PGPR-assisted phytoremediation of cadmium: an advancement towards clean environment

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One of the major problems, that the world is facing today due to rapid industrialization is environmental pollution caused by several factors, including heavy metals. Among the heavy metals, cadmium is a hazardous carcinogenic element. From contaminated soil, cadmium enters the plants through the roots and is accumulated in the harvestable (edible) parts, and thus gains entry into the food cycle. Phytoremediation plays a beneficial role in the remediation of cadmium contamination from soil, but becomes less effective with increasing toxicity. Even hyperaccumulator plants fail to perform under these conditions. Plant growth promoting rhizobacteria (PGPR), inhabitants of the plant rhizosphere, play a supporting role and promote bioremediation of soil by accumulation or transformation of contaminants, thereby enhancing plant growth and development. This article focuses on cadmium contamination and PGPR-assisted phytoremediation of cadmium-contaminated soils.

Keywords: Cadmium, phytoremediation, plant growth promoting rhizobacteria, toxicity.

INDUSTRIAL revolution is the main factor for metal pollution in the biosphere[1]. Heavy metal contamination is a serious environmental hazard for agricultural soils, plants, animals and human beings. The most toxic heavy metals are Pb, Hg, As, Cd, Sn, Cr, Zn and Cu[2]. These are a group of 65 metallic elements with density greater than 5 g/cm^3, exhibiting diverse properties with a potential to exert toxic effects on microorganisms and other forms of life. Among the heavy metals, cadmium has deleterious effects on agricultural ecosystem, environment and human health[3]. There are many sources that can cause cadmium contamination. They include use of Cd-containing sewage sludge, industrial emission, application of phosphatic fertilizers and municipal waste[4]. The heavy metals including cadmium are not degradable and persist in the soil for approximately 15–1100 years (ref. 5) and accumulate in the harvestable (edible) part of plants[6]. High accumulation rate generally causes growth inhibition and finally death of plant as well as cell[7].

Therefore, it is important to develop methods to remediate the heavy metal entry of toxic elements into the food chain. Various engineering methods (excavation, landfill, thermal treatment, leaching and electro-reclamation) presently being used are not fully satisfactory as they destroy the biotic and abiotic components of the soil, and are also technically difficult and expensive to use. According to Prasad[8], phytoremediation is defined as the use of plants to destroy, sequester and remove toxic pollutants from the environment. However, this method also has many drawbacks[8]. Therefore, phytoremediation associated with rhizospheric microorganisms has emerged as an acceptable agronomic remediation technology[9].

The relationships that exist between plants and microbes in the rhizosphere play a key role in enhancing the efficacy of phytoremediation[10] through a process known as 'bio-assisted phytoremediation'. In the soil, microorganisms present in and around the roots are called plant growth promoting rhizobacteria (PGPR); they use many types of mechanisms to promote plant growth and minimize stress. PGPR are helpful for plant growth enhancement and bioremediation of contaminated soil through sequestering or degrading heavy metals and other toxicants[11,12]. Bioremediation is, therefore, an option that offers the possibility to destroy or render harmless, various contaminants using natural biological activity. PGPR assist phytoremediation directly or indirectly through

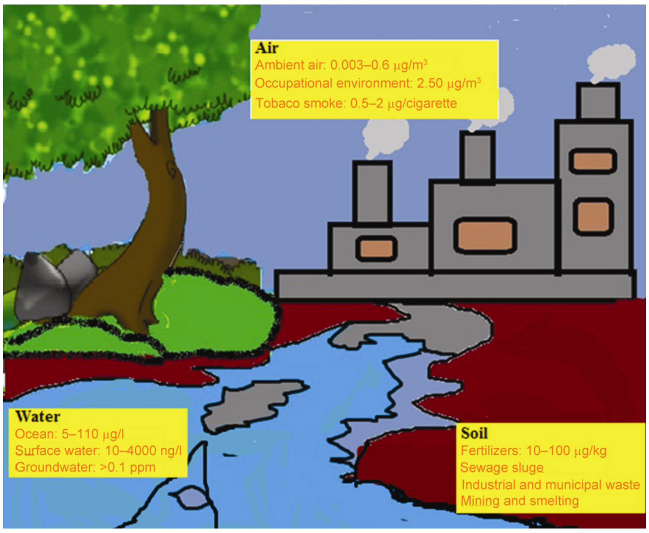

Figure 1. Level of cadmium in the environment.

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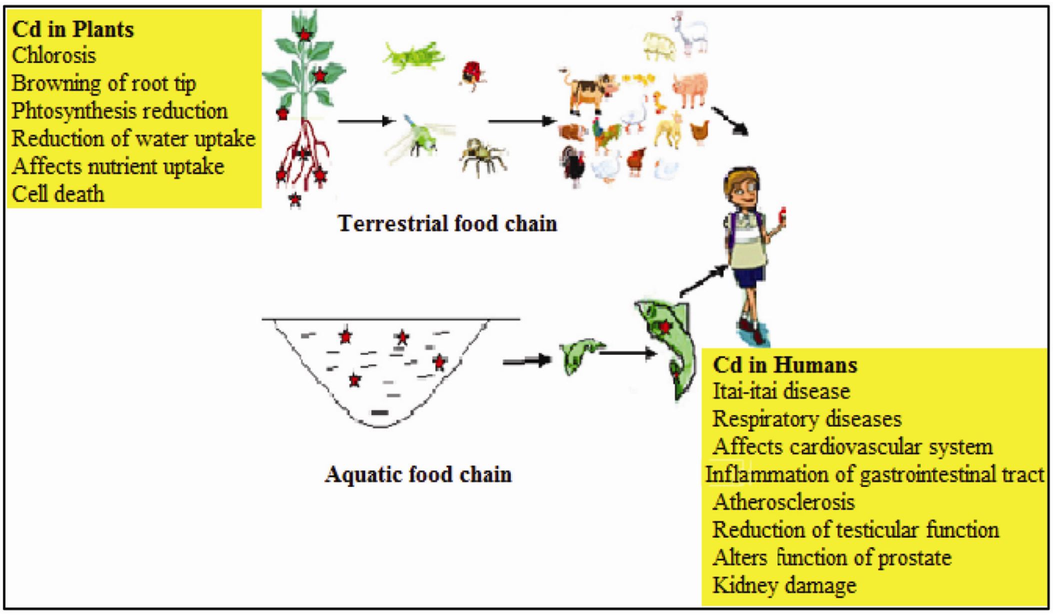

Figure 2. Movement of cadmium in the food chain and effects of the heavy metal on plants and humans.

several mechanisms, such as increased nutrient uptake, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances (hydrogen cyanide, HCN), phytohormone production (indoleacetic acid, IAA) and nitrogen fixation[11–14]. The present article focuses on the role of PGPR in remediation of cadmium from cadmium-contaminated soils and enhancement of phytoremediation in hyperaccumulator plants.

