

Sources, Microbial Interaction and Available Strategies for Remediation of Heavy Metals

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Abstract

High concentrations of heavy metals (Cadmium, Arsenic, Chromium, Lead, Mercury, Copper, Cobalt, Zinc, Nickel, and Selenium) in soil are threat to the ecosystem, human health, food safety, animal health. Heavy metal contaminants are increasing rapidly due to industrialization especially automobile industry. Previously, various techniques were developed and improved over time like encapsulation, surface capping, landfilling, soil washing, soil flushing, electro kinetic extraction, solidification, stabilization, phytoremediation, and bioremediation. These techniques minimize the contaminants by utilizing immobilization, containment and removal mechanisms. Bioremediation is a promising technique that utilizes the capability of plants and microbial resources for decontamination of ecosystem from heavy metal contaminants. Microbes have shown capability to utilize heavy metal remediation and assist plant tolerance for heavy metal accumulation. Earlier published studies have not yet completely evaluated proficiencies to large scale however, in the present review, critical analysis of reported techniques focusing on the bioremediation have been discussed. In depth analysis for the heavy metal remediation is of paramount importance of heavy metal contaminant emerging issue of soil pollution.

Keywords: Microbes, Heavy Metal, Remediation, Sources

Introduction

Heavy metals are integral part of environmental pollutants. Human activities have also contributed to the increment of environmental pollutants in addition to Natural Processes [1]. These heavy metals migrate to the cleaner areas by leaching into the soil or contaminated sewage sludge that contaminates the ecosystem. Numerous types of techniques are being used to clean up the environment but most of them are not efficient and costly [2]. Comparative to conventional chemical treatments microbial bioremediation is an exceptional technique. Heavy metal Pollution is a common problem all over the world and hazardous to human health and ecosystems with expensive cleanup costs. Due to usage of heavy metal into the industry and agriculture there is substantial amount of heavy metal is removed and discharged into the ecosystem [3]. Small Microbial interactions Distributes metals into the environment as metalloids while larger quantities interactions have a greater at environmental impact. Cellular legends have the unique nature to bind with the metals due to the ionic nature of metals and intoxicate them by relocation of essential metal from its binding site. Various Archaea and Eubacteria have capability to oxidization of Mn, Fe, Se, Co and reduction of Mn (IV), AsO₂, SeO₃, Fe, Co on a large scale and conservation of energy [4].

Heavy Metals in Soils

Heavy metals correspond to environmental problems, due to extensive distribution usage, especially its toxicity to humans and biosphere while there are some certain concentrations that crucial for living organisms [5]. In crucial elements, transition metals are having high densities >5 g cm⁻³ then other metals (Table-2.1). Essential elements are included heavy metals such as Zinc (Zn) and Iron (Fe) and toxic metals lead (Pb), Mercury (Hg) and cadmium (Cd). Soil is major food production resource and raw materials for living organism. Therefore, soil is common source of heavy metal waste release by numerous human activities [6]. Life is at risk, when the concentration of heavy metals has high concentration that cause direct or indirect effect on plants, water quality, humans health, wider ecosystems, animals, buildings and building materials and soil can be considered as Contaminated [7]. However low concentration of few metals is necessary for life such as zinc, manganese, copper, cobalt, and chromium, also known as micronutrients or trace elements. nonessential is known as toxic heavy metals such as Lead, Cadmium, Mercury, Barium, Titanium, Antimony, Uranium and Arsenic [8]. Heavy metals are released by geological processes and human activities in the soil and environment. In nature, heavy metals usually exist in the soil parent material. Parent

material weathering is the main source of soil heavy metal pollution. Microbial interactions, biosorption processes and bioremediation are the unique processes that are involved in the removal of heavy metal waste from the ecosystems nowadays [9].

Chromium

Chromium is not an important for plant developmental processes. Chromium has economically significant in industry, but there is also significant metal pollution in the environment by the usage of Cr in Chemical Processes. Chromium composites are used in wood preservatives, textile dyes, chrome plating, dyes, leather sewing, pulp and paper production. In particular, the tanning industry produces massive amounts of Chromium as Organic chromium produced by the tanneries is released directly into the environment in the form of sludge and sewage, causing great concern for the environment and human health as pollution [10]. Chromium salts are used to leather production and wastewater is flushed directly into ecosystem. Hexavalent chromium salts are not released directly into the environment and mix with various soil constituents and seep into underground drinking water sources and hazardous to humans and domestic animals [11].

