stress. Some other examples regarding the bioremediation of heavy metals by PGPB have been shown in Table 4. Thus, the application of metal detoxifying PGPB coupled with other plant growth promoting activities typically makes the entire remediation process more efficient to a great extent (Glick, 2012).

## 6. Conclusion

PGPB exhibiting multiple plant health and development enhancing traits coupled with the excellent potential to lower down the heavy metal stress in soils, may eventually find wide-ranging applications in the development of bioremediation strategies for heavy metal decontamination. In heavily contaminated soils where the metal content exceeds the limit of plant tolerance, it may be possible to treat plants with PGPB thereby stabilizing, re-vegetating, and remediating metal-polluted soils. In addition, the application of the heavy metal resistant and plant-beneficial bacteria can be considered as bioremediating tools with great economical and ecological relevance.

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