Cadmium toxicity in plants and animals

Cadmium has received special attention due to high persistent properties in the environment with an extremely long biological half-life (6–38 years in the human kidneys and 4–19 years in the liver). Cadmium level is different in soil, water and air as shown in Figure 1. It causes damage by moving up the food chain and finally accumulating in human beings and causes several damaging effects (Figure 2). The World Health Organization (WHO)[15] has set-up biotoxic limits of cadmium for human beings at 100–200 μg g^{-1} wet wt. The International Agency for Research on Cancer[16] has characterized cadmium as one of the 126 priority contaminants and the US-EPA considers it as human carcinogen. Cadmium is implicated as carcinogenic, mutagenic and teratogenic for a large number of animal species above threshold limit[17]. Cadmium poisoning could affect the kidney, cardiovascular system, liver and reproductive system, and cause renal damage, osteomalacia and lung cancer. Long-term exposure to high doses of cadmium causes itai-itai disease mainly in women and is characterized by severely impaired tubular and glomerular function and generalized osteomalacia and osteoporosis[18]. About 1–2 μg of cadmium is present in cigarette smoke; 10–20% of this is introduced into the lungs of a smoker in a complex form. It affects passive smokers as well. In passive smokers it causes the risk of sudden infant death syndrome, ear disease, asthma, respiratory illnesses, lung cancer and coronary heart diseases (Figure 3).

Storage of cadmium occurs in the liver, kidney, testis, spleen, heart, lungs, thymus, salivary glands epididymis and prostate. However, 50% of the cadmium is stored in the liver and kidneys in the form of CdMT (metallothionein) complex[19]. In kidney storage of cadmium, especially in the cortical part increases with long-time exposure to low doses (below 5 μg/g of creatinine)[15,20]. After exposure to cadmium, Cd^{2+} ions are present in the form of inorganic salts, e.g. $CdCl_2$ than as CdMT complex in the liver, kidney or bones. In urine, cadmium concentration approximately 5 μg/g of creatinine is considered as a safe limit[15]. The acidic environment (pH 4.5–5.5) of the gastro-intestinal tract is favourable for cadmium transportation with the help of proton metal cotransporter DMT1 (ref. 21). Low content of nutrients in the diet increases cadmium absorption in gastrointestinal tract.

Cadmium toxicity is also responsible for the production of reactive oxygen species (ROS) and reduction of antioxidant properties at the cellular level (Figure 4). Cadmium in the environment negatively affects biodiversity and the activity of soil microbial communities[22], and results in change in the qualitative and quantitative structure of the soil[23]. Regulatory limit of cadmium in agricultural soil is 100 ppm (ref. 24). Cadmium forms complex ions, but in a soil solution it occurs as Cd^{2+}. In plant–soil system, cadmium is the more mobile heavy metal; it easily enters into the plants and has no essential function[25]. Cadmium accumulation in plants affects root and shoot growth, inhibits nutrient uptake and homeostasis[26]. These negative effects cause physiological and morphological alteration in the cells, such as stunted growth, chlorosis and decreased reproducibility, by interacting with chlorophyll biosynthesis and biomolecules[27]. Symptoms of cadmium toxicity in plants are indicated by reduced growth, browning of root tips, chlorosis and finally death[28]. Alcantara *et al.*[29] reported that photosynthesis is affected by cadmium via inhibition of root Fe(III) reductase.

Cadmium disturbs the transport, uptake and use of various elements (K, P, Ca and Mg) and water. Reduction in absorption and transportation of nitrate from root to shoot is observed in cadmium-contaminated plants[30]. In *Silene cucubals*, reduction in the activity of nitrate reductase occurs under cadmium stress[31]. In the nodules of soyabean root, nitrogen fixation and assimilation of NH_3 are altered by cadmium toxicity[32]. It can also alter the permeability of plasma membrane and reduce water content in a cell[33]. Fodor *et al.*[34] reported that ATPase activity of plasma membrane is affected by cadmium in wheat and sunflower roots. Cadmium toxicity causes lipid peroxidation in cell membrane through reduction of functions of the membrane[34]. Cadmium toxicity also affects chloroplast metabolism by reducing the enzymes which are involved in CO_2 fixation[35].

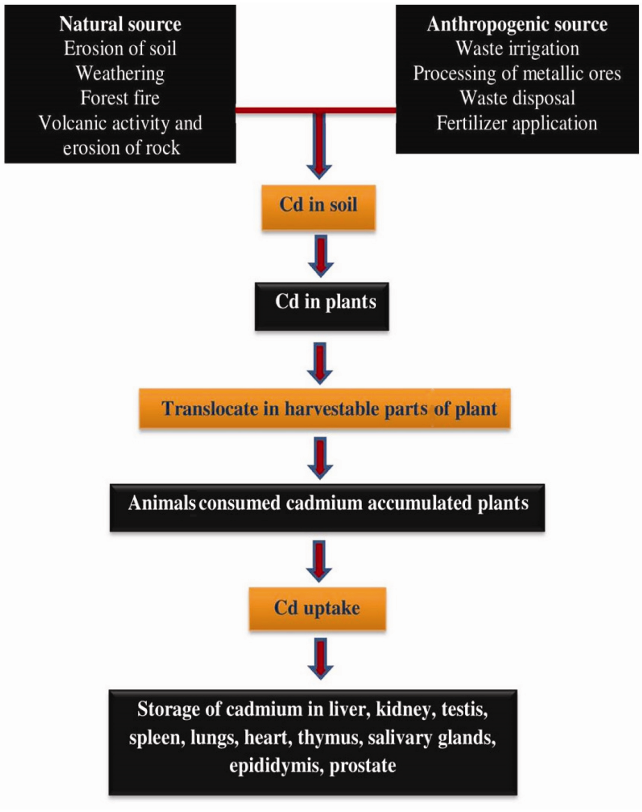

Figure 3. Transport and storage of cadmium.

Phytoremediation of cadmium and its limitations

Contamination of cadmium makes the soil unsuitable for agricultural and other uses. Therefore remediation of such soil types is important. High cost and failure or incomplete removal of heavy metals through various physicochemical and biological techniques have prompted the researchers to develop alternative low-cost methods. Phytoremediation is a novel, low cost, efficient and eco-friendly remediation strategy that has good public acceptance[36]. Many factors affect the phytoremediation efficiency such as area, contaminants, plants, etc. (Figure 5). In this process, plants accumulate high levels of contaminant heavy metals in their rhizosphere and root tissues[37]. Phytoremediation technique applied in the field using biofuel plants like maize, sunflower, soybean, barley and wheat, etc. enhances the quality of agricultural soil and make it highly relevant for agricultural use. For phytoremediation many alternate strategies can be used. The cultivation of ornamental plants, floriculture crops, tree plantations and growing of aromatic grasses was used to remediate soil[38]. However, this method is not widely accepted because of the issue of pollution transfer from soil to plant and heavy metal content in biomass[39].