Cadmium

Cadmium is representative of the Group II-B from Periodic Table and among the rare metals, which makes it rare in natural lakes and soil. The average Cd content in plants is 0.005-0.02 ppm less than 1 ppm in soil [12]. Very analogous to zinc, going through similar geochemical processes, with oxidation state is +2 (same as zinc). Cadmium is a by-product of lead and zinc mining and smelting. In recent decades, the production of cadmium has increased rapidly from 11,000 tons estimated in 1960 to estimated 19,000 tons in 1985. Cd present in nickel-cadmium batteries, dyes, semiconductors, electroplating, PVC production, various alloys, and control rods for nuclear reactors [13]. Cadmium causes soil and water pollution in smelting and mining industries, air pollution, sewage treatment plants, and fossil fuel combustion. Cadmium does not have the crucial biological activity, so it is highly toxic to any sort of life [14]. Long-term human exposure to cadmium can have drastic, multiple toxic effects, such as liver, lung, testicular damage kidney, and hypertension damage. Cadmium is also causative agent to disease named as Itai-Itai, which means "it hurts" in Japanese [15].

Copper

Copper is an important micronutrient for plant growth and also necessary for many enzyme activities, especially in metabolism of nitrogen. Copper poisoning is caused by modification of cells plasma membrane, which triggers flow of potassium or other solutes. Moreover, the similar valences and size of manganese, iron and copper, copper competes for binding site, thus interfering accumulation of other metals leading to a lack of basic [16]. Copper displace iron in the reaction center of PS-II and alter photosynthetic processes of plant. Energy conversion efficiency is therefore reduced because Copper cannot transfer energy as efficiently as Iron. Disruption of reaction center of Photosystem II causes a decrease in CO₂ assimilation, that leads to a decrease in the transpiration rate of the plant, in turn leads to a declining growth potential [17].

Lead

Lead has several lethal impacts on living organisms. Lead toxici-

ty impair plant development, cell division, seed germination, root elongation, transpiration, cell, chloroplast development, chlorophyll production and whole plant development. The gravity of hazardous effects depends on the lead concentration, exposure level, plant development stage, target plant organ, stress intensity of the plant [18]. Plants use various mechanisms for the detoxification of heavy metal i.e. metal absorption, secretion, separation, selective absorption, and complex ligand binding. environmental quality assessments are based on the lead exposed plant responses. Examples include legumes growth responses on soil contaminated with lead [19]. *Pelargonium* and *Brassica napus* are believed as lead hyperaccumulators, capable of extracting large amounts of lead from polluted soil without exposing any geomorphologic toxicity indications [20].

Arsenic

Arsenic (As) value present in the earth's crust estimated that 1.5-2 ppm. Arsenic amount in soil range from 1-40 mg/kg and the average soil arsenic concentration is approximately 5 ppm. However, in natural soils, huge amount of arsenic is usually associated with sulfide deposits and localized form of mineralization due to weathering [21]. Sources of arsenic contamination by humane activities in soil include fossil fuel burning, sulfide mining, use of pesticides containing arsenic, smelting, and copper and chromium arsenates for wood [22]. Moreover, Bangladesh, China, US and India have reported that groundwater is contaminated with arsenic. Using toxic soil, Risks of accumulation of arsenic in the food chain and possible exposure to arsenic contamination in the food chain through plant and animal uptake [23]. Arsenic is a non-essential element for plants and does not appear in specific metabolisms when administered at very low concentration. Arsenic normally exist in four oxidation states 0, +5, +3, and -3, while arsenate (As (V)) or arsenite (As (III)) are the major forms. Arsenite having a superior drastic effect on the most of species inclusive to plants [24]. Arsenic poisoning symptoms in plants usually include reduced root growth and poor seed germination. This effect may be associated to the rapid obliteration of plasma membrane, including fluidization. It has been reported that at higher concentrations, Arsenic interfere with metabolic processes and sometimes causes death of whole plant [25]. If plants survive high Arsenic exposure, experience growth retardation, severe chlorosis and nutrient deficiency, and reduced oxygen release as byproduct from photosynthesis. The critical concentration of arsenic in shoot tissue ranges from about 21- 325 ug/g, depending on species and cultivar [26].

Presence of Heavy Metals in Soil

Bio available fraction of the heavy metals corresponding of total heavy metal concentrations to the concentration of heavy metals that can be extracted by chemical agents or potentially absorbed by the plants. Generally heavy metals are less soluble so are unavailable to the plants for uptake. In natural ecosystem, the heavy metals rarely present in significant amount or bioavailable concentration for toxicity in plants [27]. Bioavailability of heavy metals is associated with the solubility of heavy metal into the soil. Well, there is another concerning matter is that —what concentration is associated with the bioavailability that is toxic to plant [28]. Bio-availability is related to the activity of heavy metal ions in soil and transferable metal proportion. However, researchers have not yet agreed upon consensus on bioavailability measurements of heavy metals in soil. While assessing accurately the risks associated with

soil pollution, the bioavailability of heavy metals rather than the overall concentration is of great importance [29]. Several fractions of the heavy metal are present in the soil such as exchangeable, soil solutions, organically bound or colloidal, residues, in primary mineral phase. Plants and animals cannot take the heavy metal pool present in the soil. The most abundant, bioavailable and possibly phytotoxic heavy metals exist in solution form and absorbed in inorganic component in soil from ion exchange sites, while other components are less absorbed by plants [30].

