Environ Chem Lett
DOI 10.1007/s10311-008-0155-0

REVIEW

Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils

Mohammad Saghir Khan · Almas Zaidi ·
Parvaze Ahmad Wani · Mohammad Oves

Received: 7 November 2007 / Accepted: 4 April 2008

Abstract Pollution of the biosphere by the toxic metals is a global threat that has accelerated dramatically since the beginning of industrial revolution. The primary source of this pollution includes the industrial operations such as mining, smelting, metal forging, combustion of fossil fuels and sewage sludge application in agronomic practices. The metals released from these sources accumulate in soil and in turn, adversely affect the microbial population density and physico-chemical properties of soils, leading to the loss of soil fertility and yield of crops. The heavy metals in general cannot be biologically degraded to more or less toxic products and hence, persist in the environment. Conventional methods used for metal detoxification produce large quantities of toxic products and are cost-effective. The advent of bioremediation technology has provided an alternative to conventional methods for remediating the metal-poisoned soils. In metal-contaminated soils, the natural role of metal-tolerant plant growth promoting rhizobacteria in maintaining soil fertility is more important than in conventional agriculture, where greater use of agrochemicals minimize their significance. Besides their role in metal detoxification/removal, rhizobacteria also promote the growth of plants by other mechanisms such as production of growth promoting substances and siderophores. Phytoremediation is another emerging low-cost in situ technology employed to remove pollutants from the contaminated soils. The efficiency of phytoremediation can be enhanced by the judicious and careful application of appropriate heavy-metal tolerant, plant growth promoting rhizobacteria including symbiotic nitrogen-fixing organisms. This review presents the results of studies on the recent developments in the utilization of plant growth promoting rhizobacteria for direct application in soils contaminated with heavy metals under a wide range of agro-ecological conditions with a view to restore contaminated soils and consequently, promote crop productivity in metal-polluted soils across the globe and their significance in phytoremediation.

Keywords Plant growth promoting rhizobacteria · Symbiotic nitrogen fixing organisms · Heavy metals · Bioremediation · Phytoremediation · Rhizoremediation · Growth regulating substances

Introduction

Release of heavy metals from various industrial sources, agrochemicals and sewage sludge present a major threat to the soil environment. Generally, heavy metals are not degraded biologically and persist in the environment indefinitely. Once accumulated in the soils, the toxic metals inversely affect the microbial compositions, including plant growth promoting rhizobacteria (PGPR) in the rhizosphere, and their metabolic activities. In addition, the elevated concentration of metals in soils and their uptake by plants adversely affect the growth, symbiosis and consequently the yields of crops (Moftah 2000; Wani et al. 2007a, 2008a) by disintegrating cell organelles, and disrupting the membranes (Sresty and Madhava Rao 1999), acting as genotoxic substance (Sharma and Talukdar 1987) disrupting the physiological process, such as, photosynthesis (Van Assche and Clijstersters 1990; Wani et al. 2007b), or by inactivating the respiration, protein synthesis and

---

M. S. Khan (✉) · A. Zaidi · P. A. Wani · M. Oves
Department of Agricultural Microbiology,
Faculty of Agricultural Sciences, Aligarh Muslim University,
Aligarh 202002, India
e-mail: khanms17@rediffmail.com

carbohydrate metabolism (Shakolnik 1984). The remediation of metal-contaminated soils thus becomes important, as these soils usually cover large areas that are rendered unsuitable for sustainable agriculture.

To circumvent the metal stress, microorganisms of agronomic importance have evolved a number of mechanisms, which they use to tolerate the uptake of heavy metal ions. Such mechanisms include (1) the pumping of metal ions exterior to the cell (2) accumulation and sequesteration of the metal ions inside the cell (3) transformation of toxic metal to less toxic forms (Wani et al. 2008b) and adsorption/desorption of metals (Mamaril et al. 1997). Due to these properties, when plant growth promoting rhizobacteria including nitrogen fixers, used as seed inoulant, were applied to soil, either treated/amended intentionally with metals or already contaminated, have shown a substantial reduction in the toxicity of metals and concomitantly improved the overall growth and yield of chickpea (*Cicer arietinum*) (Gupta et al. 2004), greengram (*Vigna radiata* L. wilczek) (Wani et al. 2007a) and pea (*Pisum sativum*) (Wani et al. 2007c). Besides their role in protecting the plants from metal toxicity, the plant growth promoting rhizobacteria are also well known for their role in enhancing the soil fertility and promoting crop productivity by providing essential nutrients (Zaidi et al. 2003, 2004; Zaidi and Khan 2006) and growth regulators (Wani et al. 2007d, e). They also promote the growth of plants by alleviating the stress induced by ethylene-mediated impact on plants (Glick et al. 2002) by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Ma et al. 2003; Uchiumi et al. 2004; Belimov et al. 2005). The use of such microbes possessing multiple properties of metal resistance/reduction and ability to promote plant growth through different mechanisms in metal-contaminated soils make them one of the most suitable choices for bioremediation studies.

The other alternative approach used to clean up the contaminated soils includes the plants, the innovative technique being known as phytoremediation (Brooks 1998). This technology involves the use of metal-accumulating plants to remove, transfer, or stabilize the contaminants from soils; but this technique is time consuming (Wenzel et al. 1999). The efficiency of phytoremediation technique is, however, influenced by the activity of rhizosphere microbes and the speciation and concentration of metals deposited into soil (Wang et al. 1989; Khan 2005a, b). For instance, use of plant growth promoting rhizobacteria *Pseudomonad* and *Acinetobacter* have shown to enhance phytoremediation abilities of non-hyperaccumulating maize (*Zea mays* L.) plants by increasing their growth and biomass (Lippmann et al. 1995). Also, plants growing in metal-stressed soils can protect themselves from metal toxicity by synthesizing antioxidant enzymes, which scavenges the toxicity of reactive oxygen species generated by plants (Cardoso et al. 2005) and by associative bacteria (Corticeiro et al. 2006) under metal stress.

Growth promotion by plant growth promoting rhizobacteria

The rhizosphere bacteria capable of aggressively colonizing plant roots and promoting plant growth are generally called as plant growth promoting rhizobacteria (Kloepper and Schroth 1978). Broadly, plant growth promoting rhizobacteria can be divided into two major groups according to their relationship with the host plants: (1) symbiotic rhizobacteria and (2) free-living rhizobacteria (Khan 2005a, b), which could invade the interior of cells and survive inside intracellular PGPR (e.g., nodule bacteria), or remain outside the plant cells, extracellular PGPR (e.g., *Bacillus, Pseudomonas, Azotobacter* etc.). These organisms affect plant growth in three different ways—(1) by synthesizing and providing particular compounds to the plants (Glick 1995) (2) facilitating the uptake of certain nutrients from environment (Çakmakçi et al. 2006) and (3) protecting plants from certain diseases (Khan et al. 2002). Generally, rhizobacteria improves plant growth by synthesizing phytohormones precursors (Perveen et al. 2002; Ahmad et al. 2008), vitamins, enzymes, siderophores, antibiotics (Burd et al. 2000; Glick 2001) and inhibiting ethylene synthesis. In addition, the rhizobacterial strains can solubilize inorganic P (Zaidi and Khan 2005, 2007; Khan and Zaidi 2007), mineralizing organic P (Ponmurugan 2006; Khan et al. 2007), improve plant stress tolerance to drought, salinity and metal toxicity, leading thereby to increased plant growth. The growth promoting substances synthesized by various rhizobacteria are summarized in Table 1. Moreover, the plant growth promoting rhizobacteria also increase the growth of plants through the synthesis of specific enzymes, which induce physiological changes in plants. For example, ethylene plays a critical role in various developmental processes, such as leaf senescence, leaf abscission, epinasty and fruit ripening (Vogel et al. 1998). Also, ethylene regulates node factor signaling and nodule formation and has primary functions in plant defense systems. Moreover, ethylene production increases as a result of plant infection by rhizobacteria (Schmidt et al. 1999). At higher concentrations, ethylene inhibits growth and development of plants (Grichko and Glick 2001); however, bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase synthesized by plant growth promoting rhizobacteria (Belimov et al. 2005; Safronova et al. 2006; Madhaiyan et al. 2006; Rajkumar et al. 2006) alleviates the stress induced by ethylene-mediated impact

Table 1 Growth regulators produced by plant growth promoting rhizobacteria

Organisms	Growth regulators	References
Azotobacter, Fluorescent pseudomona, and *Bacillus*	IAA, siderophore, ammonia, HCN, P-solubilization	Ahmad et al. (2008)
Bacillus spp.	IAA, P solubilization, siderophores, HCN, ammonia	Wani et al. (2007d)
Bacillus spp.	IAA, P solubilization, siderophores, HCN, ammonia	Wani et al. (2007e)
Azotobacter chroococcum	IAA, siderophores, HCN, ammonia	Wani et al. (2007d)
Pseudomonas and *Bacillus*	Siderophores, IAA, P-solubilization	Rajkumar et al. (2006)
Brevibacillus sp.	IAA	Vivas et al. (2006)
Bravibacterium sp.	Siderophore	Noordman et al. (2006)
Xanthomonas sp. *RJ3*, *Azomonas sp. RJ4*, *Pseudomonas sp. RJ10*, *Bacillus sp. RJ31*	IAA	Sheng and Xia (2006)
Bacillus subtilis	IAA and P-solubilization	Zaidi et al. (2006)
Bacillus sp.	P-solubilization	Canbolat et al. (2006)
Variovorax paradoxus, *Rhodococcus* sp. and *Flavobacterium* (Cd tolerant)	IAA and siderophores	Belimov et al. (2005)
Kluyvera ascorbata	Siderophore	Burd et al. (2000)
Pseudomonas fluorescens	IAA, siderophore and P-solubilization	Gupta et al. (2005)
Pseudomonas putida	Siderophore	Tripathi et al. (2005)
Sphingomonas sp, *Mycobacterium* sp, *Bacillus* sp, *Rhodococcus* sp, *Cellulomonas* sp. and *Pseudomonas* sp.	IAA	Tsavkelova et al. (2005)
Azotobacter, Fluorescen t*pseudomonas*	IAA	Ahmad et al. (2005)
Seratia spp, *Pseudomonas spp and Bacillus spp.*	IAA, P-solubilization	Wani et al. (2005)
Bacillus and *Azospirillum* sp.	IAA, P-solubilization	Yasmin et al. (2004)
Micrococcus luteus	IAA, P-solubilization	Antoun et al. (2004)
Bacillus, *Pseudomons*, *Azotobacter*, *and Azospirillum*	P-solubilization and IAA	Tank and Saraf (2003)
Pseudomonas sp.	IAA, siderophore and P-solubilization	Gupta et al. (2002)
Pseudomonas fluorescence	Siderophore	Khan et al. (2002)
Azotobacter chroococcum	Gibberellin, kinetin, IAA	Verma et al. (2001)
Azotobacter chroococcum	P-solubilization	Kumar et al. (2001)

on plants (Glick et al. 2002). The ACC of roots is metabolized by ACC deaminase to ketobutyrate and ammonia (Penrose and Glick 2001). The bacteria utilize the NH_3 evolved from ACC as a source of N and thereby restrict the accumulation of ethylene within the plant, which otherwise inhibits plant growth (Yang and Hoffman 1986; Belimov et al. 2002).