Hyperaccumulator plants should be used which have high biomass production, and enhanced metal tolerance and metal uptake potential. However, most of hyperaccumulator plants are slow-growing and usually produce limited amounts of biomass. Selection of plants either as accumulators or hyperaccumulators is important in phytoremediation[40]. Plants that accumulate metals at high concentration are called hyperaccumulators[41]. If the shoots of plants contain >100 mg Cd kg^{-1}, >1000 mg Ni, Pb and Cu kg^{-1} or >10,000 mg Zn and Mn kg^{-1} (dry wt), then they are known as hyperaccumulators[42]. Hyperaccumulation is generally expressed on a dry weight basis; about 0.2% for more toxic elements like Cd, Pb, As, Hg, Cr and above 2% for the less toxic elements like Zn, Ni and Cu. There are approximately 45 hyperaccumulator plant families and 500 plants are reported in the literature – some important families are *Brassicaceae*, *Euphorbiaceae*, *Asteraceae*, *Fabaceae*, *Lamiaceae* and *Scrophulariaceae*[43].

Practical use of hyperaccumulator plants has several advantages in phytoremediation, but some properties induce limitations. These plants generally accumulate one specific element with limited root system and this limitations makes its use irrelevant[44]. In phytoremediation, various accumulator plants have high level of contaminants in harvestable parts and incineration is used after harvesting[45]. Phytoremediation is a plant-based technology that is applicable in low-concentration areas having longer

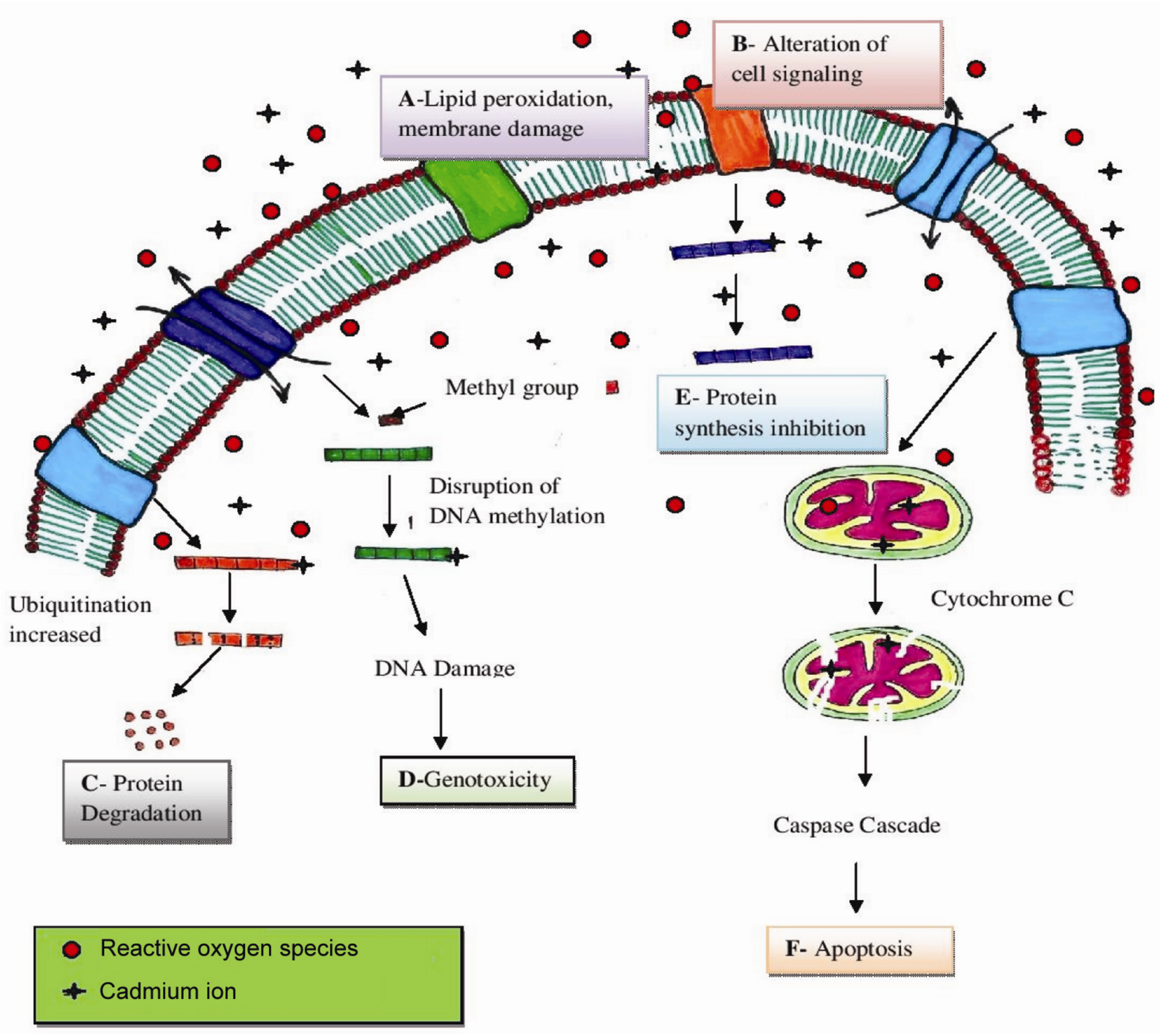

Figure 4. Mechanisms of cadmium toxicity at the cellular level.

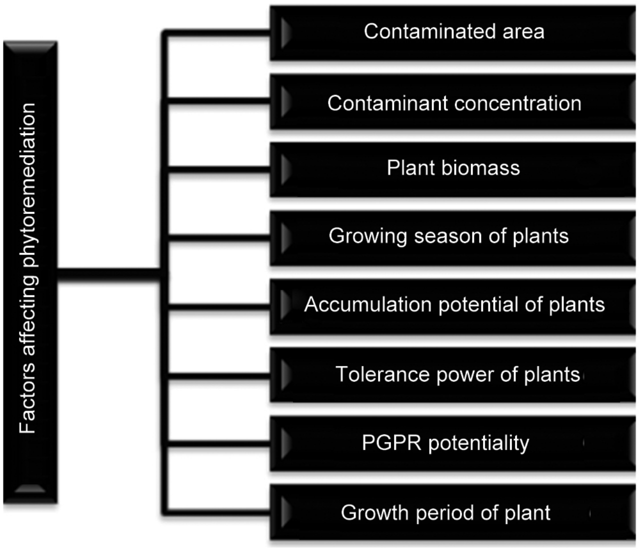

Figure 5. Factors affecting phytoremediation clean-up time.

treatment time[46]. Various mechanisms involved in this process are shown in Figure 6. Therefore, high cost is involved in traditional phytoremediation (without the involvement of microorganisms) and the owner of the polluted area does not get any benefits; he rather incurs loss. In phytoremediation, hyperaccumulator plants play an important role to enhance the removal of heavy metals from the soil through high growth rate and yield, but depletion of nutrients is responsible for reduction in plant growth under stress.