Microbial Interactions

Rhizobia bacterial species form a symbiotic relationship with leguminous plant and are responsible for converting atmospheric nitrogen by the process of nitrogen fixation into accessible forms to plant roots, such as NH_4^+ . Therefore, legumes absorb more cations parallel to anions, which acidify the surrounding rhizosphere. The pH of the soil changes and heavy metal solubility increases by the rhizobial bacterial species as the activity of nitrogen assimilation [31]. Studies have been carried out on the rhizosphere of plants growing on serpentine soils and nickel-assimilating plants. According to reports, rhizosphere bacteria increase nickel accumulation by producing siderophores to increase nickel utilization by plants. Siderophores induce the dissolution of nickel-carrying minerals, thereby indirectly promoting above-ground biomass and roots [32]. Other microorganisms in the rhizosphere include mycorrhizae, which are mutually beneficial relationship between specific soil fungi and plant roots species. It has been observed that mycorrhizae contribute in uptake of nutrients at lower metal levels. In addition, mycorrhizae can reduce metal uptake and improve plant metal stress tolerance under metal contaminated conditions [33]. For example, Red clover (*Trifolium pratense*) grows in acidic soils with high manganese concentration had lower manganese contents in shoots and roots than non-mycorrhizal plants. When this plant grown in soils with high concentrations of heavy metals including zinc, copper, manganese, nickel, chromium, mycorrhizae make plants more resistance to heavy metals [34].

Heavy Metal Transport in Plant and Soil

Ion channels are responsible for the passively absorption of heavy metals channels. The absorption mechanism depends to extent on the electrochemical gradient applicable to the transport of specific nutrients. Many selective transport routes of heavy metals in plants are now revealed, for example, phytosiderophores bound iron be transported across plasma membrane by yellow stripe 1 (YS1). Fe(III) siderophore (Fe-III-PS) transporter was synthesizes by Yellow stripe gene in maize [35]. Non-selective transporter Calcium cations through root membranes. Absorption of other metals occurs through this pathway under normal soil solution concentrations. Competition between root surface cations suggests that non-selective channels regulate both essential and non-essential absorption metals. Therefore, under excess heavy metal concentrations, competition for transport sites results absorbed heavy metals instead of macronutrients, which leads to or exacerbates nutrient cation deficiency [36]. Iron-deficient plants are sometimes found to contain higher concentrations of zinc and manganese. IRT1 protein is a common cation transporter in Arabidopsis that increases the absorption of zinc and manganese in Arabidopsis [37]. Moreover, many transporters are involved in the absorbing soil iron. Transportation carried out through roots to shoots, xylem unloading and transportation to the reproductive parts of plants,

mobilization during seeds germination, taking-up and taking-off of iron from vacuoles.

Bioremediation

Biological remediation or bioremediation is cost effective and equal friendly technology that uses microorganism plants to clean the environment or contamination off ecosystem. Biological agents like microorganism or plants degrade the pollutants of the environment by utilizing it or converting into less harmful substances. According to study reports, various organisms such as algae, fungi, bacteria and plants can effectively bioremediate pollutants. Bioremediation technology provides an alternative to traditional contaminated site remediation technologies [38]. Bioremediation uses biological means and depend on living organisms to alter contaminants and environmental condition to transform bio-functions into more accessible ways. Metabolic processes off microorganism and plants use pollutants as energy source, therefore, converting them to less harmful substance or not bioavailable for organisms [39]. Bioremediation an effective method to reduce or degrade hydrocarbons, solvents, organic compounds herbicides, nitrogen compounds, pesticides and heavy metals [40]. The term by remediator is used for the micro some that is used for the process of bioremediation. There are two categories of bio remediators. Anaerobic and aerobic microorganisms use contaminants as energy source and degrade them in the presence of oxygen while anaerobic are very less frequent and live in anaerobic conditions [41].