Among other plant growth promoting rhizobacteria, the symbiotic nitrogen fixers enhance the growth of legumes by the following: (1) providing N to the plants through N_2 fixation (Zaidi et al. 2004) (2) increasing the availability of nutrients in the rhizosphere (3) inducing increases in root surface area (4) enhancing other beneficial symbioses of the host (5) reducing or preventing the deleterious effects of phyto-pathogenic organisms (Khan et al. 2002) and (6) by the combination of modes of action. As an example of plant growth promoter, indoleacetic acid (IAA), phytohormone of the auxin series produced by many rhizobia (Abd-Alla 1994; Wani et al. 2007f, 2007g, 2008b), and its metabolically related precursor, anthranilic acid, can reductively solubilize soil Fe (III), and increase its availability via a mechanism different from that involving siderophores (Kamnev 1998; Kamnev et al. 1999b). In a study, Leinhos and Bergmann (1995) and Lippmann et al. (1995) reported that the addition of IAA to soil enhanced the uptake of iron and other elements (e.g. zinc, calcium etc.) in plant roots. Another growth promoting substance, siderophores, is a specific Fe (III)-chelating agent that makes the chelated iron unavailable to pathogenic microorganisms (Briat 1992; Braun 1997) and leads to an increase in plant health (Wang et al. 1993). Microbial siderophores are known to regulate the availability of Fe in the plant rhizosphere (Loper and Henkels 1999) and it has been found that competition for iron in the rhizosphere is controlled by the affinity of the siderophores for iron. Interestingly, the binding affinity of phyto-siderophores for iron is less than the affinity of microbial siderophores, but plants require a lower iron concentration for normal growth than do microbes (Meyer 2000). Though, several rhizobial species are known to produce growth promoting substances

Table 2 Plant growth promoting substances produced by symbiotic nitrogen fixers both in metal free and metal stressed environment

Symbiotic N_2 fixers	Heavy metal	Plant growth promoting substances	References
Rhizobium, *Bradyrhizobium*	–	P-solubilization	Abd-Alla (1994)
Bradyrhizobium japonicum	–	Siderophore	Wittenberg et al. (1996)
Rhizobium leguminosarum	–	Cytokinin	Noel et al. (1996)
Rhizobium ciceri	–	Siderophopre	Berraho et al. (1997)
Bradyrhizobium, Rhizobium	–	Siderophore	Duhan et al. (1998)
Bradyrhizobium, Rhizobium	–	IAA	Antoun et al. (1998)
Rhizobium meliloti	–	Siderophore	Arora et al. (2001)
Mesorhizobium, Bradyrhizobium sp. (*vigna*)	–	Siderophore	Khan et al. (2002)
Rhizobium	–	HCN, siderophore	Deshwal et al. (2003)
Bradyrhizobium (*Arachis*)	–	Siderophore, IAA and P-solubilization	Deshwal et al. (2003)
Rhizobium	–	P-solubilization and IAA	Tank and Saraf (2003)
Bradyrhizobium, Rhizobium	–	IAA, P-solubilization	Antoun et al. (2004)
Bradyrhizobium japonicum	–	IAA	Shaharoona et al. (2006)
Bradyrhizobium sp. RM8	Nickel, zinc	IAA, siderophore, ammonia, HCN	Wani et.al. (2007f)
Rhizobium sp. RP5	Nickel, zinc	IAA, siderophore	Wani et.al. (2007c))
Rhizobium sp. RL9	Zinc	IAA, siderophore, ammonia, HCN	Wani et.al. (2007g)
Mesorhizobium sp. RC3	Chromium (vi)	IAA, siderophore	Wani et.al. (2008b)
Mesorhizobium	–	IAA, siderophore, ammonia, HCN, P-solubilization	Ahmad et al. (2008)

under metal-free environment, the synthesis of these compounds by metal-tolerant rhizobia are limited. Nevertheless, there has been certain evidence where metals at lower concentrations either exert no harmful effect on the rhizobia or even stimulate plant growth promoting activities. For instance, *Bradyrhizobium* strain RM8, tolerant to nickel and zinc; *Rhizobium* sp. RL9, isolated from lentil nodules, tolerant to zinc; and *Rhizobium* sp. RP5, isolated from pea nodules, tolerant to zinc and nickel, produced substantial amounts of IAA (Wani et al. 2007c, f, g). The production of growth-promoting substances by metal-tolerant and natural rhizobial strains is presented in Table 2.

Biological availability of metals in soil

Heavy metals such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury are discharged from industrial operations such as smelting, mining, metal forging, manufacturing of alkaline storage batteries, and combustion of fossil fuel. Moreover, the agricultural activities like application of agrochemicals, and long-term usage of sewage sludge in agricultural practices also add significant amounts of metals to the soils (Giller et al. 1989; McGrath et al. 1995). These metals exist in bioavailable and non-bioavailable forms (Sposito 2000) whose mobility depends on two factors: (1) the metallic element that precipitates as positively charged ions (cations) and (2) the one, which makes up negatively charged component of salt. Physico-chemical properties of soils, such as cation exchange capacity (CEC), organic matter, clay minerals and hydrous metal oxides, pH and buffering capacity, redox potential and extent of aeration, water content and temperature, together with root exudates and microbial activities determines the metal availability in soils (Brown et al. 1999; Traina and Laperche 1999; Krishnamurthy 2000). The toxicity of metals within soils with high CEC is generally low even at high total metal concentrations (Roane and Pepper 2000). Under oxidized and aerobic conditions, metals are usually found in soluble cationic forms while in reduced or anaerobic conditions, as sulphide or carbonate precipitates. At low soil pH, the metal bioavailability increases due to its free ionic species, while at high soil pH it decreases due to insoluble metal mineral phosphate and carbonate formation. The mobility and bioavailability of certain metals in soils is usually in the order: Zn > Cu > Cd > Ni (Lena and Rao 1997). However, the concentration of heavy metals within all components of the ecosystems varies considerably. Coexistence and persistence of metals in soils as multiple contaminants facilitate the entry and accumulation of these pollutants into food webs and ultimately into the human diets. Contamination of agronomic soils with heavy metals (both by single or combination of metals) has thus become a global threat to the sustainability of the agro-ecosystems and therefore, is receiving considerable attention from the environmentalists. Therefore, the assessment of heavy metal bioavailability and uptake of metals by plants help in -(1) evaluating the impact of metals on beneficial rhizospheric microbes and crops grown in metal-stressed soils and (2) in predicting the application of bioremediation technologies that could be used to clean up metals from the polluted soils. The remediation of such soils therefore, requires urgent attention, so that the sustainability of crops and in turn, the food security across the globe, could be protected.

How plant growth promoting rhizobacteria combat heavy-metal stress?

Accumulation of heavy metals in the soil environment and their uptake by both plant growth promoting rhizobacteria and plants is a matter of growing environmental concern. Unlike many other pollutants, which can undergo biodegradation and produce less toxic, less mobile and/or less bio-available products, heavy metals are difficult to be removed from contaminated environment. These metals cannot be degraded biologically, and are ultimately indestructible, though the speciation and bioavailability of metals may change with variation in the environmental factors. Some metals such as, zinc, copper, nickel and chromium are essential or beneficial micronutrients for plants, animals and microorganisms (Olson et al. 2001[t3]) while others (e.g., cadmium, mercury and lead) have no known biological and/or physiological functions (Gadd 1992). However, the higher concentration of these metals has great effects on the microbial communities in soils in several ways- (1) it may lead to a reduction of total microbial biomass (Giller et al. 1998) (2) it decreases numbers of specific populations (Chaudri et al. 1993) or (3) it may change microbial community structure (Gray and Smith 2005). Thus, at high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic activities (Fig. 1) or organisms can develop resistance or tolerance to the elevated levels of metals. The ability to grow even at high metal concentration is found in many rhizospheric microorganisms including symbiotic N_2 fixing bacteria (Lakzian et al. 2002) and may be the result of intrinsic or induced mechanism (Giller et al. 1998). Tolerance may be defined as the ability to cope with metal toxicity by means of intrinsic properties of the microorganisms, while resistance is the ability of microbes to survive in higher concentrations of toxic metals by detoxification mechanisms, activated in direct response to the presence of heavy metals (Ledin 2000). Toxic heavy metals therefore, need to be either completely removed from the contaminated soil, transformed or to be immobilized, producing much less or non-toxic species. However, in order to survive and proliferate in metal contaminated soils, tolerance has to be present both in microbes and their associative hosts. For survival under metal-stressed environment, plant growth promoting rhizobacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions (Nies 1999). These mechanisms include (1) exclusion—the metal ions are kept away from the target sites (2) extrusion—the metals are pushed out of the cell through chromosomal/plasmid mediated events (3) accommodation—metals form complex with the metal-binding proteins (e.g. metallothienins, a low molecular weight proteins) (Kao et al. 2006; Umrania 2006) or other cell components (4) bio-transformation—toxic metal is reduced to less toxic forms and (5) methylation and demethylation. One or more of these defense mechanisms allows these microorganisms to function metabolically in environment polluted by metals. These mechanisms could be constitutive or inducible. The bacterial resistance mechanisms are encoded generally on plasmids and transposons, and it is probably by gene transfer or

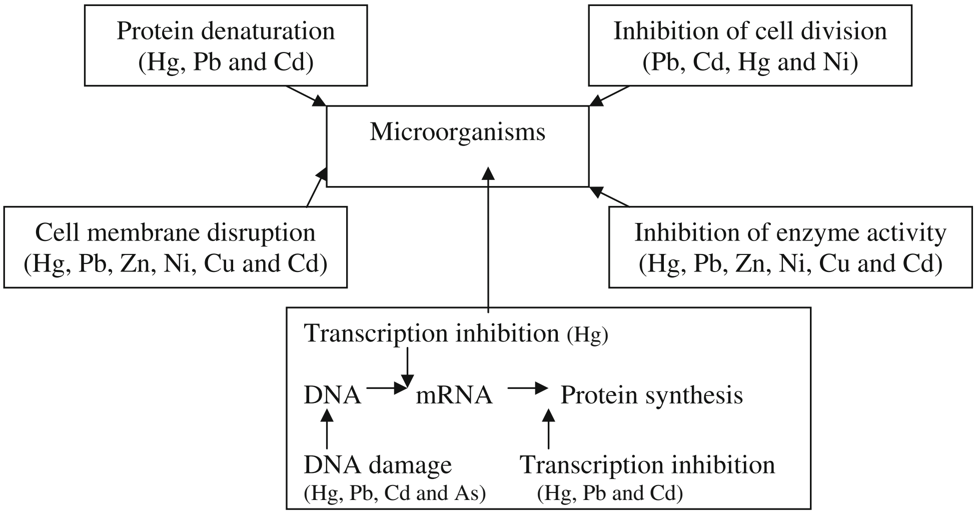


Fig. 1 Heavy metal toxicity mechanisms to microbes

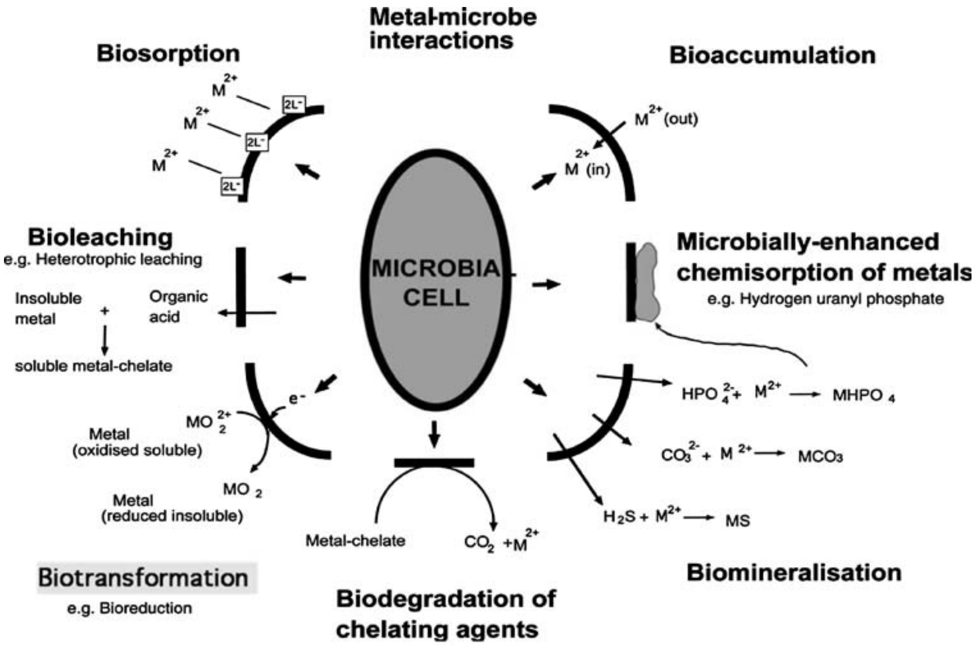


Fig. 2 Metal–microbe interactions affecting bioremediation (Tabak et al. 2005)

spontaneous mutation that bacteria acquire their resistance to heavy metals. For example, in Gram-negative bacteria (e.g. *Ralstonia eutropha*), the *czc* system is responsible for the resistance to cadmium, zinc and cobalt. The *czc*-genes encode for a cation-proton antiporter (CzcABC), which exports these metals (Nies 1999). A similar mechanism, called *ncc* system, has been found in *Alcaligenes xylosoxidans,* which provides resistant against nickel, cadmium and cobalt. On the contrary, the cadmium resistance mechanism in Gram-positive bacteria (e.g. *Staphylococcus*, *Bacillus* or *Listeria)* is through Cd-efflux ATPase. Plasmid-encoded energy dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are also reported for arsenic, chromium and cadmium resistance in other plant growth promoting rhizobacteria (Roane and Pepper 2000). The exploitation of these bacterial properties for the remediation of heavy metal-contaminated sites has been shown to be a promising bioremediation alternative (Lovley and Coates 1997; Lloyd and Lovley 2001). Though, the threshold limit of metal toxicity to soil microorganisms is not conclusive, yet the interaction between heavy metals and microbes do occur in nature. Microorganisms can interact with metals via many mechanisms (Fig. 2), some of which may be used as the basis of potential bioremediation strategies.