Role of microorganisms in the enhancement of phytoremediation

In recent years, bio-assisted phytoremediation or rhizoremediation plays an important role in decontamination of the soil. Rhizoremediation is the most emerging, eco-friendly and potentially effective process of biodegradation

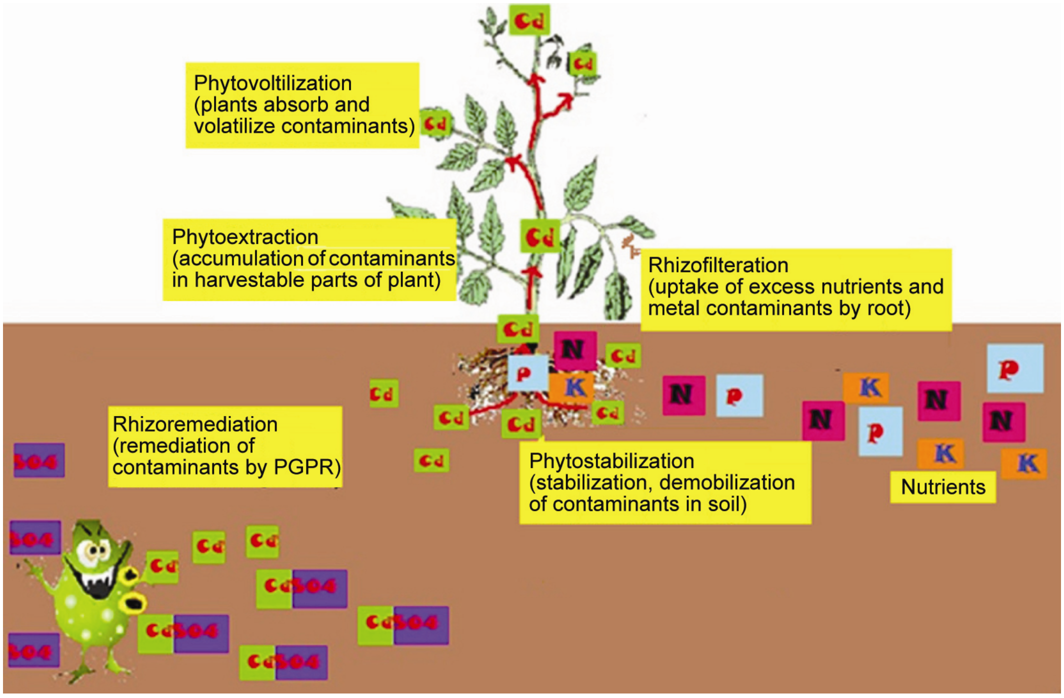

Figure 6. Mechanisms involved in the phytoremediation process.

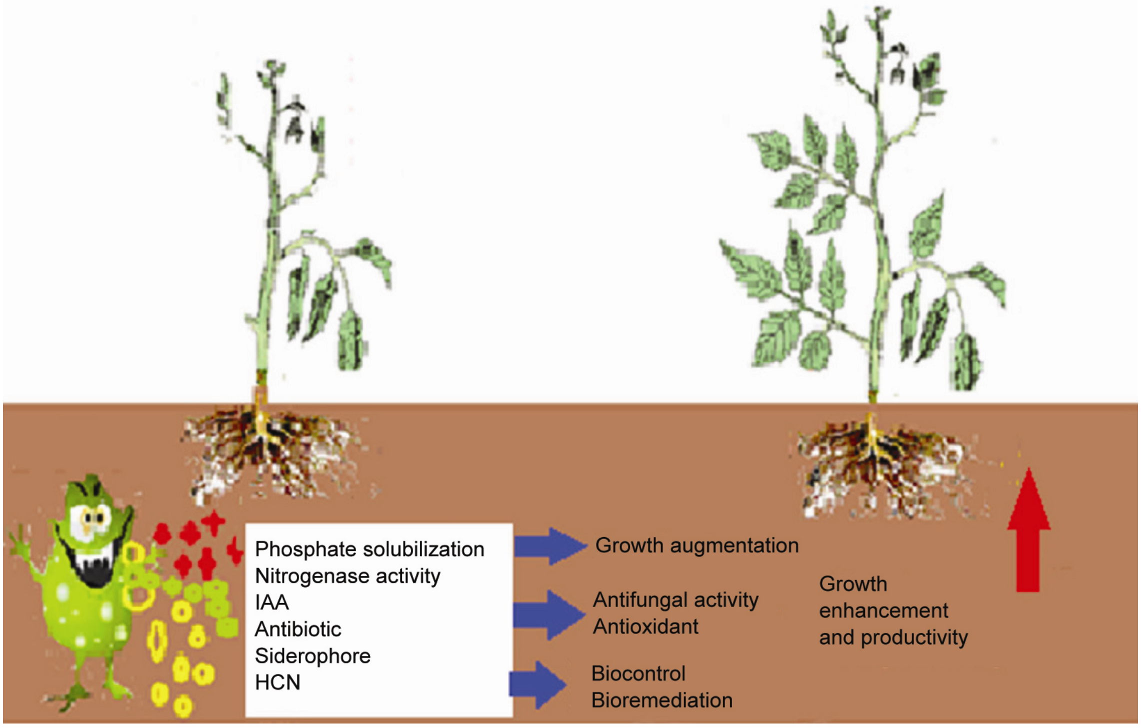

Figure 7. Mechanisms of growth promotion of plant by plant growth promoting rhizobacteria (PGPR).

of cadmium in the soil. It involves removal of specific contaminants from contaminated sites by mutual interaction of plant roots and suitable microbial species[47] (Figure 7). Rhizosphere is a micro-environment where microorganisms (PGPR) form special types of communities with plant growth promoting capabilities[48], and remove the toxic contaminants[49]. Glick[50] studied the interactions between plants and PGPR, and reported that the remediation technologies are developed by improving accumulation of metals and biomass production through

Table 1. List of phytoremediating plants and associated microorganisms with their mechanisms

Microorganisms	Plants	Metals	Mechanisms	Reference
Kluyvera ascorbata	Canola	Ni	Increased biomass; ACC deaminase	70
Psuedomonas	Canola	Cu	Increased biomass; IAA	69
Brevibacillus sp.	*Trifolium pratense*	Pb	Decreased lead uptake; IAA	71
Enterobacter aerogenes, *Rahnella aquatilis*	Indian mustard	Ni, Cr	Increased biomass and metal uptake; IAA, siderophores, ACC deaminase, phosphate solubilization	72
Achromobacterxyl osoxidans	Indian mustard	Cu	Increased root and shoot length and biomass; ACC deaminase, phosphate solubilization, IAA	73
Flavobacterium sp.	*Orychophragmus violaceus*	Zn	Increased root length, biomass, metal uptake	74
Bacillus edaphicus	Indian mustard	Pb	Increased biomass; IAA, siderophores, ACC deaminase	75
Pseudomonas putida	Canola	Ni	Increased seed germination and biomass; siderophores, IAA, ACC deaminase	76
Enterobacter sp.	Indian mustard	Ni, Zn, Cr	Increased biomass and metal uptake; IAA, siderophores, ACC deaminase, phosphate solubilization	77
Bacillus subtilis	Indian mustard	Ni	Increased nickel uptake; IAA, phosphate solubilization	78
Bacillus licheniformis, *Bacillus biosubtyl*, *Bacillus thurnigiensis*	Indian mustard	Se, Cd, Cr	Increased metal uptake depending upon specific metal–bacteria combination; mechanism unknown	79
Pseudomonas putida	Canola	Ni	Increased biomass in the field; IAA, ACC deaminase	80

the activities of rhizospheric microorganisms. According to Gadd[51], many types of bacteria improve the mobilization and immobilization of metals and tolerance power of the plants, but only few types of interactions between rhizospheric microbes and hyperaccumulating crops are important for decontamination purpose (Table 1). According to Amico *et al.*[52] soil bacteria transform metals into simple form by different types of mechanisms. Various types of soil microorganisms (PGPR) involved in rhizospheric biodegradation, and natural substances that are released by the plant roots increase the activity of these types of microorganisms[53].