In-Situ Bioremediation

Two types of bioremediation strategies: i. *ex-situ*, and ii. *in-situ*. *In situ* bioremediation is a process that does not require the excavation of contaminated soil. Generally, it is used to decompose pollutant in saturated soils. [42]. Beneficial microorganisms used to destroy chemicals in contaminated environments and is cost effective than conventional techniques. Biosparging, bioaugmentation, and bioventing are examples of in-situ bioremediation techniques [43]. Bioventilation participating in the use of low airflow to provide the oxygen needed to keep microorganisms functioning. Bioventilation is commonly used to treat organic pollutants in aerobic conditions. Bio ventilation accelerates natural processes because it provides less airflow, which promotes the growth of naturally occurring soil microorganism [44]. Biosparging involves the injection of oxygen into a saturated zone under pressure to the transfer of unstable (volatile) compounds, and the biological decomposition occurs by natural microorganisms. Biosparging is comparatively inexpensive and easy to install, and oxygen can be rapidly distributed throughout the site to amplify microbial activity [45]. Bio-Augmentation includes natural microbial strains and genetically engineered variants for treatment of soil contaminations. however, maintenance of this system difficult because it needs to be monitored to ensure complete elimination of contaminants. To optimize the microbe's efficiency externally in an uncontrolled environment is challenging to attain and evaluate [46].

Ex-Situ Bioremediation

Ex-situ bioremediation implicates the removal of contaminants from the soil for treatment, which may occur at other sites both off-site or on site. The sample is transferred to elsewhere and there be significant potential risks in extracting and transporting hazardous materials. Therefore, Ex-situ bioremediation is generally considered less profitable than in situ [47]. Technologies and techniques

like land farming, composting and biopiling are involved in the Ex-situ Bioremediation. Composting a controlled process that decomposed organic matter at high temperatures by microorganisms to produce organic and inorganic by-products. Temperature of the optimized compost is between 55 and 65 °C [48]. The addition of post-composting modifiers generally increases the volume of material, the addition of post-composting modifiers usually increases the volume of material, and this is a limitation of the technology. Landfarming is a process in which contaminated soil spread on a thin layer on the soil surface and contaminants would be degraded by aerobic microbes [49]. Mixture of microorganism and contaminants are thoroughly mixed and microbes often added in mixture to achieve rapid degradation or increase the interface between contaminants and microorganisms. The technique of landfarming requires a large area of land, which limits the application of this technology. Biopiling is a technique in which aeration is used to pile the soil and mix it with microorganisms. To prevent solar heating, spillage, and evaporation, the stack must be covered. Contaminants usually condense into carbon dioxide and water. Biological composting is like piling, but in the end of the soil the artificially aerated [50].

Phytoremediation

Phytoremediation is a process of bioremediation that uses micro-organisms and green plants to extract, absorb or detoxify contaminants. Plants are capable of absorbing, collecting, crude oil, removing or decomposing solvents, toxic pollutants and heavy metals [51]. Phytoremediation is environment friendly, cost-effective and clean technology designed primarily for the treatment of large and dispersed contaminated sites. There have been several successful cases where phytoremediation being applied and proven to be effective in recovering a polluted industrial environment. Several techniques may be used in phytoremediation on sites where inorganic compounds and heavy metal are present depending on the nature of the contaminants involved [52].

(a) Phytostimulation: beneficial microorganisms that are able to degrade contaminants from the soil are promoted by the roots of plant roots and these microorganisms used as source of carbon in roots of plants [53].

(b) Phytodegradation: organic contaminants mineralized contaminants are detoxified by specific type of enzymes by specific species of the plant [54].

(c) Rhizovolatilization: uses the enzymatic potential of different plants and related with rhizosphere micro-organisms that convert pollutant into volatile molecules that may be discharge released into environment without causing damage. Heavy metals and new PGPR strains are taken by the roots and transformed to smaller hazardous forms before being discharged into environment [55].

(d) Phytoextraction: phytoaccumulation or phytoextraction is a technique that utilizes the plants ability to extract or accumulate certain types of heavy metals, translocate metal concentrating them in biomass. Phytoaccumulation or phytoremediation are technique used to reduce concentration of poisonous metals present in soils, due to this they give accurate results in forestry, agriculture, gardening and various other fields [56].

(e) Phytoimmobilization: plants, commonly combined with other soil additives, are used in phytoimmobilization to reduce the transmission of contaminants to other ecosystem components, as well as food webs and food chains. "Stable" inorganic or organic compounds are usually added to plant lignin and soil humus. The basic goal of phytostabilization is in-situ stability, not metal degradation. This method is particularly useful when treating low-level, extensive, and scattered polluted regions [57].