Bioremediation: a natural method for the restoration of derelict soils

Conventional approaches employed for the remediation of metals from contaminated sites are presented in Fig. 3. These methods include, (1) land filling—the excavation, transport and deposition of contaminated soil in a permitted hazardous waste land (2) fixation—the chemical processing of soil to immobilize the metals, usually followed by treatment of the soil surface to eliminate penetration by water and (3) leaching—using acid solutions as proprietary leaching agent to distort and leach metals from soil followed by the return of clean soil residue to site (Krishnamurthy 2000). The applications of these processes are sometimes restricted, due to the technological or economical constrains. Therefore, the search for alternative methods to restore polluted soils in an inexpensive, less labor intensive, safe and environment friendly manner is required. Such an alternative method is bioremediation, which is defined as the action of microbes or other biological systems to degrade/transform environmental pollutants under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Muller et al. 1996). Bioremediation can be applied in situ without the removal and transport of contaminated soils and without the disturbance of soil matrix or can be applied ex situ to soil at the site, which has been removed from the site via excavation.

Therefore, managing the microbial populations in the rhizosphere by using microbial inoculum consisting of a consortium of plant growth promoting rhizobacteria and symbiotic nitrogen fixers as allied colonizers and biofertilizers, could provide plants with benefits crucial for ecosystem restoration on derelict lands (Khan 2004). These microorganisms may be indigenous to a contaminated area (intrinsic bioremediation) or can be isolated from elsewhere and then introduced into the contaminated sites

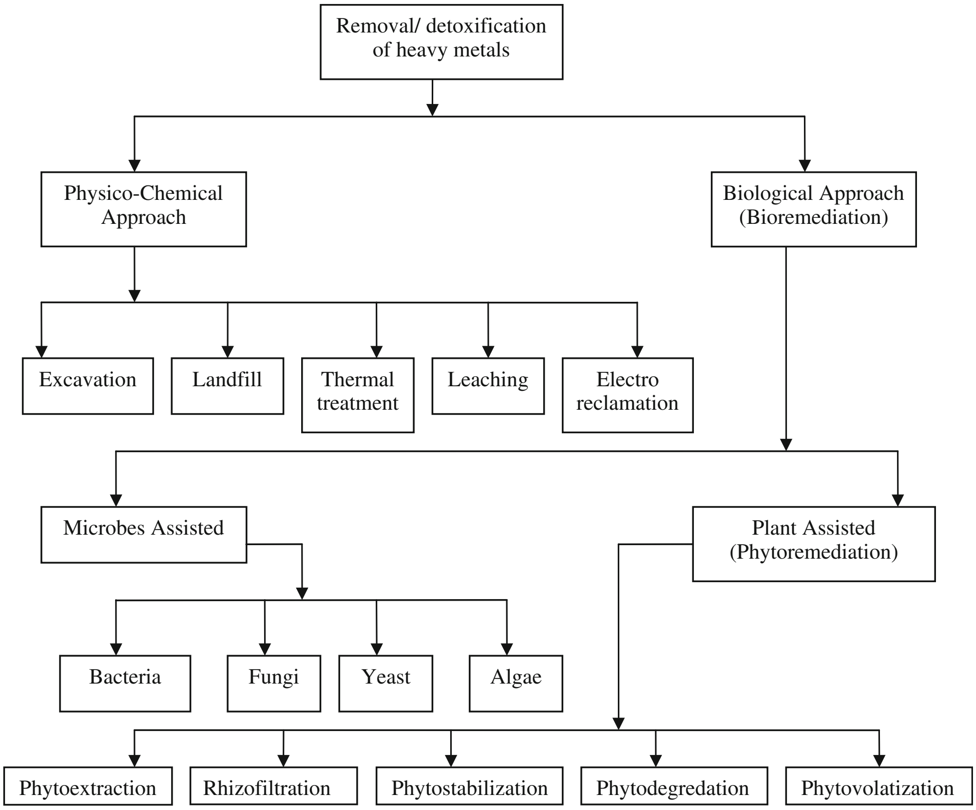


Fig. 3 Approaches employed for the remediation of heavy metals from contaminated soil

(bioaugmentation). Bioremediation depends on the functionality of organisms in the rhizosphere and the environmental conditions amenable for their growth. Advances in understanding the role of microorganisms in such processes, together with the ability to fine-tune their activities using the tools of molecular biology, has led to the development of novel or improved metal bioremediation processes.

Advantages and limitations of bioremediation

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it is inexpensive, low-technology technique, which generally has a high public acceptance and can consistently be carried out on contaminated sites, often without affecting the fertility of soils or the metabolic activities of microbes. This property of remediation help to avoid the transport of waste, off site and consequently the potential threats to human health and the environment that could arise during transportation. Furthermore, bioremediation can be useful for remediation of variety of contaminants leading to the complete destruction and when the contaminants are transformed/degraded, the toxicity of contaminants declines. Bioremediation technologies also have certain disadvantages, like the products of biodegradation may be more persistent or toxic than the parent compound; since biological processes are often specific, they require active and specific microbial communities whose success depends on nutrient status of soil, and levels of contaminants in the sites to be remediated; it is a time-consuming process and has problems in transferring of success from lab to field environment. Since, bioremediation seems to be a good alternative to conventional clean up technologies, research in this field is rapidly increasing. However, there is still urgent need of molecular engineering of microbes, so that they could be manipulated for better performance and wider application under diverse agro-climatic conditions.

Plant growth promoting rhizobacteria-assisted remediation of heavy metals

Rhizosphere, with high concentration of nutrients exuded from the roots, attracts more bacteria compared to non-rhizosphere soils (Penrose and Glick 2001). These bacteria including plant growth promoting rhizobacteria, in turn facilitate the growth of the plant and this phyto-bacteria system has been proved to be more effective in minimizing the bioavailability and biotoxicity of heavy metals (Khan 2005a, b; Wani et al. 2008b). The plant growth promoting rhizobacteria, though, has been used largely as a growth-promoting agent in agronomic practices; substantial emphasis is being placed on them in order to exploit their bioremediation potential as well. The removal of metals from contaminated soils by plant growth promoting rhizobacteria can be carried out by artificial introduction of viable population to contaminated sites (bioaugumentation), stimulation of viable native microbial population (biostimulation), biotransformation, bioreduction, bioaccumulation, and biosorption. In recent times, new metal treatment and recovery techniques based on biosorption have been explored using both dead and living microbial biomass with considerable success. Generally, prokaryotic microbes accumulate metals by binding them as cations to the cell surface in a passive process. In this context, biosorption of metals by the plant growth promoting rhizobacterial strains has been studied extensively (Volesky and Holan 1995; Lloyd and Macaskie 2000). For example, Hernandez et al. (1998), isolated three species of bacteria belonging to family enterobacteriaceae, which were capable of accumulating nickel and vanadium. Other potential alternative technologies currently in use involving surface complexation for metal removal include ion exchange and microprecipitation. In a study, cadmium, copper, selenium and zinc were reported to be biosorbed by *Streptococcus faecalis*, *Streptococcus aureus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Serratia marscecens*, in the mixtures of Gram positive and Gram negative bacteria. Generally, in Gram-positive bacteria, surface complexation occurs between organic P groups in cell surface teichoic acid and metal contaminants. For example, uranium (VI) phosphate solids are the least soluble of all the uranium (VI) solid phases. By contrast, Gram-negative bacteria appear to have a lesser ability to sorb uranium, possibly because they lack these cell-surface organic P groups. One of the most common surface structures found both in bacteria and archaea is a crystalline proteinaceous surface layer called the S-layer, which attenuates the sorption ability of Gram-positive bacteria.

Furthermore, heavy metals in general, cannot be destroyed biologically and hence, persist in the environment. However, microorganisms can transform a wide variety of multivalent metals that pose major threat to the environment. In this regard, numerous strains of plant growth promoting rhizobacteria possessing metal reducing ability have been identified (Faisal and Hasnain 2005). As an example, among the different forms of chromium, the hexavalent form of chromium is more toxic and carcinogenic (McLean and Beveridge 2001) due to its high solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular macromolecules (Kamaludeen et al. 2003). Reduction of toxic hexavalent chromium to trivalent form of chromium is considered as a useful process for remediation of chromium contaminated soil environments. The reduction/detoxification of hexavalent chromium by microbes is a low cost process and environmentally safe approach and provides a viable option to protect the soil environment from chromium toxicity. In this regard, numerous chromium reducing plant growth promoting rhizobacteria, for example, *Ochrobacterium intermedium* (Faisal and Hasnain 2005), *Pseudomonas* sp. (Rahman et al. 2007), *Bacillus* spp. (Wani et al. 2007h) and *Mesorhizobium* sp. (Wani et al. 2008b) have been reported.

Recently, the inoculation effects of plant growth promoting rhizobactera *Methylobacterium oryzae* strain CMBM20 and *Burkholderia* sp. strain CMBM40, isolated from rice (*Oryza sativa*) tissues, on toato (*Solanum tuberosum*), grown in nickel and cadmium-treated soil was studied (Madhaiyan et al. 2007). These bacterial strains significantly reduced the toxicity of both metals in tomato and promoted the plant growth under gnotobiotic and pot culture conditions. It was concluded from this study that, the bacterial strains reduced the uptake and consequent translocation of these metals to shoots and also synthesized phytohormones and ACC deaminase, which together accounted for increased growth of the test plant. In other study, a strain of *Pseudomonas maltophilio* transformed the mobile and toxic form of chromium (Cr VI) to non-toxic and immobile form (Cr III) and also minimized the mobility of other toxic ions, such as Hg^{2+}, Pb^{2+} and Cd^{2+} (Blake et al. 1993; Park et al. 1999). From these and other studies, it seems reasonable to believe that the plant growth promoting rhizobacteria could be developed as inoculants to increase plant biomass and thereby to stabilize, re-vegetate and re-mediate metal-polluted soils. Recent examples of the bioremediation of heavy metals by plant growth promoting rhizobacteria are shown in Table 3. Despite all these, there are certain issues that need to be addressed. These are, (1) how do microorganisms induce changes in the rhizosphere and influene metal accumulation? (2) How do microbes select a particular metal for removal/detoxification from a pool of multiple metals in the contaminated sites? and, (3) how do microorganisms mobilize and affect the transfer of metals to different organs of plants?