PGPR were first used to promote the growth of plants, now they play a relevant role in remediation of cadmium-contaminated soils. PGPR assist in phytoremediation by the production of soluble minerals such as phosphorus and potassium[54], siderophore for iron and heavy metal chelation[14], phytohormones such as IAA and cytokinin[55], ACC deaminase for lowering stress ethylene[56], EPS and osmoprotectants[57], rhamnolipid[58] and immobilization of heavy metals[59]. Rhizobacteria such as *Pseudomonas cepacia*, *P. fluorescens* and *Streptomyces aurantiacus* were reported to increase crop yield up to 25% more than control[60]. Indian mustard and canola (*Brassica campestris*) seeds were grown in the presence of a PGPR strain in Ni, Pb and Zn-contaminated regions[61]. According to Belimov *et al.*[48], growth of canola (*Brassica napus*) plant improved on inoculating recalcitrant PGPR. Rhamnolipid is a commercially available amphiphilic biosurfactant produced by *Pseudomonas aeroginosa* (Table 2). It is extensively used in remediation of the soil through extraction of cadmium or other metals[58].

AMF are important endophytic fungi living in the roots of most terrestrial plants. They reduce metal toxicity to plants through decreasing translocation of heavy metals and their concentration[62]. In stress condition increasing interaction of plants and microbes enhances the availability of metal as well as growth of plant. According to Idris *et al.*[63] metal mobility and availability to the plants are enhanced by rhizospheric microorganisms releasing chelating agents, acidification, phosphate solubilization and redox changes. PGPR affect the bioavailability of cadmium and other metals by secretion of various metabolites such as siderophore, rhamnolipid, EPS and organic acids like oxalic acid, malic acid and citric acid, which chelate the cadmium ions and reduce their toxicity (Figure 8). Vulcanizing bacteria produce H_2S and alter the bioavailability of cadmium by precipitation of metal[64].

Cadmium bioavailability in rhizosphere region can be reduced by application of clay and modified clay minerals to cadmium and other heavy metal contaminated sites[65]. This method reduce cadmium toxicity in the plants as well as the rhizosphere region by accumulation of the heavy metal. Mycorrhizal species improve the bioavailability of toxic metals by affecting the root–rhizosphere system[66]. Siderophore, an iron-chelating complex synthesized by the PGPR helps in chelating iron,

Table 2. List of cadmium hyperaccumulating plants and associated microorganisms

Microorganisms	Plants	Mechanisms	Reference
Variovoraxparadoxus, *Rhodoccus* sp., *Flavobacterium* sp.	Canola	Increased root length; IAA, siderophores, ACC deaminase	61
Pseudomonas putida KNP9	Mung bean	Increased biomass and decreased metal uptake; siderophores	81
Rhizosphere bacteria	Graminaceae grasses	IAA, siderophore, ACC deaminase	52
Pseudomonas putida	Sunflower	Increased cadmium uptake and decreased toxicity; bacterium expresses a metal-binding peptide	64
Pseudomonas brassicacearum, Pseudomonas marginalis	Pea	Increased biomass and nutrient uptake; ACC deaminase	82
Pseudomonas sp., *Bacillus* sp.	Canola	Increased biomass and metal uptake; IAA	83
Mesorhizobium huakuii	Chinese milk vetch	Increased metal accumulation; bacterium expresses phytochelatin and metallothionein	84
Burkholderia cepacia	*Sedum alfredii*	Increased biomass, metal uptake and translocation of metal to shoots	85
Bacillus sp.	Canola, Corn, Sudan grass, tomato	Some increased biomass and cadmium uptake, IAA, siderophore, biosurfactant production	86
Pseudomonas aeruginosa	Black gram	Increased biomass and rooting, and decreased cadmium uptake; IAA, siderophore, ACC deaminase, phosphate solubilization	87
Pseudomonas sp, *Bacillus* sp.	Tomato	Increased root length, aboveground biomass and aboveground metal; siderophore, IAA, ACC deaminase	88
Streptomyces tendae	Sunflower	Decreased metal uptake and increased iron content; siderophores	89

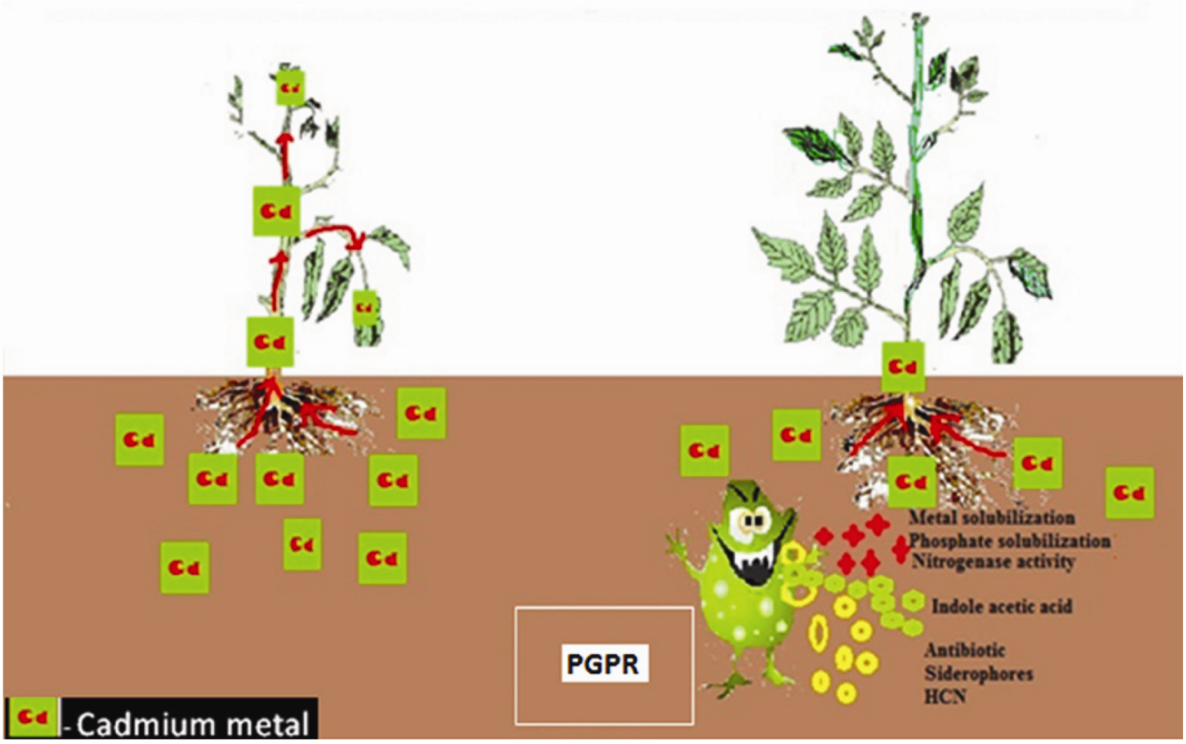

Figure 8. Mechanisms of PGPR action in the improvement of phytoremediation.

especially under cadmium and other heavy metal stress conditions. Microbial siderophore is more potent than phyto-siderophore for chelation of iron and heavy metals. So, under stress conditions, where phyto-siderophores fail to sequester iron for the plants, microbial siderophores help the plants avoid chlorotic conditions of leaves by improving chlorophyll synthesis through chelation of iron.

