# Plant Growth-Promoting Bacteria: A Good Source for Phytoremediation of Metal-Contaminated Soil

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**Abstract** Phytoremediation is a sustainable technique for the removal of contaminants from the polluted environments, but the removal of contaminants by plants and microorganisms together is more effective than phytoremediation alone. Phytoremediation is enhanced with the involvement of microorganisms in soil as well as in water. Rhizosphere microorganisms develop beneficial interactions with plants which ultimately results in increased plant growth and improved phytoremediation of heavy metals. Microorganisms play a vital role in mobilization and immobilization of metal contaminants from the environment for availability to different plants. Different microorganisms produce different metabolites which interact and make complexes with the contaminants and decrease their levels of toxicity by transforming them to less toxic state.

**Keywords** Phytoremediation • Plants • Microorganisms • Contaminants • Rhizobacteria • Metals

## 1 Introduction

Industrialization is increasing with increased global economy during past century, due to which a dramatically increased level of anthropogenic chemical release is observed into the atmosphere. Predominant pollutant includes halogenated hydrocarbons, aromatic hydrocarbons, petroleum hydrocarbons, salts, solvents, heavy metals, and pesticides. These pollutants are causing stress to environment as well as to human health [1–3]. Phytoremediation is a plant-mediated viable technique for the removal of contaminants from the environment. Phytoremediation of polluted environment are usually occurred by phytodegradation, phytoextraction, phytovolatilization, phytostabilization, rhizodegradation, and rhizofiltration [4, 5]. The process of phytoremediation depends on the capability of plants for accumulating or metabolizing the metal contaminants to less toxic state. The accumulation, degradation, or uptake of pollutants differs from species to species.

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The selection of plants for phytoremediation process is usually based on growth and yield of plant, tolerance towards contaminants, rate of accumulation, root formation, and transpiration [4]. Phytoremediation has several advantages as compared to other remediation techniques, such as fewer costs for installation and maintenance, less environmental disruption, and some valuable side effects including biofuel production and sequestration of carbon [6, 7]. Soil polluted with combination of contaminants is difficult to treat; different strategies are required to remediate different contaminants [8, 9]. Mixed contamination of different toxic materials is causing huge number problems all over the word in soil, sediments, and water [9, 10]. The process of degradation depends on microbial population present at polluted site [11].

## 2 Plant Growth-Promoting Bacteria as Tool for Phytoremediation

Microorganisms are present ubiquitously in environment. They can grow in and bear the extreme environmental conditions. Rhizosphere is an important environment for different microbes including protozoa, algae, fungi, and bacteria [12]. Plants develop advantageous relationships with microorganisms which can help in improving growth and yield of plants. Endophytic microorganisms live inside the tissues of plants without causing damage to host. Their application can result in increased availability of nutrients and metals to plants, cause reduction in their level of toxicity, and promote plant growth for better accumulation of heavy metal contaminants [13, 14]. The uses of plants in combination with contaminant tolerant/resistant plant growth-promoting bacteria are helpful in possible cleanup of polluted soils [14]. Effectiveness of bioremediation is influenced by different factors which affect each other in complex ways; these factors include environmental conditions and characteristics of contaminants [9]. Different evidences suggest that roots of plants stimulate the microenvironment by releasing secretions, which influence the microbial pool in rhizosphere soil [15, 16].

In soil rhizosphere, phytoremediation efficacy is enhanced by the activities of different microorganisms by the following two methods: (1) by direct enhancement of phytoremediation, microbes associated with plants improve translocation of contaminants/metal or decrease their availability/mobility in rhizosphere, (2) by indirect enhancement of phytoremediation, microorganisms enhance tolerance towards metals in plants or by improving biomass production in plant to arrest or remove the contaminants. Beneficial microorganisms play an important role to mobilize metal contaminants, and the metabolites produced by these microorganisms are less toxic, biodegradable, and their in situ production is possible in rhizosphere soils. Plant growth stimulating components include fixation of atmospheric nitrogen, phosphate solubilization, production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, growth hormones, and siderophore by microorganisms associated with plants for better plant growth in polluted soils [13, 14, 17–19]. Nodule forming bacteria also affect plants by enhancing plant growth and development of roots, which improves tolerance/resistance towards a number of ecological stresses [19–22]. Some examples of microorganisms involved in enhanced phytoremediation of metals are summarized in Table 1.