Rhizoremediation by symbiotic nitrogen fixing organisms

The use of plants for rehabilitation of heavy-metal-contaminated soil is an emerging area of interest because it provides an ecologically sound and safe method for restoration and remediation of polluted soils. Although numerous plant species are capable of hyperaccumulation of heavy metals, the technology is not adequate for remediating sites with multiple contaminants. A meaningful

Table 3 Examples of plant growth promoting rhizobacteria used in bioremediation studies

Bacteria	Plant	Heavy metals	Conditions	Role of PGPR	References
Methylobacterium oryzae, Berknolderia sp.	*Lycopersicon esculentom*	Ni, Cd	Gnotobiotic and pot culture experiments		Madhaiyan et al. (2007)
Azotobacter chroococcum HKN-5	*Brassica Juncea*	Pb, Zn	Experiments in greenhouse	Stimulated plant growth	Wu et al. (2006a)
Bacillus megaterium HKP-1	*Brassica Juncea*	Pb, Zn	Experiments in greenhouse	Protected plant from metal toxicity	Wu et al. (2006a)
Bacillus mucillaginosus HKK-1	*Brassica Juncea*	Pb, Zn	Experiments in greenhouse	Protected plant from metal toxicity	Wu et al. (2006a)
Bacillus subtilis SJ-101	*Brassica Juncea*	Ni	Experiments in growth chamber	Facilitated Ni accumulation	Zaidi et al. (2006)
Xanthomonas sp. RJ3, Azomonas sp. RJ4,Pseudomonas sp. RJ10, Bacillus sp. RJ31	*Brassica napus*	Cd	Experiment in pots	Stimulated plant growth and increased cadmium accumulation	Sheng and Xia (2006)
Pseudomonas sp, *Bacillus* sp.	Mustard	Cr (VI)	Pot experiment	Stimulated plant growth and decreased Cr (VI) content	Rajkumar et al. (2006)
Ochrobactrum, Bacillus cereus	Mungbean	Cr (VI)	Experiment in pots	Lowers the toxicity of chromium to seedlings by reducing Cr (VI) to Cr (III)	Faisal and Hasnain (2006)
Kluyvera ascorbata SUD165 *Kluyvera ascorbata* SUD165	Indian mustard, canola, tomato	Ni, Pb, Zn	Experiments in 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)
Brevundimonas Kro13	None	Cd	Culture media	Sequestered cadmium directly from solution	Robinson et al. (2001)
Brevibacillus	*Trifolium repens*	Zn	Pot experiment	Enhanced plant growth and nutrition of plants and decreased zinc concentration in plant tissues	Vivas et al. (2006)
Variovox paradoxus, Rhodococcus sp, *Flavobacterium*	*Brassica juncea*	Cd	Experiment in Petri dishes	Stimulating root elongation	Belimov et al. (2005)
Pseudomas fluorescens	Soybean	Hg	Experiment in greenhouse	Increased plant growth	Gupta et al. (2005)
Ochrobactrum intermedium	Sunflower	Cr (VI)	Experiment in pots	Increased plant growth and decreased Cr(VI) uptake	Faisal and Hasnain (2005)
Pseudomonas sp.	Soybean, mungbean, wheat	Ni, Cd, Cr	Experiment in pots	Promotes growth of plants	Gupta et al. (2002)
Brevundimonas Kro13	None	Cd	Culture media	Sequestered cadmium directly from solution	Robinson et al. (2001)

solution could be to combine the advantages of microbe-plant symbiosis within the plant rhizosphere into an effective cleanup technology. Symbiosis between plants, especially legumes and their symbionts (rhizobia) has long been studied by rhizobiologists. The rhizosphere is an area encircling the plant root system, which is characterized by enhanced biomass productivity. The exudation of nutrients by plant roots creates a nutrient-rich environment in which microbial activity is increased. Rhizosphere bacteria obtain nutrients such as, organic acids, enzymes, amino acids, and complex carbohydrates, exuded from roots. In addition, the mucigel secreted by root cells, lost root cap cells, or the decay of complete roots provides nutrients to rhizosphere microbes. In return, the bacteria convert nutrients into available forms of mineral for uptake by plants. For example, chickpea (*C. arietinum*) inoculated with phosphate-solubilizing bacteria and *Mesorhizobium ciceri* were demonstrated to have increased growth, symbiosis and yield through enhanced solubilization of phosphate and availability of sufficient quantity of N to the legume (Zaidi and Khan 2007; Wani et al. 2007e). Furthermore, the root tips provide a steady-state redox condition and a structural surface for bacterial colonization. Researchers have exploited this symbiotic relationship for rhizoremediation technologies. The combination of bioaugmentation and phytoremediation resulting in rhizoremediation (Anderson et al. 1993) could solve some of the problems of derelict land, encountered during the application of both techniques individually. In this process, the root exudates released from various plants stimulate the growth and metabolic activities of nodule bacteria that in turn very effectively remove the contaminants from polluted sites (Glick 2004; Zhuang et al. 2007). Through various studies, it has conclusively been proved that the rhizobial population acquires resistance, when grown in the soils contaminated heavily with metals (Pereira et al. 2006), though the information on metal tolerance by rhizobia at the molecular and cellular levels is limited. However, in recent times the interest in rhizobia for their role in remediation of heavy metals has greatly increased due to the fact that it influence the solubility, bioavailability and mobility of metals in both rhizosphere and within their legume host and help to maintain N pool of soils and legumes.

Rhizobia grow slowly for long periods in soil, but if they infect compatible legume hosts, they can grow rapidly and successful infection by a single bacterium can lead to the formation of a nitrogen-fixing nodule on the root of legumes. Moreover, once symbiosis is established, metals may accumulate in nodules. This would be an alternative and less expensive method to remove metals from the soil. The use of *Rhizobium* legume symbiotic interaction has therefore, been suggested as a tool for rhizoremediation of metals in derelict soils (Nie et al. 2002), because the symbiotic relationship between leguminous plants and rhizobia could be exploited for the improvement of plant abilities by introducing genetically engineered rhizobia to plant roots. Recombinant rhizobia in each nodule on a root of a legume are advantageous for the expression of foreign genes that help to sequester metals in contaminated soil. For example, *Mesorhizobium huakuii* subsp. rengei strain B3 (Murooka et al. 1993; Nuswantara et al. 1999) is the N_2 fixing bacterium that establishes a symbiotic relationship with *Astragalus sinicus*, the legume that has been used as green manure in rice (*O. sativa*) fields in China and Japan, and formed nitrogen-fixing root nodules (Chen et al. 1991). It would be advantageous if this plant could be used to increase N and, at the same time, to remove metals from soil. In other studies, the gene encoding metal-binding protein, tetrameric metallothionein (MTL4) (Hong et al. 2000) or arabidopsis phytochelatin synthase (PCS) (Rauser 1995; Zenk 1996; Cobbett 2000), was introduced into *M. huakuii* subsp. Rengei strain B3 (Sriprang et al. 2002, 2003) which expressed under the control of a bacteroid-specific promoter, nifH or nolB (Ruvkun et al. 1982; Perret et al. 1999). Resultant recombinant strain enhanced the accumulation of cadmium in free-living cells. In another study, the most suitable plant species for rhizoremediation showed that leguminous plant such as alfalfa is suitable (Shann and Boyle 1994). This is probably due to their ability to harbor large number of bacteria on their root systems. Rhizoremediation, however, depends on factors such as primary and secondary metabolites and colonization and establishment of rhizobia, survival and ecological interaction with other organisms in the rhizosphere.

Yet in another study, when greengram plants inoculated with metal tolerant *Bradyrhizobium* was grown in sandy clay loam soils exposed to different levels of nickel and zinc, the bioinoculant significantly enhanced the plant growth and symbiosis and reduced the uptake of nickel and zinc by plant organs (Wani et al. 2007f). Thus, the overall increase in inoculated greengram plants in metal-contaminated soils was suggested to be possibly due to the reduction in the toxicity of metals, or by the sufficient availability of N and phytohormones synthesized by the inoculants strain, to the plants. Moreover, the phytohormone (e.g., giberellin) is reported to reduce the effect of high concentration of certain metals (e.g., cadmium) on the growth of non-inoculated soybean (*Glycine max*) (Ghorbanli et al. 1999). In a similar study, the application of metal-tolerant rhizobia as a seed bioinoculant reduced metal toxicity and through their PGP activities promoted the growth of lentil (*Lens esculentum*) (Wani et al. 2007g), and chickpea (Wani et al. 2008b) in metal treated soils. In a recent study, *Rhizobium* sp. RP5, tolerant to nickel and zinc was reported to protect pea (Wani et al. 2007c) plants from nickel (Fig. 4) and zinc (Fig. 5) toxicity without affecting the metal uptake by roots, shoots and grains. The plant growth promoting effect in the presence of metals was suggested to be due to the reasons as discussed earlier, in addition to their metal-reducing potential through adsorption/desorption mechanism (Mamaril et al. 1997).

Exposure of heavy metals and other adverse environmental factors can disrupt cellular homeostasis and enhance the production of several activated species of oxygen such as, superoxide, singlet-oxygen, H_2O_2 and hydroxyl radicals, which constitute an important aspect of the oxygen problem in different organs of legumes. For instance, nodules have a high capacity to produce these damaging chemicals because of the high rates of respiration, the strong reducing conditions required to reduce N_2, the tendency of leghaemoglobin to auto-oxidize and the likely ability of nitrogenase to directly reduce oxygen (Dalton 1995). Moreover, plants and nitrogen-fixing rhizobia possess the efficient defense system that allows the scavenging of reactive oxygen species. One such enzyme is the glutathione reductase, which detoxifies the H_2O_2 via

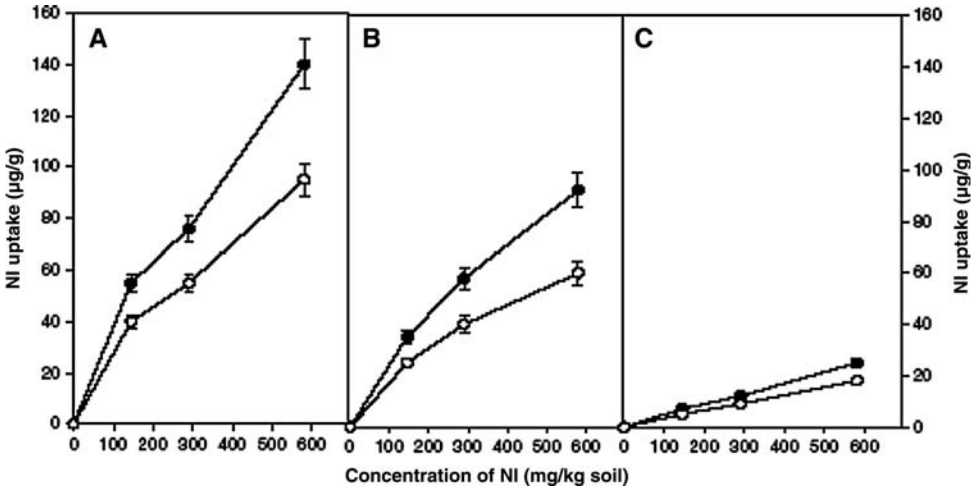


Fig. 4 Concentration of nickel in (**a**) roots and (**b**) shoots at 90 and (**c**) grains at 120 days after seeding pea in the absence (*filled circle*) and presence (*open circle*) of bioinoculant strain RP5 with different levels of nickel. The values indicate the mean ± SD of three replicates