(f) Rhizofiltration: is the utilization of terrestrial plants in the aquatic system to deposit, concentrate, and absorb contaminants. Some industrial and agricultural effluents were treated by using rhizofiltration process. Plants having the ability to accumulate huge amounts of heavy metals through natural processes has found to be used to accumulate heavy metal pollutants, as well as investigated for metal removal effectiveness [58]. These plants are known as hyperaccumulators and are present in areas where the concentration of metals in the soil is high. These plants growth is relatively slow and reaches only a small size. Restoration of the site depends upon the concentration and types of metals found at that site these plants can take up to 20 to 15 years for recovery of site [59]. This period is normally too short for practical implementation. However, studies on these plants should highlight species that grow rapidly and accumulate more biomass, as well as tolerant various heavy metals. In addition, presence of metals in the bioavailable portion of the soil determines the efficacy of phytoremediation. As a result, it's important for scientists to look at the bioavailability and uptake of overlapping target minerals by plants [60]. If goal of studies is to identify optimal hyperaccumulators, the main objectives of the study should include (1) assessing the effects of metal stress on useful microorganism or rhizosphere plants, (2) evaluating the implementation of bioremediation process for heavy waste treatment. metals from contaminated soil.

About 400 terrestrial plant species have been identified as heavy metals hyperaccumulators and might be used as bioagents in heavy metal phytoextraction. The effectiveness of phytoaccumulation determine by number of parameters, such as the amount of heavy metal uptake and the decrease in phytotoxicity by increasing biomass production [61]. Several researches have indicated that hyperaccumulators is unsuitable for lowering phytotoxicity due to the less production of biomass and relatively slow growth of soils contaminated with heavy metals. This limitation can be overcome by utilizing rhizobacteria as a bioinoculant that promotes plant development. A unique mechanism of potential for effective plant-microbiota interactions need to be explored [62].

Rhizoremediation

Rhizoremediation a soil decontamination process by rhizobacteria, bacteria living in plants rhizospheres. Microbiota and plant symbiotic advantages in the plant rhizosphere can be integrated into an efficient soil remediation technique, a relatively new approach with the potential to give effective bioremediation [63]. To resist the stress of heavy metals in polluted soils, certain microorganisms have developed mechanisms that can be utilized to reduce heavy metal absorption (Figure 1). Rhizoremediation mechanisms including different step such as

a. Extrusion: plasmid/ chromosomal mediated events pump ions of metal to outside the cell, where these ions of metal held at such

a safe area from cells;

b. Exclusion: heavy metals ions are expelled from the cell and kept in an enclosed bay.

c. Accommodation: complex is our form by the metal binding protein or cellular components put the metals i.e., the collection and uptake of ions in cell;

d. Biotransformation: when a poisonous metal rendered low poisonous through transformation;

e. Desorption / adsorption and f. Methylation or demethylation of heavy metals. This protective mechanism allows resistance microbes to survive metabolically in environments contaminated with various heavy metals.

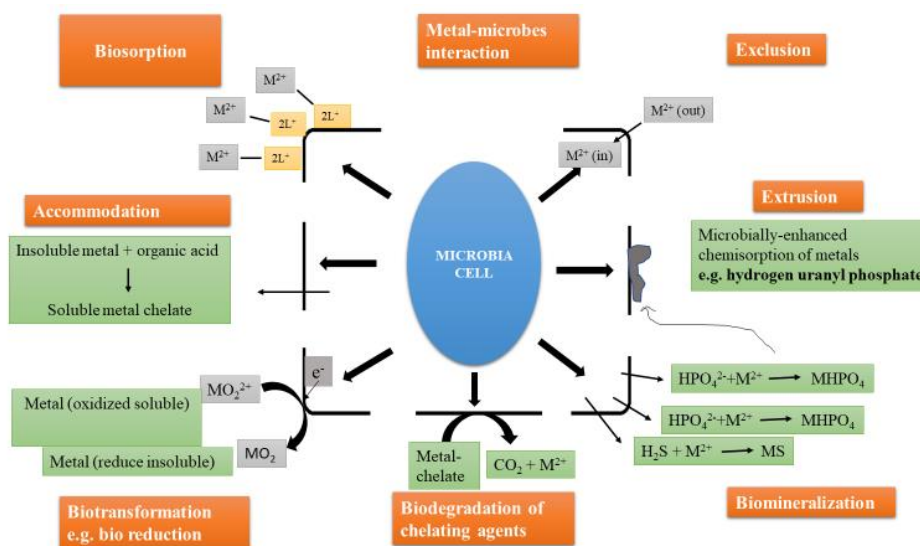


Figure 1: Resistance Mechanism in Soil Microbes Against Metal

Plant Growth Promoting Rhizobacteria (PGPR)