**Table 1** Phytoremediation of some heavy metals by microorganisms associated with plants

| Microorganism | Metal uptake | Plant | Reference |
|---|---|---|---|
| *Bacillus subtilis* | Ni | *Brassica juncea* | [61–67] |
| *Klebsiella* sp. and *Enterobacter* sp. | Zn, Pb and Cd | *Brassica napus* | [68] |
| *Burkholderia* sp. | Zn and Cd | *S. alfredii* | [5, 69] |
| *P. tolaasii*, *P. fluorescens*, and *Mycobacterium* sp. | Cd | *Brassica napus* | [70] |
| *G. diazotrophicus* | Zn | – | [71] |
| *Pseudomonas* sp. | Cd | *Solanum nigrum* | [72] |
| *Psychrobacter* sp. | Ni | *Helianthus annuus* and *Ricinus communis* | [13] |
| *Arthrobacter nitroguajacolicus* | Ni | *A. serpyllifolium* | [63] |
| *P. fluorescens* and *Chromobacterium violaceum* | Cu and Ni | – | [53] |
| *Pseudomonas aeruginosa* | Cr and Pb | *Zea mays* | [26] |
| *Eleocharis acicularis* | Zn, Cu, Pb and As | – | [73] |
| *Sanguibacter* sp. | Cd | *Nicotiana tabacum* | [74] |
| *Stenotrophomonas* sp., *Comamonas* sp., and *Pseudomonas* sp. | As | *Pterisvittata* | [64] |
| *Pseudomonas putida* | Cd | *Helianthus annuus* | [75] |

# 3 Activities of Microorganisms for Phytoremediation

## *3.1 Siderophore Production*

Microorganisms associated with plants are able to produce iron chelators also termed as siderophores, due to iron deficiency in rhizosphere. These compounds have low molecular weights ranging from 400 to 1000 Da, having high affinity for making complexes with iron as well as with other metals including cadmium, copper, aluminium, zinc, gallium, lead, and indium [19, 23–25]. They also contain some functional groups, which can be generally divided into three major groups on the basis of chemical nature of oxygen ligands donating moieties to coordinate with iron. These functional groups include carboxylates, hydroxamates, and catecholates. Siderophores dissolve inaccessible forms of minerals containing heavy metals by forming complexes with them. Therefore, these microorganisms are known to play significant role in phytoextraction of heavy metals [26–29]. Microbial siderophore production depends on different factors such as availability of iron, nutrients, pH, and type and level of heavy metals present in soil.

For example, *Pseudomonas aeruginosa* is a good example of rhizosphere bacteria which produces pyochelin and pyoverdine, and as a result it increases the bioavailability of Pb and Cr in soil [26, 30]. Similarly, Siderophore producing *Streptomyces tendae*-F4 enhanced the cadmium uptake in sunflower plant. Siderophore production by mycorrhizal fungi has also been reported [31, 32].

Braud et al. [33] reported increased production of pyoverdine in the presence of Ni, Cr, and Cu. Ectomycorrhizal fungi isolated from *Pinus radiata* including *Rhizopogon luteolus*, *Suillus luteus*, and *Scleroderma verrucosum* are reported for producing hydroxamate and catecholate siderophores under iron deficiency in environment. Such reports suggested that siderophore producing microorganisms can be inoculated to plants for improved uptake of heavy metal by plants.