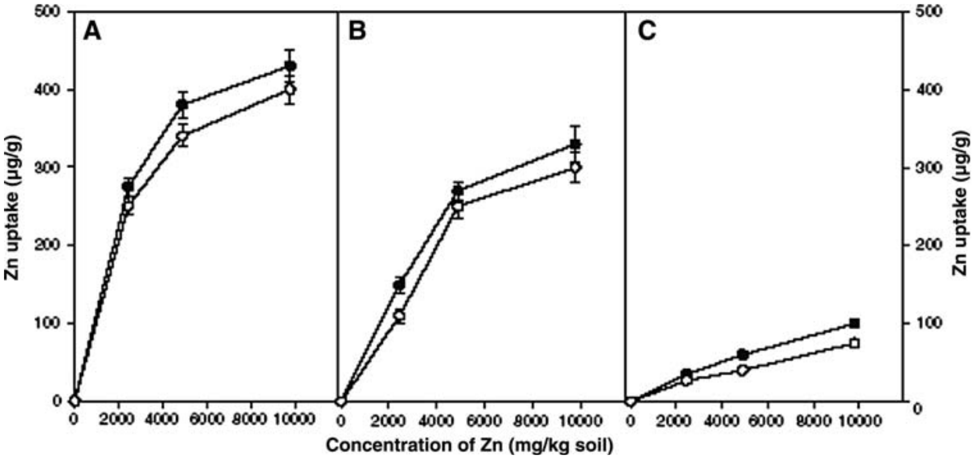


Fig. 5 Concentration of zinc in (**a**) roots and (**b**) shoots at 90 and (**c**) grains at 120 days after seeding pea in the absence (*filled circle*) and presence (*open circle*) of bioinoculant strain RP5 with different levels of zinc. The values indicate the mean ± SD of three replicates

the ascorbate-glutathione cycle (Azevedo et al. 1998). In this context, the glutathione reductase activity has been detected in the roots and nodules of inoculated pea plants, grown in soils treated with nickel (Fig. 6) and zinc (Fig. 7). Generally, concentration-dependent increase in glutathione reductase activity of roots and nodules was observed for nickel and zinc in inoculated and non-inoculated plants (Wani et al. 2007c). However, the glutathione activity in general, was found more in roots of both rhizobium inoculated and un-inoculated plants grown in nickel and zinc-stressed soil suggesting that the higher concentration of these metals has probably induced the oxidative stress and generation of reactive species of oxygen , leading to the synthesis of antioxidant enzymes, which might have played a pivotal role in protecting the pea plants from the oxidative stress, as also reported for other legumes (Cardoso et al. 2005; Ana et al. 2006; Lima et al. 2006). Furthermore, the enhanced growth of inoculated legumes under metal stress could also be due to the synthesis of glutathione reductase or other detoxifying agents by rhizobia (Figueira et al. 2005; Corticeiro et al. 2006). These results could play an important role in developing biotechnological strategies for metal bioremediation procedures and open novel prospective for the restoration of polluted soil using legume-*Rhizobium* symbiosis.

Phytoremediation

Phytoremediation is an environment friendly and visually attractive technology that involves the use of plants to clean up pollutants from the contaminated soil environment (Cunningham et al. 1995). The sensitivity or tolerance of plants toward metals is influenced greatly by plant species and genotypes. Broadly, plants can be grouped into three categories: (1) excluders (2) indicators and (3) accumulators (Fig. 8). Among these, the plants belonging to excluder groups are sensitive to metals over a wide range of soil concentrations and survive through restriction mechanisms while indicators show poor control over metal uptake and transport processes and correspondingly respond to metal concentrations in soils. Grasses (e.g.,

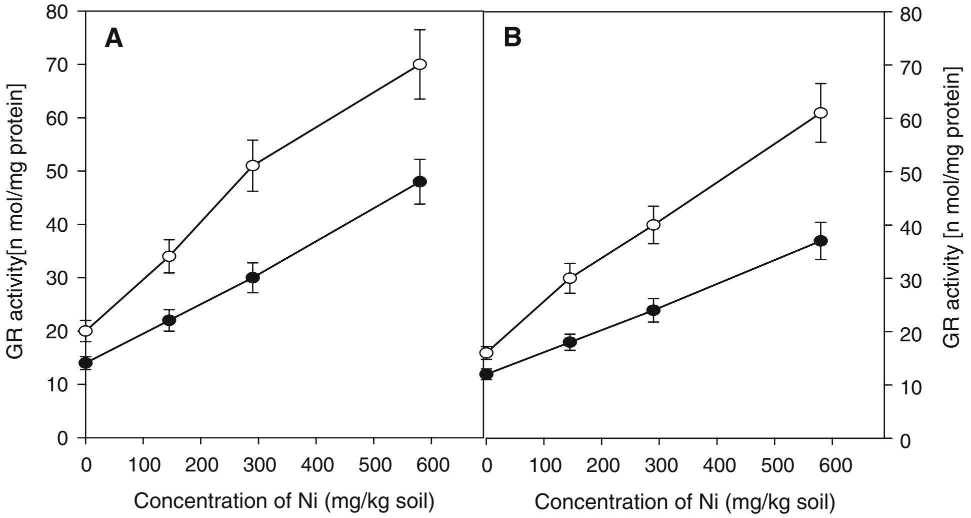


Fig. 6 Glutathione reductase activity in (**a**) roots and (**b**) nodulbes at 90 days after seeding pea in the absence (*filled circle*) and presence (*open circle*) of bioinoculant strain RP5 with different levels of nickel. The values indicate the mean ± SD of three replicates

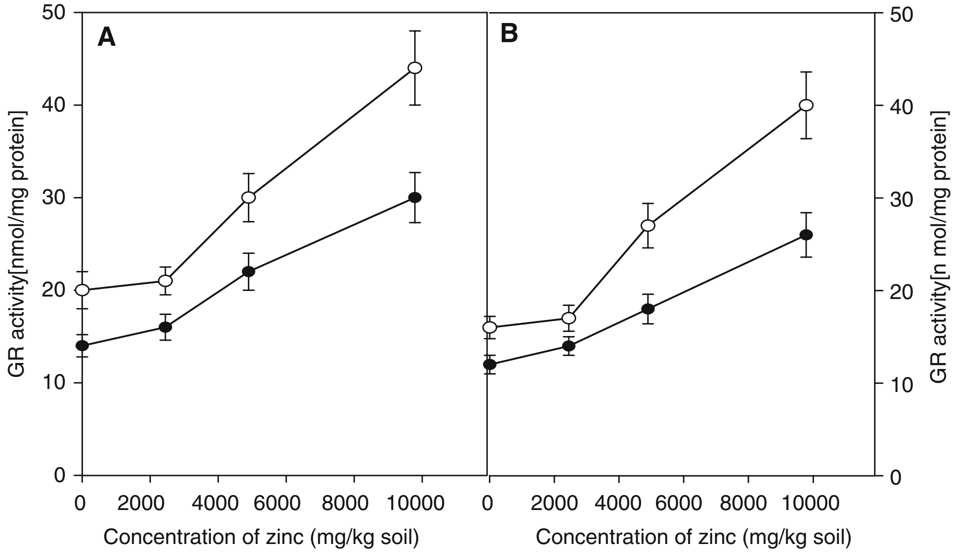


Fig. 7 Glutathione reductase activity in (**a**) roots and (**b**) nodules at 90 days after seeding pea in the absence (*filled circle*) and presence (*open circle*) of bioinoculant strain RP5 with different levels of zinc. The values indicate the mean ± SD of three replicates

sudangrass, bromegrass, fescue, etc.) and grain and cereal crops (e.g. corn, soybean, wheat, oats, etc.) are included in the excluder and indicator groups, respectively. Plants in the accumulator group do not prevent metals from entering the roots and hence, have evolved specific mechanisms for detoxifying high concentrations of metal accumulated in the cells. Common plants included in this group are tobacco (*Nicotiana tabacum* L.), the mustard (*Brassica campestris*) and members of compositae families (e.g. lettuce, spinach, etc.). Among these accumulators there are certain plants, which possess exceptionally high metal-accumulating capacity, hyper-accumulators that allow them to survive and even thrive in heavily contaminated soils. The term hyper-accumulator was introduced for the first time by Brooks et al. (1977) to describe plants which in their natural habitats were capable of accumulating more than 1,000 mg Ni kg^{-1} dry weight of shoots. This limit of metal accumulation is also applied to other metals (e.g., cobalt, copper and lead), whereas for cadmium and zinc, the threshold limit is about 100 and 10,000 mg kg^{-1} shoots dry weight (Brooks 1998; Baker et al. 2000). Most of the commonly known heavy metal accumulators belong to the brassicaceae or fabaceae family (Kumar et al. 1995). However, currently more than 400 plant species have been reported as hyper-accumulator plants and a considerable number of species show the capacity to accumulate two or more elements (Zayad et al. 1998; Chaudhry et al. 1998; Hayes et al. 2003). Among the metal-accumulating plants, Indian mustard (*Brassica juncea* L. Czern) is one of the most promising species, which has attracted considerable attention because of its ability to grow in heavily polluted soil together with its capacity for metal ion accumulation (Blaylock and Huang 2000). Generally, hyper-accumulator plants accumulate one to

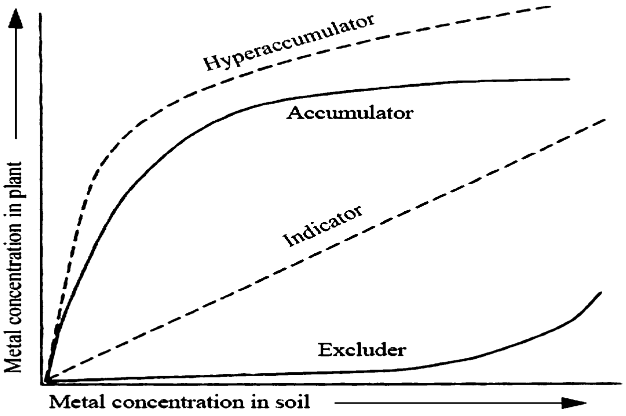


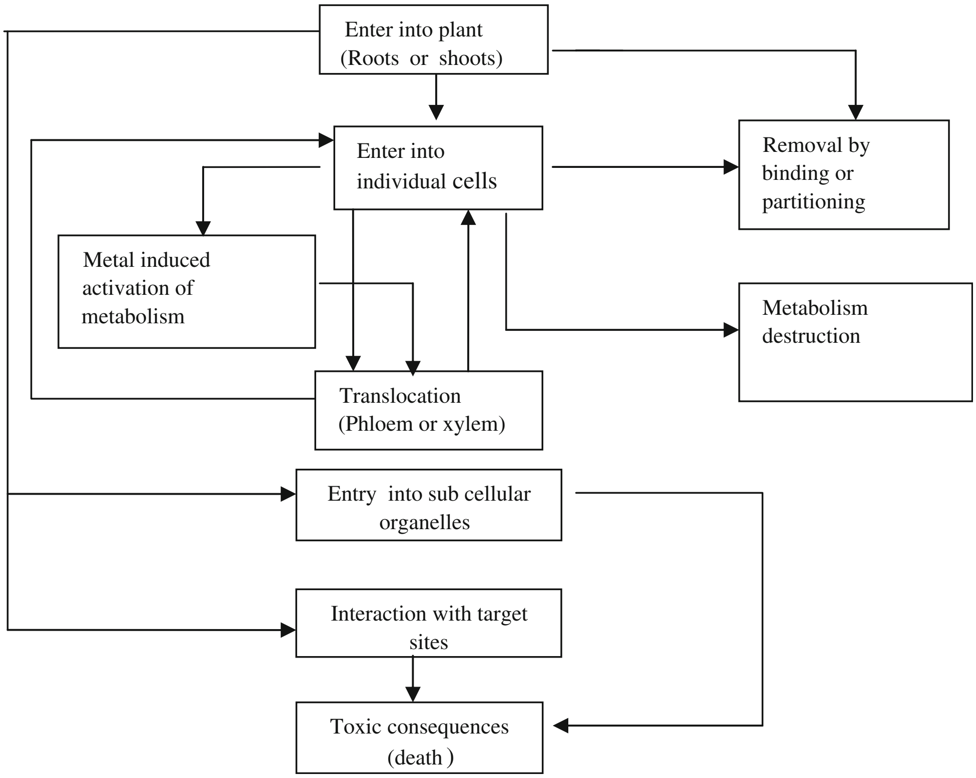
Fig. 8 Strategies of metal uptake by plants depending on the concentration of metal in soil (adapted from Adriano 2001)

threefold higher concentrations of metals than do the non-hyper-accumulator plants (Baker et al. 1994; Shen et al. 1997). Phytoremediation technology has certain advantages and disadvantages as well. The advantages include the following: (1) it is a low cost and low energy and environment friendly ecotechnology (2) it is far less disruptive to the soil environment (3) it avoids excavation and is socially acceptable and (4) since it involves the use of plants, it is easy to implement and maintain. The following are the disadvantages: (1) it is time-consuming due to slow growth rate of plants (2) it is affected by the change in agro-climatic conditions (3) plant biomass after remediation requires proper disposal (4) the contaminants may enter again into soil due to litter formation by the metal accumulating plants and (5) the root exudates of hyper accumulators may enhance the solubility of pollutants and consequently may increase the distribution of metals in the soil environment. Thus, to make phytoremediation a viable technology, we need to search for plants which grow faster with extensive root system, have a capacity to produce high amount of biomass, have lower level contaminant uptake ability, and is capable to accumulate higher amounts of contaminants or engineer common plants with hyperaccumulating genes.