Growth promoting rhizobacteria include several bacteria growing in the rhizosphere of plant that stimulates plant growth by several mechanisms in soil. Microorganisms promote plant cell development by interacting with the plant's roots under different stress conditions due to heavy metal. Colonization and Localization of rhizosphere bacteria in roots have implications for plant beneficial aspects [64]. Several agricultural systems based on PGPR for improvement of yield and crop quality. For instance, the symbiosis of legume-rhizium converts atmospheric nitrogen (N) into a form used by plants, which is an important part of the nitrogen-cycle. However, legumes are important source of protein for animals and human worldwide, using rhizobium inoculants in legumes provides effective Nitrogen fixation and has been in the fixing Nitrogen from a century. At the same time, inoculants markets are developed in Thailand and Myanmar [65]. Moreover, its use in agricultural systems, the properties of PGPR can be exploited in other systems, such as stabilization and remediation of contaminated land. Several PGPRs have also been shown to protect their host plants from the toxic effect's heavy metals and from pathogenic microorganisms [66]. Many techniques convert toxins into less noxious forms, reduced mobile forms and bioavailable outcome, while the removal of heavy metals in pollutant soil and complex ecosystem is complex or somewhat complicated [67]. Research show heavy metals that not be biodegraded due to the specificity and bioavailability of heavy metals change environmental factors, especially Copper, Zinc, Nickel and Chromium. Similarly, Copper,

Zinc, Nickel and Chromium promote beneficial characteristics in plant roots, but microbial community total biomass is effected by the higher concentration of these metals, thus altering the structure and activity of microbe's community [68].

Use of Heavy-Metal Tolerant Bacteria as PGPR

It is now known that many bacteria have increased tolerance or resistance to heavy metals. Many rhizosphere microorganisms have potential to grow and survive large concentrations of heavy metals. Microbial tolerance to poisonous heavy metals is the ability of microbes to overcome metal toxicity by activating mechanisms directly in response to high concentrations of heavy metal, whereas resistance is the ability of heavy metals detoxification of microbes by activating mechanisms directly in response to high concentrations of heavy metal [69]. Toxic forms of heavy metals must be completely removed or altered from the contaminated soil or immobilized in a way that makes them safe. To survive heavy metals in a stressful environment, PGPRs developed a variety of mechanisms by which they mobilize, immobilize and transform heavy metals, thus rendering them as inactive [70].

PGPR mechanisms including

- Metal ions exclusion from the target region
- Accumulation of metal ions into complexes compatible with either metal binding proteins such as metallothioneines or ligands,
- Extrusion of metal ions from cells through plasmid/chromosome-mediated events

- d). Demethylation and methylation processes
 e). Biotransformation of heavy metals to less toxic forms. This mechanism let microorganism to be metabolically active in contaminated soil. Interest in the potential of these organism to decontaminate the area with heavy metals is rising, and the results of their application are encouraging and inspiring [71].

Synergistic Interaction of PGPR and Plants in Heavy Metal Remediation

Several types of studies indicated plant-microbe's interactions, but most research only highlighted interactions of plant-pathogen. In Recent studies microbial ecology focused rhizospheric bacteria were investigated for the detoxification or decontamination of the heavy metal contaminated soil [72]. Babalola well documented the potential of growth promoting Rhizosphere microorganisms and utilized for reducing heavy metal stress and inducing stress tolerance in plants. The synergic effect of Root-associated microorganisms developed relationships with roots, that increase uptake of nutrients by improve plant activity and soil quality [73]. Bacteria cooperate with plant roots and encourage growth in several ways. Several bacterial species are pathogenic and inhibit plant growth. In the PGPR system, bacteria enhance plant growth without influencing soil conditions. Some microbial communities could absorb metals and may be used in bioremediation of pollutant sites [74]. During the process, PGPR enhances phytoextraction process by solubility, altering availability, heavy metal transport, nutrient transfer, reformation into less harmful forms, reducing chelate release, as well as soil pH alteration. Considering PGPR produced metabolites, siderophores play significant role in heavy metals ac-

cumulation and mobilization [75]. Soil Inoculation with *Pseudomonas aeruginosa* effectively distributes chromium and leads into soil solution and can serve as both model organism and pathogen. Although not yet successful in the field, inoculation of rhizospheric seeds / soils with metal-mobilizing bacteria to improve phytoextraction in polluted soils is valuable and leaves considerable holes for future research [76].

Role of PGPR in Heavy Metal Contaminated Soil

In the laboratory and in greenhouse studies, the prospective application of PGPR has generated promising results; however, responses in the field have been mixed. PGPR associated with plants promotes plant growth and restores contaminated soil as well [77]. Studies have shown that PGPR plays a key role in plants by enhancing tolerance and growth of plants under heavy metal stress conditions. In Nickel-pollutant soil, PGPR *Bacillus subtilis* strain SJ-101 (Heavy metal tolerant) was found to increase *Brassica juncea* growth [78].