There are some reports which oppose the idea of microbe-assisted increased uptake of metals by plants. For example with siderophores producing *Pseudomonas aeruginosa*-KUCd1 inoculation, decrease in uptake of cadmium was observed in shoots and roots of *Brassica juncea* and *Cucurbita pepo* [34]. Similar results were reported [35] when siderophores producing nickel-resistant *Pseudomonas* were inoculated to chickpea plants; decreased nickel uptake but increased growth of plant was observed. Siderophore positive bacterial strains are not always involved in enhanced uptake of metal by plants [36, 37]. The possible reason behind this conflict in reported results might be the plants capability to uptake heavy metals, which depends on several other factors such as availability of metal, type of plant, and its capacity of transporting metals towards shoot. In plants, root activities play an important role in metal uptake by releasing root exudates, which is influenced by the properties of soil, and also affects the nutrients, pH, and diversity of microorganisms associated with plants [38]. The synthesis of siderophores production by microbes and their importance in transport and tolerance towards metals have been studied well [24], but their interaction with plants in metal-contaminated soil still needs more attention. Siderophore production can also increase or decrease the harmful effects of metals in microbes [18]. Yasin et al. [39] also reported increased uptake of iron and selenium through bio-fortification of wheat when inoculated with *Bacillus pichinotyi* strain.

### 3.2 Organic Acid Production

Microorganisms associated with plants produce organic acids having low molecular mass. These compounds contain CHO and carboxyl groups having molecular mass of up to 300 Da. They play an important role in solubilization and mobility of heavy metals and other minerals in soil [40, 41]. Organic acid bind with metals by making complexes with them, and their stability depends on various factors which include the nature of acid, binding form of heavy metal, and pH of soil [19, 40, 42]. Organic acid production by microorganisms associated to plants play a significant role in improving the mobility of essential ions and nutrients to be taken up by plants. Mobilization of heavy metals or ions can be correlated with improved release of organic acids including acetic acid, oxalic acid, succinic acid, tartaric acid, and formic acid [43]. Among the microbially produced organic acids, oxalic acid, citric acid, and gluconic acid have gained more importance because they increase the bioavailability of heavy metals.

According to a recent study, zinc solubilizing *Gluconacetobacter diazotrophicus* produced a derivative of gluconic acid (5-ketogluconic acid) which helps in zinc solubilization under in vitro conditions by solubilizing $Zn_3(PO_4)_2$, ZnO, and $ZnCO_3$. In a study related to *Sedum alfredii* (Zn/Cd hyperaccumulating plant), inoculation with Zn/Cd-resistant rhizosphere bacteria significantly improved the concentration of water soluble zinc and cadmium as compared to untreated control. Mycorrhizal fungi also produce organic acids in rhizosphere and play an important role in mobilization of heavy metals. *Oidiodendron maius* has also been studied for the production of citric and malic acid for improved production of Zn [44]. Similarly, *Beauveria caledonica* and *Aspergillus niger* also facilitate the release of zinc and lead from pyromorphodite through the production of acids for increased uptake by plants [45, 46]. These reports highlight the importance of organic acid producing microorganisms to promote phytoextraction in polluted environments. The formation of complexes between organic acid and metal is influenced by type and concentration of organic acid, physical and chemical properties of soil, and presence of minerals in the environment [19]. Properties of rhizosphere soil including buffering capacity, sorption, metal complexation, and biodegradation may change organic acid profile and behavior [18, 47].

### *3.3 Production of Biosurfactant*

Petroleum hydrocarbons are most common amongst the persistent organic contaminants found in shoreline and estuaries, which is causing great concerns [48]. Another essential metabolite having potential to improve mobility of metal and phytoremediation is biosurfactant production by microorganisms. It is an amphiphilic molecule having hydrophobic (nonpolar) tail and a hydrophilic (ionic/polar) head. Hydrophilic moiety contains proteins or peptides, and hydrophobic group comprises of hydroxylated, saturated, or unsaturated alcohols and fatty acids. Biosurfactant production by microorganisms increases the solubility and bioavailability of heavy metals in soil by making complexes with them on soil interface to desorb from the environment. These surfactant producing microbes play an important role in mobilization of heavy metals in contaminated soils [19, 49]. An experiment was conducted to check the petroleum degradation by tall fescue, and for this purpose three bacterial strains were used including *Pseudomonas* sp. (SB), *Klebsiella* sp. (D5A), and *Streptomyces* sp. (KT) having potential of biosurfactant production, plant growth enhancement, and petroleum degradation. The results showed that palmitic acid production by microorganisms was most critical for petroleum removal by phytoremediation [50]. A study revealed that *Enterobacter ludwigii* can efficiently colonize the endosphere and rhizosphere of alfalfa, birdsfoot trefoil, and Italian ryegrass. It also contained alkane hydroxylase due to which they actively degrade hydrocarbons in estuaries [51].