How plants help to restore degraded soil?

Roots are the first organ of plants to come in contact with heavy metals in contaminated soils and after uptake by the roots, metals are translocated to different organs of plants (Fig. 9). In soils heavily contaminated with metals, plants suffer various injuries leading to the death of plants (Rout and Das 2003; Wani et al. 2006) by inactivation of photosynthesis (Wani et al. 2007b), synthesis of proteins and DNA (Asada 1994), stomatal action and generation of free radicals (Breen and Murphy 1995), as presented in Fig. 10. However, in order to survive in the metal polluted soils, plants could accumulate, sequester, or synthesize metal-binding complexes and phytochelatins; a simple γ-glutamyl peptides (Grill et al. 1985) whose formation in response to

Fig. 9 *Flow chart* showing the sequence of events from metal entry into a plant to death of the plant



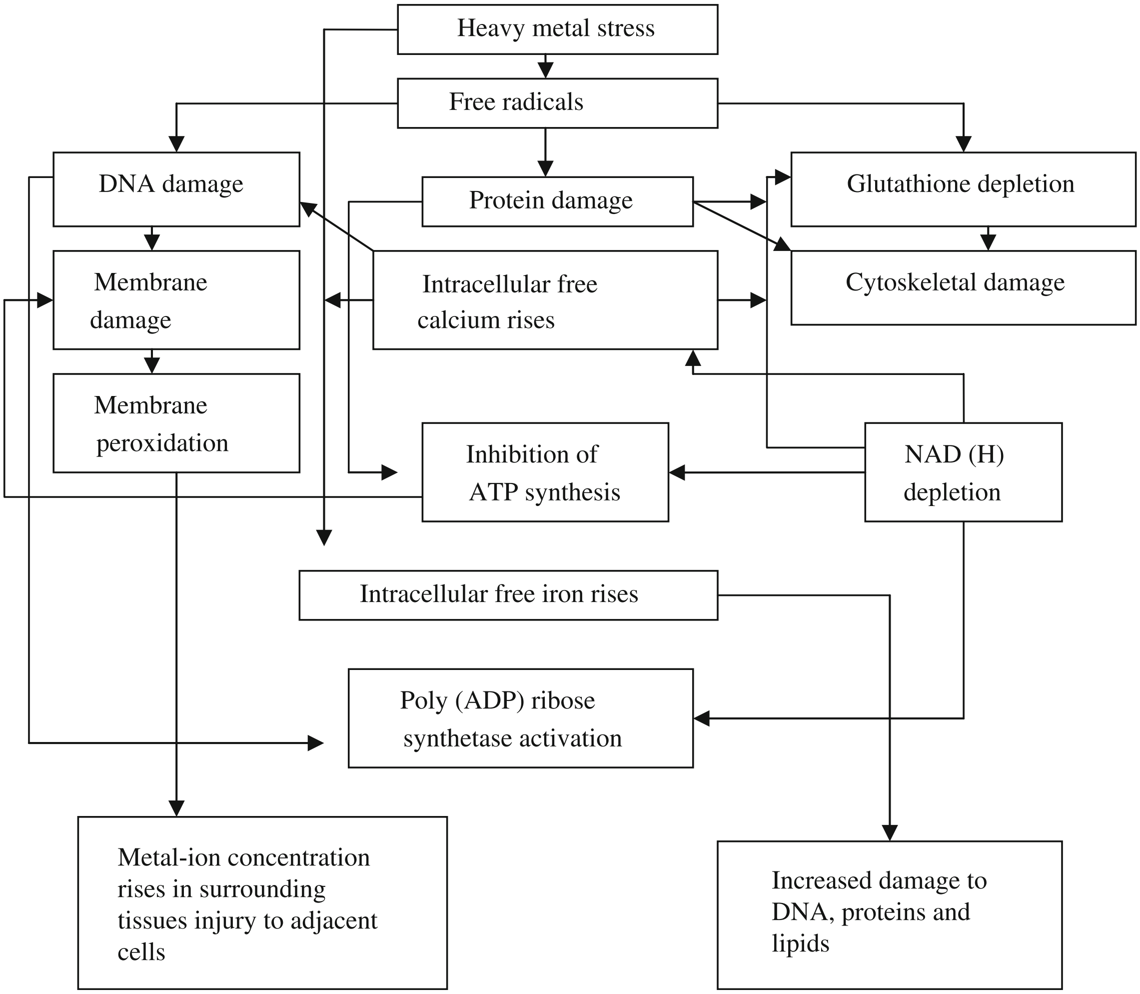


Fig. 10 Metabolism of plants damaged by heavy metals

the challenge of heavy metals is one of the few truly adaptive stress responses observed in plants. Generally, plants adopt one or combination of the following mechanisms in order to protect themselves from metal toxicity. Such process could be—(1) phytoextraction—this is a low-cost technique through which metal is removed or concentrated in to plant parts. This process produces a mass of plants and contaminants (usually metals) that can be transported for disposal or recycling (2) phytodegradation or rhizodegradation—metals are degraded by the proteins or enzymes produced by plants and their associated microbes (3) rhizofiltration—metal is absorbed by plant roots (4) phytostabilization—in this technique, metals are immobilized and the mobility and bioavailability of metals to plant roots are reduced. Leachable constituents are adsorbed and form a stable complex with plant structure from which the contaminants will not re-enter the environment and (5) phytovolatization—volatization of pollutants by plants from the soil in to atmosphere. Phytoremediation techniques in general, are applied to a large field sites where the cost of other remediation methods are high or not practicable.

Plant growth promoting rhizobacteria affecting phytoremediation

Contaminated soils are often nutrient poor or sometimes nutrient deficient, due to the loss of beneficial microbes. However, such soils can be made nutrient rich by applying metal-tolerant microbes, especially the plant growth promoting rhizobacteria, which would provide not only the essential nutrients to the plants growing in the contaminated sites but would also play a major role in detoxifying heavy metals (Mayak et al. 2004) and thus help plants capable of remediating heavy metals (Glick 2003). For example, plant growth promoting rhizobacterium *Kluyvera ascorbata* SUD165 isolated from metal contaminated wetland near Sudbury, Ontario, Canada, when applied to soils amended with nickel, zinc, lead and chromate have shown to increase the growth of canola (*Brasica rapa*) while protecting the plants from nickel toxicity (Burd et al. 1998). Similarly, nickel resistant *K. ascorbata* protected tomato (*Lycopersicon esculentum* L.), Indian mustard (*B. campestris*) and canola plants when grown in soils supplemented with nickel lead and zinc (Burd et al. 2000). Moreover, the growth promoting rhizobacteria *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* sp. stimulated root elongation of Indian mustard seedlings either in the presence or absence of toxic cadmium (Belimov et al. 2005) suggesting that these bacterial strains could be develop as inoculants to improve growth of the metal-accumulating Indian mustard in the presence of toxic cadmium concentration and for the development of plant inoculant systems useful for phytoremediation of polluted soils. Similarly, the canola plants inoculated with *Enterobacter cloacae*, when grown in the presence of arsenates, grew to a significantly greater extent than non-transformed canola plants (Nie et al. 2002). In yet other studies, *O. intermedium* and *Bacillus cereus* protected greengram plants against chromium toxicity (Faisal and Hasnain 2006) while inoculation of *O. intermedium* improved the overall growth of sunflower (*Helianthus annus*), when grown in metal amended soils (Faisal and Hasnain 2005). In other studies, metal tolerant growth promoting rhizobacteria has also shown a substantial protection to plants against metal toxicity, and consequently improved the growth, symbiosis and seed yield of plants (Chaudri et al. 2000; Wani et al. 2007c; Wani et al. 2008b). The increase in the growth of agronomically important crops grown in metal-stressed soils by applying metal tolerant rhizobacteria was attributed to the ability of rhizobacterial strains to mitigate the toxic effects of metals using mechanisms as discussed earlier besides providing plants with the sufficient amounts of growth promoting substances. It may therefore, be advisable for growers to inoculate plants with such rhizobacterial microbes in order to increase plant biomass and thereby stabilize, revegetate and restore/remediate heavy metal polluted soils.

Conclusion

Remediation of metal-polluted soils using biological systems (both microbes and plants) is an emerging area of interest and has shown a substantial progress in situ, which needs to be further consolidated through field trials under different agro-climatic zones of the world. Understanding the mechanistic basis of the physical, chemical and biological rhizosphere processes and the interactions between hyperaccumulators and non-accumulators and plant growth promoting rhizobacteria will be important in modeling better the full impact of phytoremediation in the restoration of derelict lands. Furthermore, the remediation of heavy metal contaminated sites using rhizobacteria is an exciting area of research, since these organisms can easily and inexpensively be mass-produced. Therefore, the molecular engineering of both microbes and plants with desired genes would help immensely to enhance the efficiency of growth promoting rhizobacteria mediated or plant-based remediation of contaminated soils. However, to make bioremediation a successful option for the remediation of contaminated soils, some of the problems need to be critically addressed. Such problems are—(1) why do plant growth promoting rhizobacteria fail to perform in comparatively extreme environments? (2) How do rhizobacteria colonize plant roots and interact selectively with other indigenous microflora? (3) How do the remediation effects will change under field conditions? (4) Research is needed to investigate various aspects of metal accumulation by plant organs and, (5) we also need to understand the mechanisms involved in mobilization and transfer of metals in order to develop further strategies and optimize the phytoremediation process. These are some of the challenges, which need urgent attention of the scientists before the potential of bioremediation in remediating the metal polluted soils could be appreciated.

References

Abd-Alla MH (1994) Solubilization of rock phosphates by *Rhizobium* and *Bradyrhizobium*. Folia Microbiol 39:53–56

Adriano DC (2001) Trace elements in terrestrial environments. Biogeochemistry, bioavailability and risks of metals. Springer-Verlag, New York, p 866

Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk J Biol 29:29–34

Ahmad F, Ahmad I, Khan MS (2008) Screening of free—living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ana IGL, Sofia CC, Etelvina MAPF (2006) Glutathione-mediated cadmium sequestration in *Rhizobium leguminosarum*. Enz Microb Technol 39:763–769

Anderson TA, Guthrie EA, Walton BT (1993) Bioremediation in the rhizosphere. Environ Sci Technol 27:2630–2636

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effects on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Llande R (2004) Potential of *Rhizobium* and *Bradyrhizobioum* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes. Plant Soil 204:57–67

Arora NK, Kang SC, Maheshwari DK (2001) Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr Sci 81:673–677

Asada K (1994) Production and action of active oxygen species in photosynthetic tissues In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, Boca Raton, pp 77–104

Azevedo RA, Alas RM, Smith PJ, Lea PJ (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild type and a catalase deficient mutant of barley. Physiol Plant 104:280–292

Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal accumulating plants. Resour Conserv Recycling 11:41–49

Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Baelos G (eds) Phytoremediation of contaminated soil and water. CRC Press, Boca Raton, pp 85–107

Belimov AA, Safroonova VI, Mimura T (2002) Response of spring rape to inoculation with plant growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. Can J Microbiol 48:189–199

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

Berraho EL, Lesueur D, Diem HG, Sasson A (1997) Iron requirement and siderophore production in *Rhizobium ciceri* during growth on an iron-deficient medium. World J Microbiol Biotechnol 13:501–510

Blake RC, Choate DM, Bardhan S, Revis N, Barton LL, Zocco TG (1993) Chemical transformation of toxic metals by a Pseudomonas strain from a toxic waste site. Environ Toxicol Chem 12:1365–1376

Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals using plants to clean-up the environment. Wiley, New York, pp 53–70

Breen AP, Murphy JA (1995) Reaction of oxyl radicals with DNA. Free Rad Biol Med 18:1033–1077