Siderophores also form complexes with heavy metals like Cd, Al, Cu, Ga, Pb, Zn, radionuclides, including U and Np (refs 67, 68). Binding of the siderophore to a metal increases the soluble metal concentration and hence bacterial siderophores help alleviate the stress imposed on plants by high levels of these heavy metals in the soil[14]. Ethylene is important for normal plant development, as well as for their response to stress. However, high levels of ethylene lead to inhibition of root elongation. PGPR strains possessing ACC deaminase activity get bound to seeds or roots of seedlings and can reduce the amount of plant ethylene by breaking it into ammonia and alpha ketogutarate, thereby reducing the extent of its inhibition on root elongation[56]. Growth of crop plant is improved by PGPR that help in decreasing the plant stress related to phytoremediation methods[69]. Selection of highly potential microbial combination is a big challenge for developing phytoremediation strategies. Once PGPR are established in the rhizospheric zone, native plants do not require fertilizers, pesticides or excess water; they restore wetlands and other habitats, and are helpful for creating natural parks, sanctuaries and other green areas.

Conclusion

In the natural environment, cadmium has several deleterious effects on the diversity of flora, fauna and microbial communities. Contamination of agricultural soils by cadmium results in its easy entry into the food chain, thereby affecting animal and human health. Various conventional strategies discussed in this article have some disadvantages and even green technology using plants alone, sometimes fails. Under these conditions, PGPR assist plants in remediation of cadmium from the contaminated environment (abiotic stress management) by production of various metabolites. These metabolites relieve plants from stress by various mechanisms such as supplying nutrients like iron and phosphate, lowering of stress ethylene, promotion of apical growth, etc. in normal as well as under stressed conditions. PGPR also help in biotic stress management in the rhizospheric zone of plants (indirect plant growth promotion–hydrogen cyanide production, rhamnolipids and other biosurfactants for biocontrol of pathogens) and enhance plant growth and biomass (direct plant growth promotion by modulating plant growth hormones and solubilizing phosphate, sequestering nutrients like iron, nitrogen, phosphorus and other essential minerals from the environment) which are key factors for further extraction of cadmium from contaminated soils by plants. Siderophores synthesized by these PGPR also improve bioavailability under normal as well as cadmium or other heavy metal stress conditions. Microorganisms isolated from cadmium metal stress sites are more adapted to peculiar soil environment and can be commercially and effectively used to assist in phytoremediation. On the basis of the above discussions, we can conclude that PGPR-assisted phytoremediation technique (Rhizoremediation) for treating cadmium-contaminated sites/soils is useful with high acceptance compared to other methods.

1. Patorczyk-Pytlik, B. and Spiak, Z., Effect of liming on the availability of cadmium for plants. *Zesz. Nauk. Kom. Człowiek i Środowisko*, *PAN*, 2000, **26**, 219–225.
2. Gosh, S., Wetland macrophytes as toxic metal accumulators. *Int. J. Environ. Sci.*, 2010, **1**(4), 523–528.
3. Wagner, G. J., Accumulation of cadmium in crop plants and its consequences to human health. *Adv. Agron.*, 1993, **51**, 173–212.
4. Lima, A. I. G., Pereira, S. I. A., de Almeida Paula Figueira, E. M., Caldeira, G. C. N. and de Matos Caldeira, H. D. Q., Cadmium detoxification in roots of *Pisum sativum* seedlings: relationship between toxicity levels, thiol pool alterations and growth. *Environ. Exp. Bot.*, 2006, **55**, 149–162.
5. Kabata Pendias, A. and Pendias, H., *Trace Elements in Soils and Plants*, CRC Press, Florida, USA, 2nd edn, 1992.
6. Baker, A. J., Reeves, R. D. and Hajar, A. S. M., Heavy metal accumulation and tolerance in British population of the metallophyte *Thlaspi caerulescens* J & C. Presel (Brassicaceae). *New Phytol.*, 1994, **129**, 61–68.
7. Khan, S. and Khan, N. N., Influence of lead and cadmium on the growth and nutrient concentration of tomato (*Lycopersicon esculentum*) and egg-plant (*Solarium melongena*). *Plant Soil*, 1983, **74**, 387–394.
8. Prasad, M. N. V., Phytoremediation of metal–polluted ecosystems: hype for commercialization. *Russ. J. Plant Phys.*, 2003, **50**, 686–700.
9. Helmisaari, H.-S., Salemaa, M., Derome, J., Kiikkilä, O., Uhlig, C. and Nieminen, T. M., Remediation of heavy metal contaminated forest soil using recycled organic matter and native woody plants. *J. Environ. Qual.*, 2007, **36**, 1145–1153.
10. Glick, B. R., Plant growth-promoting bacteria: mechanisms and applications. *Science*, 2012, **2012**, 15.
11. Ahemad, M., Implications of bacterial resistance against heavy metals in bioremediation: a review. *Int. Integr. Omics. Appl. Biotechnol. J.*, 2012, **3**, 39–46.
12. Ahemad, M. and Malik, A., Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater. *Bacteriol. J.*, 2011, **2**, 12–21.
13. Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I., Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann. Microbiol.*, 2010, **60**, 579–598.
14. Rajkumar, M., Ae, N., Prasad, M. N. V. and Freitas, H., Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.*, 2010, **28**, 142–149.
15. World Health Organization, Evaluation of certain food additives and contaminants. Thirty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 776, 1989.
16. International Agency for Research on Cancer, Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry

[M]. In *Monographs on the Evaluation of Carcinogenic Risks to Humans*, WHO Press, Lyon, France, 1994, vol. 58, p. 444.