### 3.4 Glycoprotein and Polymeric Substances Production

Microorganisms associated with plants play a significant role in decreased mobility of toxic contaminants in soil by producing extracellular polymeric substances (EPS), muco-polysaccarides, and proteins [19]. According to a study conducted on plants inoculated with EPS releasing *Azotobacter*s pp, inoculation resulted in the immobilization of Cr and Cd by 21.9 mg $g^{-1}$ and 15.2 mg $g^{-1}$, respectively [52]. *Azotobacter* inoculation has also been reported for reduced uptake of metal by wheat plant. The production of glomalin (insoluble glycoprotein) by arbuscular mycorrhizal fungi can bind heavy metals in metal-contaminated soils. The concentration of immobilized heavy metals depends on the quantity of glomalin produced by mycorrhizal fungi, more the production of glomalin the strain become more appropriate for phytostabilization. Although arbuscular mycorrhizal fungi can immobilize the heavy metals by the releasing glomalin, but its complete structure and mechanism which lead to decreased uptake of metals by plants is still poorly understood [18].

### 3.5 Oxidation and Reduction Reaction

A number of microorganisms associated to plants can change the mobilization of toxic metals by reduction or oxidation reactions. From the phytoextraction view point, oxidization of metals by microorganisms is getting more importance. For example, sulfur oxidation by microbes present in rhizosphere soil showed increased mobility and uptake of Cu by plants tissues in polluted soils [53]. Sulfur and iron oxidizing bacteria have been reported for increased availability of heavy metals in soil by the production of acids [54]. Plant-associated chromium tolerating *Cellulosimicrobium cellulans* strain inoculation in green chilly resulted in decreased uptake of chromium in root as well as shoot by 56 and 37 %, respectively, in Cr(VI) polluted soil. Abou-Shanab et al. [55] reported lower Cr accumulation in water hyacinth shoots by chromium reducing bacteria [18]. *Streptomyces* sp. M-7 has also been reported to remediate lindane (pesticide) and Cr(VI) in contaminated places [56].

### 3.6 Biosorption

Plant-associated microorganisms also have the potential to enhance mobilization of metal by means of biosorption mechanism. Biosorption is the process of metal adsorption by microorganisms either by dependent or independent metabolisms [13, 19]. A number of studies have been reported for decreased metal uptake by plants due to microbial biosorption processes. For example, according to a study conducted to assess the mobilization of metal by bacterial inoculation, it was found that *Burkholderia* sp. and *Magnaporthe oryzae* reduced the concentration of Cd and Ni accumulation in tomato plant [57]. Likewise, Vivas et al. [58] reported that *Brevibacillus* sp. inoculation in *Trifolium repens* resulted in reduced Zn accumulation in shoots as compared to untreated control plants due to enhanced biosorption of Zn. From these reports, it can be concluded that metal binding microbes are able to restrict or reduce the bioavailability of metals in plants. Similarly, mycorrhizal fungi has also been studied for biosorption activities; they act as a barrier for translocation of metals in plants. Investigation of pine seedling showed that treatment with *Lactarius rufus*, *Amanita muscaria*, and *Scleroderma citrinum* resulted in reduced translocation of Pb, Cd, or Zn in shoots in comparison with untreated plants [59]. Large surface area of mycorrhizal fungi helps in increased adsorption capacity for metals in polluted soils. Extracellular and intracellular components of fungal cell wall may also arrest/immobilize toxic ions inside the plant roots [18, 60].

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