Briat JF, Lebrun M (1999) Plant responses to metal toxicity. C. R. Acad Sci III, Sciences de la 322:43–54

Braun V (1997) Avoidance of iron toxicity through regulation of bacterial iron transport. Biol Chem 378:779–786

Brown GE Jr, Foster AL, Ostergren JD (1999) Mineral surfaces and bioavailability of heavy metals: a molecular-scale perspective. Proc Natl Acad Sci USA 96:3388–3395

Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimen of indicator plants. J Geochem Explor 7:49–57

Brooks RR (1998) Geobotany and hyperaccumulators. In: Brook RR (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, pp 55–94

Burd GI, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Burd GI, Dixon DG, Glick BR (1998) A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Çakmakçi R, Dönmez F, Aydm A, Şahin F (2006) Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol Biochem 38:1482–1487

Canbolat MY, Bilen S, Cakmakci R, Sahin F, Aydin A (2006) Effect of plant growth promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fertil Soils 42:350–357

Cardoso PF, Priscila LG, Rui AG, Leonardo OM, Ricardo AA (2005) Response of *Crotalaria junceae* to nickel exposure. Braz J Plant Physiol 17:267–272

Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation–focusing on hyperaccumulator plants that remediate metal-contaminated soils. Aust J Ecotoxicol 4:37–51

Chaudri AM, McGrath SP, Giller KE, Rietz E, Sauerbeck DR (1993) Enumeration of indigenous *Rhizobium leguminosarum* biovar trifolii in soils previously treated with metal-contaminated sewage sludge. Soil Biol Biochem 25:301–309

Chaudri AM, Allain CM, Barbosa-Jefferson VL, Nicholson FA, Chambers BJ, McGrath SP (2000) A study of the impacts of Zn and Cu on two rhizobial species in soils of a long term field experiment. Plant Soil 22:167–179

Chen W, Li GS, Qi YL, Wang ET, Yuan HL, Li L (1991) *Rhizobium huakuii* sp. nov. isolated from the root nodules of *Astragalus sinicus*. Int J Syst Bacteriol 41:275–280

Cobbett CS (2000) Phytochelatins and theirs roles in heavy metal detoxification. Plant Physiol 123:825–832

Corticeiro CS, Lima AIG, Figueira EMAP (2006) The importance of glutathione in oxidative status of Rhizobium leguminosarum biovar viciae under cadmium stress. Environ Microbiol Technol 40:132–137

Cunningham SD, Berri WR, Haung JW (1995) Phytoremediation of contaminated soil. Trends Biotech 134:393–397

Dalton DA (1995) Antioxidant defenses of plants and fungi. In: Ahmad S (ed) Oxidative stress and antioxidant defenses in biology. Chapman & Hall, New York, pp 298–355

Deshwal VK, Pandey P, Kang SC, Maheshwari DK (2003) Rhizobia as a biological control agent against soil borne plant pathogenic fungi. Ind J Expt Biol 41:1160–1164

Duhan JS, Dudeja SS, Khurana AL (1998) Siderophore production in relation to N_2 fixation and iron uptake in Pigeon Pea–*Rhizobium* symbiosis. Folia Microbiol 43:421–426

Faisal M, Hasnain S (2005) Bacterial Cr (VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. Biotechnol Lett 27:943–947

Faisal M, Hasnain S (2006) Growth stimulatory effect of *Ochrobactrum intermedium* and *Bacillus cereus* on *Vigna radiata* plants. Lett Appl Microbiol 43:461–466

Figueira EMAP, Lima AIG, Pereira SIA (2005) Cadmium tolerance plasticity in *Rhizobium leguminosarum* bv. *v*iciae: glutathione as a detoxifying agent. Can J Microbiol 5:11–16

Gadd GM (1992) Metals and microorganisms: a problem of definition. FEMS Microbiol Lett 100:197–204

Ghorbanli M, Kaveh SH, Sepehr MF (1999) Effect of cadmium and gibberellin on growth and photosynthesis of *Glycine max*. Photosynthetica 37:627–631

Giller KE, McGrath SP, Hirsch PR (1989) Absence of nitrogen fixation in clover grown on soil subject to long-term contamination with heavy metals is due to survival of only ineffective *Rhizobium*. Soil Biol Biochem 21:841–848

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial process in agricultural soils: a review. Soil Biol Biochem 30:1389–1414

Glick BR, Penrose DM, Li JA (2002) Model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J Theor Biol 190:63–68

Glick BR (1995) The enhancement of plant growth by free-livingbacteria. Can J Microbiol 41:109–117

Glick BR (2001) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotech Adv 21:383–393

Glick BR (2004) Bacterial ACC deaminase and the alleviation of plant stress. Adv Appl Microbiol 56:291–312

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase containing plant growth promoting rhizobacteria. Plant Physiol Biochem 39:11–17

Grill E, Winnacker EU, Zenk MH (1985) Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230:674–676

Gupta A, Meyer JM, Goel R (2002) Development of heavy metal resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI4014 and their characterization. Curr Microbiol 45:323–332

Gupta DK, Rai UN, Sinha S, Tripathi RD, Nautiyal BD, Rai P, Inouhe M (2004) Role of *Rhizobium* (CA-1) inoculation in increasing growth and metal accumulation in *Cicer arietinum* L. growing under fly-ash stress condition. Bull Environ Contam Toxicol 73:424–431

Gupta A, Rai V, Bagdwal N, Goel R (2005) In situ characterization of mercury resistant growth promoting fluorescent pseudomonads. Microbiol Res 160:385–388

Hayes WJ, Chaudhry RT, Buckney RT, Khan AG (2003) Phytoaccumulation of trace metals at the Sunny Corner mine, New South Wales, with suggestions for a possible remediation strategy. Aust J Ecotoxicol 9:69–82

Hernandez A, Mellado RP, Martinez JL (1998) Metal accumulation and vanadium induced multidrug resistance by environmental isolates of *Escherichia herdmanni* and *Enterobacter cloacae*. Appl Env Microbiol 64:4317–4320

Hong SH, Gohya M, Ono H, Murakami H, Yamashita M, Hirayama N, Murooka Y (2000) Molecular design of novel metalbinding oligomeric human metallothioneins. Appl Microbiol Biotechnol 54:84–89

Kamaludeen SP, Megharaj M, Juhasz AL, Sethunathan N, Naidu R (2003) Chromium–microorganism interactions in soil remediation implications. Rev Environ Contam Toxicol 178:1076–1084

Kamnev AA (1998) Reductive solubilization of Fe (III) by certain products of plant and microbial metabolism as a possible alternative to siderophore secretion. Doklady Biophys (Moscow) 358–360:48–51

Kamnev AA, Kuzmann E, Perfiliev Yu D, Vanko GV, Ve'rtes A (1999b) Mossbauer and FTIR spectroscopic studies of iron *anthranilates*: coordination, structure and some ecological aspects of iron complexation. J Mol Struct 482/483:703–711

Kao PH, Huang CC, Hseu ZY (2006) Response of microbial activities to heavy metals in a neutral loamy soil treated with biosolid. Chemosphere 64:63–70

Khan AG (2004) Mycotrophy and its significance in wetland ecology and wetland management. In: Wong MH (ed) Developments in ecosystems, vol 1. Elsevier, Northhampton, pp 97–114

Khan AG (2005a) Mycorrhizas and phytoremediation. In: Willey N (ed) Method in biotechnology-phytoremediation: methods and reviews. Humana Press, Totowa

Khan AG (2005b) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan MS, Zaidi A (2007) Synergistic effects of the inoculation with plant growth promoting rhizobacteria and arbuscular mycorrhizal fungus on theperformance of wheat. Turk J Agric For 31:355–362

Khan MS, Zaidi A, Aamil M (2002) Biocontrol of fungal pathogens by the use of plant growth promoting rhizobacteria and nitrogen fixing microorganisms. Ind J Bot Soc 81:255–263

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate solubilizing microorganisms in sustainable agriculture:a review. Agron Sustain Dev 27:29–43

Kloepper JW, Schroth MN (1978) Plant growth promoting rhizobacteria on radishes, fourth international conference on plant pathogen bacteria, vol 2. Angers, France, pp 879–882

Krishnamurti GSR (2000) Speciation of heavy metals: an approach for remediation of contaminated soils, in remediation engineering of contaminated soils. In: Wise DL, Trantalo DJ, Cichon EJ, Inyang HI, Stottmeister U (eds) Marcel Dekker Inc, New York, pp 693–714

Kumar V, Behl RK, Narula N (2001) Establishment of phosphate solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol Res 156:87–93

Lakzian A, Murphy P, Turner A, Beynon JL, Giller KE (2002) *Rhizobium leguminosarum* bv. viciae populations in soils with increasing heavy metal contamination: abundance, plasmid profiles, diversity and metal tolerance. Soil Biol Biochem 34:519–529

Ledin M (2000) Accumulation of metals by microorganisms-processes and importance for soil system. Earth Sci Rev 51:1–31

Leinhos V, Bergmann H (1995) Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. 2. Root-growth promotion and nutrient accumulation of maize (*Zea-mays* L.) by inoculation with indole-3-acetic acid (IAA) producing Pseudomonas strains and by exogenously applied IAA under different water-supply conditions. Angew Bot 69:37–41

Lena QM, Rao GN (1997) Heavy metals in the environment. J Environ Qual 26:264

Lima AIG, Pereira SAI, Figueira EMAP, Caldeira GCN, Caldeira HDQM (2006) Cadmium detoxification in roots of *Pisum sativum* seedlings: relationship between toxicity levels, thiol pool alterations and growth. Env Exp Bot 55:149–162

Lloyd JR, Lovley DR (2001) Microbial detoxification of metals and radionuclides. Curr Opin Biotechnol 12:248–253

Lloyd JR, Macaskie LE (2000) Bioremediation of radioactive metals. In: Lovley DR (ed) Environmental microbe–metal interactions. ASM Press, Washington, pp 277–327

Lippmann B, Leinhos V, Bergmann H (1995) Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. 1. Changes in root morphology and nutrient accumulation in maize (Zea-mays L.) caused by inoculation with indole-3-acetic acid (IAA) producing Pseudomonas and Acinetobacter strains or IAA applied exogenously. Angew Bot 69:31–36

Loper JE, Henkels MD (1999) Utilization of heterologous siderophore enhances levels of iron available to *Pseudomonas putida* in rhizosphere. Appl Environ Microbiol 65:5357–5363

Lovley DR, Coates JD (1997) Bioremediation of metal contamination. Curr Opin Biotechnol 8:285–289

Madhaiyan M, Poonguzhali S, Ryu JH, Sa T (2006) Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carbxylate deaminase containing *Methylobacterium fujisawaense*. Planta 224:268–278

Madhaiyan M, Poonguzhali S, Sa T (2007) Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*lycopersicon esculentum* L.). Chemosphere 69:220–228

Mamaril JC, Paner ET, Alpante BM (1997) Biosorption and desorption studies of chromium (iii) by free and immobilized *Rhizobium* (BJVr 12) cell biomass. Biodegradation 8:275–285

Mayak S, Tirosh S, Glick BR (2004) Plant growth promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Physiol 166:525–530

McGrath SP, Chaudri AM, Giller KE (1995) Long-term effects of metals in sewage sludge on soils, microorganisms and plants. J Ind Microbiol 14:94–104

McLean J, Beveridge TJ (2001) Chromate reduction by *Pseudomonad* isolated from a site contaminated with chromated copper arsenate. Appl Environ Microbiol 67:1076–1084

Meyer JM (2000) Pyoverdines: pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* species. Arch Microbiol 137:135–142

Moftah AE (2000) Physiological response of lead polluted tomato and eggplant to the antioxidant ethylene diurea. Menufiya Agric Res 25:933–955

Muller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In: Ronald LC, Crawford DL (eds) Bioremediation: principles and applications. Cambridge University Press, Cambridge, pp 125–194

Murooka Y, Xu Y, Sanada H, Araki M, Morinaga T, Yokota A (1993) Formation of root nodules by *Rhizobium huakuii* biovar rengei bv. nov. on *Astragalus sinicus* cv. Japan J Ferment Bioeng 76:38–44