17. Degraeve, N., Carcinogenic, teratogenic and mutagenic effects of cadmium. *Mutat. Res.*, 1981, **86**, 115–135.
18. Inaba, T. *et al*., Estimation of cumulative cadmium intake causing itai-itai disease. *Toxicol. Lett.*, 2005, **159**(2), 192–120.
19. FAO/WHO, 2011. JECFA cadmium evaluation, draft toxicological monograph, as submitted by WHO, to be published as: Safety evaluation of certain contaminants in food, 2011.
20. Gonick, H. C., Nephrotoxicity of cadmium and lead. *Ind. J. Med. Res.*, 2008, **128**, 335–352.
21. Ryu, D. Y., Lee, S. J., Park, D. W., Choi, B. S., Klassen, C. D. and Park, J. D., Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. *Toxicol. Lett*., 2004, **152**, 19–25.
22. McGrath, S. P., Effects of heavy metals from sewage sludge on soil microbes in agricultural ecosystems. In *Toxic Metals Soil–Plant Systems* (ed. Ross, S. M.), Wiley, New York, 1994, pp. 247–273.
23. Giller, K., Witter, E. and McGrath, S. P., Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil Biol. Biochem*., 1998, **30**, 1389–1414.
24. Salt, D. E., Blaylock, M., Kumar, N. P. B. A., Dushenkov, V., Ensley, D., Chet, I. and Raskin, I., Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology*, 1995, **13**, 468–474.
25. Lehoczky, E., Marth., P., Szabados, I., Palkovics, M. and Lukacs, P., Influence of soil factors in the accumulation of cadmium by lettuce. *Commun. Soil Sci. Plant Anal.*, 2000, **31**, 11–14.
26. Sanità di Toppi, L. and Gabbrielli, R., Response to cadmium in higher plants. *Environ. Exp. Bot.*, 1999, **41**, 105–130.
27. Van Assche, F., Cardinaels, C. and Clijsters, H., Induction of enzyme capacity in plants as a result of heavy metal toxicity; dose–response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. *Environ. Pollut*., 1988, **52**, 103–115.
28. Guo, J., Dai, X., Xu, W. and Ma, M., Over expressing GSHI and AsPCSI simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. *Chemosphere*, 2008, **72**, 1020–1026.
29. Alcantara, E., Romera, F. J., Canete, M. and De La Guardia, M. D., Effects of heavy metals on both induction and function of root Fe(III) reductase in Fe-deficient cucumber (*Cucumis sativus* L.) plants. *J. Exp. Bot.*, 1994, **45**, 1893–1898.
30. Hernandez, L. E., Carpena-Ruiz, R. and Garate, A., Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J. Plant Nutr.*, 1996, **19**, 1581–1598.
31. Mathys, W., Enzymes of heavy metal-resistant and non-resistant populations of *Silenecu cubalus* and their interactions with some heavy metals *in vitro* and *in vivo*. *Physiol. Plant*, 1975, **33**, 161–165.
32. Balestrasse, K. B., Benavides, M. P., Gallego, S. M. and Tomaro, M. L., Effect on cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. *Funct. Plant Biol*., 2003, **30**, 57–64.
33. Costa, G. and Morel, J. L., Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiol. Biochem.*, 1994, **32**, 561–570.
34. Fodor, A., Szabo-Nagy, A. and Erdei, L., The effects of cadmium on the fluidity and H?-ATPase activity of plasma membrane from sunflower and wheat roots. *J. Plant Physiol.*, 1995, **14**, 787–792.
35. De Filippis, L. F. and Ziegler, H., Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of Euglena. *J. Plant Physiol.*, 1993, **142**, 167–172.
36. Turan, M., and Esringu, A., Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb and Zn. *Plant Soil Environ*., 2007, **53**, 7–15.
37. Hayes, W. J., Chaudhry, R. T., Buckney, R. T. and Khan, A. G., Phytoaccumulation of trace metals at the Sunny Corner mine, New South Wales, with suggestions for a possible remediation strategy. *Aust. J. Ecotoxicol.*, 2003, **9**, 69–82.
38. Gupta, A., K., Verma, S. K., Khan, K. and Verma, R. K., Phytoremediation using aromatic plants: a sustainable approach for remediation of heavy metals polluted sites. *Environ. Sci. Technol.*, 2013, **47**, 10115–10116.
39. Gomes, H. I., Phytoremediation for bioenergy: challenges and opportunities. *Environ. Technol. Rev.*, 2012, **1**(1), 59–66.
40. Zhang, S., Chen, M., Li, T., Xu, X. and Deng, L., A newly found cadmium accumulator *Malva sinensis* Cavan. *J. Hazard. Mater.*, 2010, **173**, 705–709.
41. Visoottiviseth, P., Francesconi, K. and Sridokchan, W., The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environ. Pollut.*, 2002, **118**, 453–461.
42. Baker, A. J. M. and Brooks, R. R., Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. *Biorecovery*, 1989, **1**, 81–126.
43. Ghosh, M. and Singh, S., A review on phytoremediation of heavy metals and utilization of its byproducts. *Asian J. Energy Environ.*, 2005, **3**, 214–231.
44. Begonia, M. T., Begonia G. B., Ighoavodha, M. and Gilliard, D., Lead accumulation by tall fescue (*Festuca arundinacea* Schreb) grown on a lead contaminated soil. *Int. J. Environ. Res. Public Health*, 2005, **2**, 228–233.
45. Oh, K., Hu, X. F., He, C. Q., Yonemochi, S. and Shi, F., Perspective on application of phytoremediation technology in remediation of contaminated soils. In Proceedings of the 2011 World Congress on Engineering and Technology, Shanghai, China, 2011, pp. 532–535.
46. Wenzel, W. W., Adriano, D. C., Salt, D. and Smith, R., Phytoremediation: a plant–microbe-based remediation system. In *Bioremediation of Contaminated Soils* (eds Adriano, D. C. *et al*.), Madison, WI: ASA, CSSA and SSSA, Agronomy Monographs 37, 1999, pp. 457–508.
47. Kuiper, I., Lagendijk, E. L., Bloemberg, G. V. and Lugtenberg, B. J., Rhizoremediation: a beneficial plant–microbe interaction. *Mol. Plant–Microbe Interact.*, 2004, **17**, 6–15.
48. Belimov, A. A. *et al*., Characterization of plant growth-promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. *Can. J. Microbiol.*, 2001, **47**, 642–652.
49. Belimov, A. A. and Dietz, K.-J., Effect of associative bacteria on element composition of barley seedlings grown in solution culture at toxic cadmium concentrations. *Microbiol. Res.*, 2000, **155**, 113–121.
50. Glick, B. R., Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.*, 2003, **21**, 383.
51. Gadd, G. M., Heavy metal accumulation by bacteria and other microorganisms. *Experientia*, 1990, **46**, 834–840.
52. Amico, E. D., Cavalca, L. and Andreoni, V., Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. *FEMS Microbiol. Ecol.*, 2005, **52**, 153–162.
53. Walker, T. S., Bais, H. P., Grotewols, E. and Vivanco, J. M., Root exudation and rhizosphere biology. *Plant Physiol.*, 2003, **132**(1), 44–51.
54. Erkovan, I., Giillap, M. K., Dasci, M. and Koc, A., Effects of phosphorus fertilizer and phosphorus solubilizing bacteria application on clover dominant meadow: yield and botanical composition. *Turk. J. Field Crops*, 2010, **15**(1), 12–17.
55. Egamberdiyeva, D. and Hoflich, G., Influence of growth promoting bacteria on the growth of wheat at different soil and temperatures. *Soil Biol. Biochem.*, 2003, **35**, 973–978.