It has been reported that many species of rhizobium tolerate heavy metals and promote plant growth in the excessive exposure of heavy metals. For example, *Bradyrhizobium* strain RM8 was found to be tolerant to Nickel and Zinc, *Rhizobium* sp. RL9 isolated from lentil nodules was effective against Zinc, and *Rhizobium* sp. extracted from pea nodules RP5 was found to be tolerant to Zinc and Nickel, significant amount of indoleacetic acid was also produced by these species [79]. Various strains of PGPR that restore heavy metal toxicity have been described in the literature (Table-1).

Table 1: Examples of PGPR Ability to Tolerate A Variety of Heavy Metals in Plants [80]

PGPR	Heavy metals	Plant
<i>Sinorhizobium</i> Pb002	Pb	<i>Brassica juncea</i>
<i>Rhizobacteria</i>	Cd	Wheat and barley
<i>Bacillus subtilis</i> SJ- 101	Ni	Indian Mustard
<i>Bradyrhizobium japonicum</i> CB1809	As	Soybean
<i>Bacillus</i> sp. RJ31	Cd	<i>Brassica napus</i>
<i>Pseudomonas & Bacillus</i> sp.	Cr	Mustard
<i>Brevibacillus</i>	Zn	<i>Trifolium repens</i>
<i>Pseudomonas putida</i> KNP9	Pb & Cd	Mung bean
<i>Rhizobium</i> sp. RL9	Zn	Lentil
<i>Pseudomonas</i> sp.	Ni	Chickpea
<i>Pseudomonas</i> sp. RJ10	Cd	<i>Brassica napus</i>
<i>Rhizobacterium</i> sp. D14	As	<i>Populus deltoids</i> LH05-17
<i>Bradyrhizobium</i> sp. (<i>Vigna</i>) RM8	Ni	<i>Vigna radiate</i>
<i>Rhizobium</i> sp. RP5	Zn & Ni	Pea

Several strains of rhizobacteria help to alter the toxicity of heavy metals. PGPR, *Acinetobacter* and *Pseudomonas* strains increased Zinc, Magnesium, Potassium, Calcium, Phosphorus, and Iron uptake by plants. *Pseudomonas* was able to create siderophores and flourish in nickelcontaminated soil, according to certain rhizobacteria uptake in heavy metal.

Some strains of rhizobacteria can reduce heavy metals. For example, silver fern (*Pityrogramma calomelanos*) accumulated Arsenic have also been reduced by some rhizobacteria. Rhizosphere microbes collected from *P. calomelanos* roots significantly increased Arsenic concentration and plant biomass, indicating that these rhizobacteria enhance Arsenic *Pteris vittata* fern is also reported as

hyper Arsenic accumulator, and inoculation with Arsenic-reducing bacteria expressed increment in plant biomass upto 53% and Arsenic uptake by 44% [81]. A similar analysis revealed that Bradyrhizobium japonicum CB1809's growth-promoting effects stimulated soybean plant growth on arsenic-contaminated growing media. However, Bradyrhizobium inoculation did not increase plant Arsenic uptake, indicating that the bacteria have substantial potential for in situ phytostabilization [82].

Recently, different growth-promoting bacterial strains of Rhizobacterium *Azospirillum lipoferum* *Agrobacterium radiobacter*, and *Arthrobacter mysorens* have been isolated from lead and cadmium stressed barley plants [83]. Variations among plants of the strain were assessed barley plants by contaminated and uncontaminated soil conditions. Inoculated barley plants flourished with better growth and nutrient uptake than control plants when planted in lead and cadmium contaminated soil. From this study, it was concluded that bacteria that causes the growth of infected plants, reduced the concentration of Pb and Cd in barley plants. In a similar investigation rhizobacteria that tolerance chromium was secluded by rhizosphere of chromium containing soil. This bacterium was inoculating *Vigna radiata* in Chromium contaminated soil, or infected plants have larger root, elevated biomass, and shoot length compared to uninoculated plants growing on the same soil.

Bacteria Possessing ACC Deaminase Activity for Stress Alleviation

Several mechanisms by PGPR were reported by investigations to alleviate plant growth: plant growth hormone production, plant nutrient uptake efficiency enhancement, and protection of host plant from pathogens [84]. Cereals with inoculated PGPRs have shown better uptake of nutrient, increase plant height, higher nitrogen content in tissues, larger root, greater size of leaf, and overall plant biomass. Bacteria including (ACC + ACC deaminase positive) are one of PGPR groups degrade the precursor of ethylene namely 1-aminocyclopropane-1-carboxylic acid (ACC). The ACC + bacteria can reduce ACC and ethylene levels 2-4 times and thus improving growth of plant under abiotic stresses (salinity and heavy metals) [85]. ACC deaminase bacteria has molecular weight of approximately 35-42 kDa and is a multimeric enzyme. ACC is sulfhydryl enzyme that utilizes pyridoxal-5-phosphate as an essential cofactor [86]. D-serine and D-cysteine substrates for ACC deaminase enzymes are also documented, while L-serine and Lalanine can compete with ACC deaminase. ACC deaminase can cleave ACC constituents, inclusive to cyclopropane ring; As a result, ammonia and ketobutyrate are formed. Enzymes are mostly function in the bacterial cytoplasm, whereas plant systems catalyze and take ACC from bacterial cells by enzyme [87].