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

Nie L, Shah S, Burd GI, Dixon DG, Glick BR (2002) Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiol Biochem 40:355–361

Noel TC, Sheng C, Yost CK, Pharis RP, Hynes MF (1996) *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: direct growth promotion of canola and lettuce. Can J Microbiol 42:279–283

Noordman WH, Reissbrodt R, Bongers RS, Rademaker ILW, Bockelmann W, Smit G (2006) Growth stimulation of *Brevibacterium* sp. by siderophores. J Appl Microbiol 101: 637–646

Nuswantara S, Fujie M, Yamada T, Malek W, Inaba M, Kaneko Y, Murooka Y (1999) Phylogenic position of *Mesorhizobium huakuii* subsp. rengei, a symbiont of *Astragalus sinicus* cv. Japan J Biosci Bioeng 87:49–55

Olson JW, Mehta NS, Maier RJ (2001) Requirement of nickel metabolism protein HypA and HypB for full activity of both hydrogenase and urease in *Helicobacter pylori*. Mol Microbiol 39:176–182

Park CH, Keyhan M, Matin A (1999) Purification and characterization of chromate reductase in *Pseudomonas putida*. Gen Meet Am Soc Microbial 99:536–548

Penrose DM, Glick BR (2001) Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth promoting bacteria. Can J Microbiol 47:368–372

Pereira SIA, Lima AIG, Figueira EMAP (2006) Heavy metal toxicity in *Rhizobium leguminosarum* biovar *viciae* isolated from soils subjected to different sources of heavy metal contamination: effect on protein expression. Appl Soil Ecol 33:286–293

Perret X, Freiberg C, Rosenthal A, Broughton WJ, Fellay R (1999) High-resolution transcriptional analysis of the symbiotic plasmid of *Rhizobium* sp. NGR234. Mol Microbiol 32:415–425

Perveen S, Khan MS, Zaidi A (2002) Effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiatus*). Ind J Agric Sci 72:421–423

Ponmurugan PGC (2006) In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. Afr J Biotechnol 5:340–350

Rahman M, Gul S, Haq MZ (2007) Reduction of chromium(vi) by locally isolated *pseudomonassp*. C-171. Turk J Biol 31:161–166

Rajkumar M, Nagendran R, Kui Jae L, Wang Hyu L, Sung Zoo K (2006) Influence of plant growth promoting bacteria and Cr (vi) on the growth of Indian mustard. Chemosphere 62:741–748

Rauser WE (1995) Phytochelatins and related peptides. Structure, biosynthesis, and function. Plant Physiol 109:1141–1149

Roane TM, Pepper IL (2000) Microorganisms and metal pollution, in environmental microbiology. In: Maier RM, Pepper IL, Gerba CB (eds) Academic Press, London, p 55

Robinson B, Russell C, Hedley, Clothier B (2001) Cadmium adsorption by rhizobacteria: implications for New Zealand pastureland. Agric Ecosyst Environ 87:315–321

Rout GR, Das P (2003) Effect of metal toxicity on plant growth and metabolism: I, zinc. Agronomie 23:3–11

Ruvkun GB, Sundaresan V, Ausubel FM (1982) Directed transposon Tn5 mutagenesis and complementation analysis of *Rhizobium meliloti* symbiotic nitrogen fixation genes. Cell 29:551–559

Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root associated bacteria containing1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Shaharoona B, Arshad M, Zahir ZA (2006) Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean. Lett Appl Microbiol 42:155–159

Shakolnik MY (1984) Trace elements in plants. Elsevier, New York, pp 140–171

Shann JR, Boyle JJ (1994) Influence of plant species on in situ rhizosphere degradation. In: Anderson TA, Coats JR (eds) Bioremediation through rhizosphere technology. American Chemical Society, Washington DC, pp 70–81

Sharma A, Talukdar G (1987) Effects of metals on chromosomes of higher organisms. Environ Mutagen 9:191–226

Shen ZG, Zhao FJ, McGrath SP (1997) Uptake and transport of zinc in the hyper accumulator *Thlaspi caerulescens* and the non-hyperacumulator *Thlaspi ochroleucum*. Plant Cell Environ 20:898–906

Sheng XF, Xia JJ (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64:1036–1042

Sriprang R, Hayashi M, yamashita M, Ono H, Saeki K, Murooka Y (2002) A novel bioremediation system for heavy metals using the symbiosis between leguminous plant and genetically engineered rhizobia. J Biotechnol 99:279–293

Sposito FG (2000) The chemistry of soils. In: Maier RM, Pepper IL, Gerba CB (eds) Environmental microbiology. Academic Press, London, pp 406

Sriprang R, Hayashi M, Ono H, Takagi M, Hirata K, Murooka Y (2003) Enhanced accumulation of Cd2+ by Mesorhizobium transformed with a gene for phytochelatin synthase from Arabidopsis. Appl Env Microbiol 69:1791–1796

Stresty EV, Madhava Rao KV (1999) Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeonpea. Environ Exp Bot 41:3–13

Tabak HH, Lens P, van Hullebusch ED, Dejonghe W (2005) Developments in bioremediation of soils and sediments polluted with metals and radionuclides—1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport. Rev Env Sci Biotech 4:115–156

Tank N, Saraf M (2003) Phosphate solubilization, exopolysaccharide production and indole acetic acid secretion by rhizobacteria isolated from Trigonella graecum. Ind J Microbiol 43:37–40

Traina SJ, Laperche V (1999) Contaminant bioavailability in soils, sediments, and aquatic environments. Proc Natl Acad Sci USA 96:3365–3371

Tripathi M, Munot HP, Shouche Y, Meyer JM, Goel R (2005) Isolation and functional characterization of siderophore producing lead and cadmium resistant *Pseudomonas putida* KNP9. Curr Microbiol 50:233–237

Tsavkelova EA, Cherdyntseva TA, Netrusov AI (2005) Auxin production by bacteria associated with orchid roots. Microbiology 74:46–53

Uchiumi T, Oowada T, Itakura M, Mitsui H, Nukui N, Dawadi P, Kaneko T, Tabata S, Yokoyama T, Tejima T, Saeki K, Oomori H, Hayashi M, Maekawa T, Sriprang R, Murooka Y, Tajima S, Simomura K, Nomura M, Suzuki A, Shimoda S, Sioya K, Abe M, Minamisawa K (2004) Expression islands clustered on symbiosis island of m*esorhizobium loti* genome. J Bacteriol 186:2439–2448

Umrania VV (2006) Bioremediation of toxic heavy metals using acidothermophilic autotrophes. Biores Technol 97:1237–1242

Van Assche F, Clijsters H (1990) Effect of metals on enzyme activity in plants. Plant Cell Environ 13:195–206

Verma A, Kukreja K, Pathak DV, Suneja S, Narula N (2001) In vitro production of plant growth regulators (PGRs) by *Azorobacter chroococcum*. Ind J Microbiol 41:305–307

Vivas A, Biro B, Ruiz-Lozano JM, Barea JM, Azcon R (2006) Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn toxicity. Chemosphere 52:1523–1533

Vogel JP, Woeste KE, Theologis A, Kieber JJ (1998) Recessive and dominant mutations in the ethylene biosynthetic gene ACS5 of Arabidopsis confer cytokinin insensitivity and ethylene overproduction, respectively. Plant Biol 95:4766–4771

Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250

Wang PC, Mori T, Komori K, Sasatsu M, Toda K, Ohtake H (1989) Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. Appl Environ Microbiol 55:1665–1669

Wang Y, Brown HN, Crowley DE, Szaniszlo PJ (1993) Evidence for direct utilization of a siderophore, ferroxamine B, in axenically grown cucumber. Plant Cell Env 16:579–585

Wani PA, Khan MS, Zaidi A (2006) An evaluation of the effects of heavy metals on the growth, seed yield and grain protein of lentil in pots. Ann Appl Biol (Suppl TAC) 27:23–24

Wani PA, Khan MS, Zaidi A (2007a) Cadmium, chromium and copper in greengram plants. Agron Sustain Dev 27:145–153

Wani PA, Khan MS, Zaidi A (2007b) Impact of heavy metal toxicity on plant growth, symbiosis, seed yield and nitrogen and metal uptake in chickpea. Aust J Expt Agric 47:712–720

Wani PA, Khan MS, Zaidi A (2007c) Effect of metal tolerant plant growth promoting Rhizobium on the performance of pea grown in metal amended soil. Arch Environ Conatm Toxicol. doi:10.1007/00244-9097-y

Wani PA, Khan MS, Zaidi A (2007d) Co-inoculation of nitrogen fixing and phosphate solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. Acta Agron Hung 55:315–323

Wani PA, Khan MS, Zaidi A (2007e) Synergistic effects of the inoculation with nitrogen fixing and phosphate-solubilizing rhizobacteria on the performance of field grown chickpea. J Plant Nutr Soil Sci 170:283–287

Wani PA, Khan MS, Zaidi A (2007f) Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (*vigna*) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70:36–45

Wani PA, Khan MS, Zaidi A (2007g) Impact of zinc-tolerant plant growth promoting rhizobacteria on lentil grown in zinc-amended soil. Agron Sustain Dev. doi:10.1051/agro-2007048

Wani PA, Khan MS, Zaidi A (2007h) Chromium reduction, plant growth promoting potentials and metal solubilization by *Bacillus* sp. isolated from alluvial soil. Curr Microbiol 54:237–243

Wani PA, Khan MS, Zaidi A (2008a) Effect of heavy metal toxicity on growth, symbiosis, seed yield and metal uptake in pea grown in metal amended soil. Bull Environ Contam Toxicol. doi:10.1007/s00128-008-9383-z

Wani PA, Khan MS, Zaidi A (2008b) Chromium reducing and plant growth promoting *Mesorhizobium* improves chickpea growth in chromium amended soil. Biotechnol Lett 30:159–163

Wani PA, Zaidi A, Khan AA, Khan MS (2005) Effect of phorate on phosphate solubilization and indole acetic acid (IAA) releasing potentials of rhizospheric microorganisms. Ann Plant Prote Sci 13:139–144

Wenzel WW, Adriano DC, Salt D, Smith R (1999) Phytoremediation: a plant-microbe-based remediation system. In: Adriano DC, et al. (eds) Bioremediation of contaminated soils. Agronomy monographs 37. ASA, CSSA and SSSA, Madison, pp 457–508

Wittenberg JB, Wittenberg BA, Day DA, Udvardi MK, Appleby CA (1996) Siderophore bound iron in the peribacteroid space of soybean root nodules. Plant Soil 178:161–169

Wu CH, Wood TK, Mulchandani A, Chen W (2006a) Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72:1129–1134

Yang SF, Hoffman NE (1986) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol 35:155–189

Yasmin S, Rahman M, Hafeez FY (2004) Isolation, characterization and beneficial effects of rice associated plant growth promoting bacteria from Zanzibar soils. J Basic Microbiol 44:241–252

Zaidi A, Khan MS (2005) Interactive effect of rhizospheric microorganisms on growth, yield and nutrient uptake of wheat. J Plant Nutr 28:2079–2092

Zaidi A, Khan MS (2006) Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on greengram-*Bradyrhizobium* symbiosis. Turk J Agric For 30:223–230

Zaidi A, Khan MS (2007) Stimulatory effect of dual inoculation with phosphate solubilizing microorganisms and arbuscular mycorrhizal fungus on chickpea. Aust J Exp Agric 47:1014–1022

Zaidi A, Khan MS, Aamil M (2004) Bioassociative effect of rhizospheric microorganisms on growth, yield and nutrient uptake of greengram. J Plant Nutr 27:599–610

Zaidi A, Khan MS, Amil M (2003) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Eur J Agron 19:15–21

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 64:991–997

Zayad A, Lytle CM, Qian JH, Terry N (1998) Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206:293–299

Zenk MH (1996) Heavy metal detoxification in higher plants: a review. Gene 179:21–30

Zhuang X, Chen J, Shin H, Bai Z (2007) New advances in plant growth promoting rhizobacteria for bioremediation. Environ Intern 33:406–413