56. Glick, B., Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol. Res.*, 2014, **169**(1), 30–39.
57. Upadhyay, S. K., Singh, J. S. and Singh, D. P., Exopolysaccharide producing plant growth promoting rhizobacteria under salinity condition. *Pedosphere*, 2011, **21**(2), 214–222.
58. Mulligan, C. N., Recent advances in the environmental applications of biosurfactants. *Curr. Opin. Colloid Interface Sci.*, 2009, **14**, 372–378.
59. Ma, Q. Y., Traina, S. J., Logan, T. J. and Ryan, J. A., Effects of aqueous Al, Cd, Cu, Fe(II), Ni, and Zn on Pb immobilization by hydroxyapatite. *Environ. Sci. Technol.*, 1994, **28**, 1219–1228.
60. Hernandez, A. N., Hernandez, A. and Heydrich, M., Seleccion de rizobacterias asiciadas al cultivo del maiz. *Cultivos Trop.*, 1995, **16**, 5–8.
61. Belimov, A. A., Hontzeas, N., Safronova, V. I., Demchinskaya, S. V., Piluzza G., Bullitta, S. and Glick, B. R., Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.*, 2005, **37**, 241.
62. Redon, P. O., Béguiristain, T. and Leyval, C., Influence of *Glomus intraradices*on Cd partitioning in a pot experiment with *Medicago truncatula* in four contaminated soils. *Soil Biol. Biochem.*, 2008, **40**, 2710–2712.
63. Idris, R. *et al.*, Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thalspi goesingense*. *Appl. Environ. Microbiol.*, 2004, **70**, 2667–2677.
64. Wu, C.H., Wood, T. K., Mulchandani, A. and Chen, W., Engineering plant–microbe symbiosis for rhizoremediation of heavy metals. *Appl. Environ. Microbiol.*, 2006, **72**, 1129–1134.
65. Biswas, B., Sarkar, B., Mandal, A. and Naidu, R., Heavy metal-immobilizing organoclay facilitates polycyclic aromatic hydrocarbon biodegradation in mixed-contaminated soil. *J. Hazard Mater.*, 2015, **298**, 129–137.
66. Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S. and Hayes, W. J., Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 2000, **41**, 197–207.
67. Neubauer, U., Furrer, G., Kayser, A. and Schulin, R., Siderophores, NTA, and citrate: potential soil amendments to enhance heavy metal mobility in phytoremediation. *Int. J. Phytoremediat*, 2000, **2**, 353–368.
68. Kiss, T. and Farkas, E., Metal-binding ability of desferrioxamine B. *J. Inclus. Phenom. Mol.*, 1998, **32**, 385–403.
69. Burd, G. I., Dixonand, D. G. and Glick, B. R., A plant growth promoting bacterium that decreases nickel toxicity in seedlings. *Appl. J. Environ. Microbiol.*, 1998, **64**, 3663.
70. Reed, M. and Glick, B., Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. *Can. J. Microbiol.*, 2005, **51**, 1061–1069.
71. Vivas, A., Vorosm, A., Biro, B., Barea, J. M., Ruiz-Lozano, J. M. and Azcón, R., Beneficial effects of indigenous Cd-tolerant and Cd-sensitive *Glomus mosseae* associated with a Cd-adapted strain of *Brevi bacillus* sp. in improving plant tolerance to Cd contamination. *Appl. Soil Ecol.*, 2003, **24**, 177–186.
72. Kumar, K. V., Srivastava, S., Singh, N. and Behl, H. M., Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *J. Hazard. Mater.*, 2009, **170**, 51–57.
73. Ma, Y., Rajkumar, M. and Freitas, H., Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. *J. Hazard. Mater.*, 2009, **166**, 1154–1161.
74. He, C. Q. *et al.*, Effect of Zn-tolerant bacterial strains on growth and Zn accumlation in *Orychophragmus violaceus*. *Appl. Soil Ecol.*, 2010, **44**, 1–5.
75. Sheng, X., He, L., Wang, Q., Ye, H. and Jiang, C., Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in cadmium-amended soil. *J. Hazard. Mater.*, 2008, **155**, 17–22.
76. Rodriguez, H., Vessely, S., Shah, S. and Glick, B. R., Isolation and characterization of nickel resistant *Pseudomonas* strains and their effect on the growth of non-transformed and transgenic canola plants. *Curr. Microbiol.*, 2008, **57**, 170–174.
77. Kumar, K. V., Singh, N., Behl, H. M. and Srivastava, S., Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. *Chemosphere*, 2008, **72**, 678–683.
78. Zaidi, S., Usmani, S., Singh, B. R. and Musarrat, J., Significance of *Bacillus subtilis* SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. *Chemosphere*, 2006, **64**, 991–997.
79. Hussein, H. S., Optimization of plant–bacteria complex for phytoremediation of contaminated soils. *Int. J. Bot.*, 2008, **4**, 437–443.
80. Farwell, A. J. *et al.*, The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. *Plant Soil*, 2006, **288**, 309–318.
81. Tripathi, M., Munot, H., Shouche, Y., Meyer, J. M. and Goel, R., Isolation and functional characterization of siderophore-producing lead- and cadmium-resistant *Pseudomonas putida* KNP9. *Curr. Microbiol.*, 2005, **50**, 233–237.
82. Safranova, V. I., Stepanok, V. V., Engqvist, G. L., Alekseyev, Y. V. and Belimov, A. A., Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fertil. Soils*, 2006, **42**, 267–272.
83. Sheng, X.-F. and Xia, J.-J., Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere*, 2006, **64**, 1036–1042.
84. Ike, A., Sriprang, R., Ono, H., Murooka, Y. and Yamashita, M., Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant rhizobia with the MTL4 and the PCS genes. *Chemosphere*, 2007, **66**, 1670–1676.
85. Li, W. C., Ye, Z. H. and Wong, M. H., Effects of bacteria on enhanced metal uptake of the Cd/Zn hyperaccumulating plant, *Sedum alfredii*. *J. Exp. Bot.*, 2007, **58**, 4173–4182.
86. Sheng, X.-F., Xia, J.-J., Jiang, C.-Y., He, L.-Y. and Qian, M., Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environ. Pollut.*, 2008, **156**, 1164–1170.
87. Ganesan, V., Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. *Curr. Microbiol.*, 2008, **56**, 403–407.
88. He, L.-Y., Chen, Z.-J., Ren, G.-D., Zhang, Y.-F., Qian, M. and Sheng, X.-F., Increased cadmium and lead uptake of a cadmium hyperaccumulator tomato by cadmium-resistant bacteria. *Exotoxicol. Environ. Saf.*, 2009, **72**, 1343–1348.
89. Dimpka, C. O., Merten, D., Svatos, A., Büchel, G. and Kothe, E., Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. *J. Appl. Microbiol.*, 2009, **107**, 1687–1696.

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