Various types of bacteria have ACC enzyme, include gram-negative and gram-positive bacteria, endophytic and rhizobia bacteria. ACC + bacteria indole acetic acid that stimulates plant biomass production [88]. Higher ethylene levels reduction mechanisms in ACC + bacteria are described in Figure 2. Plant roots and number of nodes and node mass in chickpea by ACC-deaminase containing PGPRs, and these bacteria can regulate ethylene and increase nutrient availability stated that ACC + bacteria reduced ethylene negative effects on plants under stress and normal conditions due to ACC deaminase activity. Seedling length and root elongation were better with ACC bacteria. Reduced ethylene production in

soybean roots by inoculation of ACC⁺ containing Brady rhizobium japonicum was also reported, consequently reducing the negative effects of ethylene on nodule formation [89].

ACC⁺ bacteria can enhance plant heavy metal tolerance as well as floods and phytopathogenic fungi tolerance. Under drought stress, plants inoculated with strains of bacterial had higher fresh and dry weights than control plants. Peanut plant growth responses in roots in early growth phases by ACC deaminase activity was also reported [90]. In late stage of plant growth, activity of PGPR of ACC + bacteria assist plant biomass production and yield increment by producing siderophore, solubilizing phosphorus and nitrogen fixation. In Consequence of PGPR activity, nodule formation and nutrient availability are increased. ACC⁺ bacteria also increase plant height and shoot Nitrogen and Phosphorus contents, as well as increase plant resistance to salinity by reducing salinity-induced ethylene biosynthesis [91].

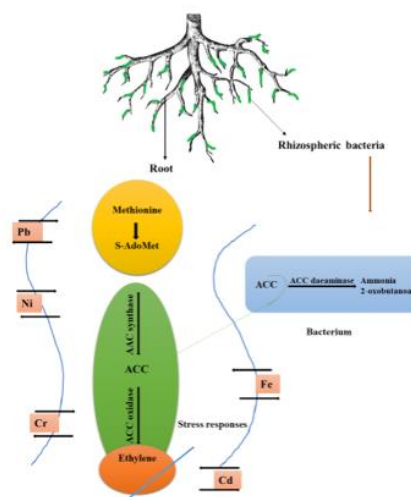


Figure 2: Schematic Model for The Process of Reducing Ethylene Levels in Plants-Roots Via Bacteria Having 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase

Many strains of ACC⁺ bacteria have been discovered. *Pseudomonas fluorescens* is one of them that increases plant root and shoot elongation. *Pseudomonas putida* support growth and seed germination in canola under salinity condition by ACC-deaminase and *Azospirillum brasilense* present to be an ACC⁺ species which improve shoot and root growth [92].

Hormones as Stress Releasing Agents in Plants

Phytohormone production ability is process that promoting plant growth. Plant growth promoting hormones are gibberellins, abscisic acid, auxin and cytokinin's [93]. These hormones can be synthesized not only by higher plants but also by bacteria. Naturally occurring auxins namely indole-3-acetic acid (IAA) have a wide range of physiological effects, including plant development and growth regulation [94]. Auxins associated with division, proliferation, inhibition of root growth, root initiation, phototropism, apical dominance, geotropism, and increasing growth rate. The largest auxin-producing bacteria, found in rhizosphere, are strains belonging to different genera include *Bradyrhizobium*, *Azobacter*, *Rhizobium*, *Azospirillum*, *Enterobacter*, *Bacillus*, and *Pseudomonas* [95]. Above mentioned bacteria are auxin secreting that help-

ful for plant growth as an endogenous pool.

Gibberellins a group of endogenous plant hormones that promote plant development, seed germination, stem elongation and persuade physiological changes include fruit and flowering formation. It is also known to be a mediator of particular environmental signals, e.g. light quality and photoperiod. In the 1950s, gibberellic acids (GAs) were used to recover maize and pea dwarf mutants from *Gibberella fujikuroi* cultures. Four GAs (GAI, GAIL, GAIII and GAIV) have been recognize in bacteria such as *Azospirillum lipoferum*, *Bacillus pumilus*, *Azospirillum brasilense*, *Herbospirillum seropedicae*, *Bacillus licheniformis*, *Rhizobium phaseoli* and *Acetobacter diazotrophicus* [96].

Cytokinins are N6-substituted aminopurines and also endogenous plant hormones regulate growth. Cytokinin's are organic compound affects plant developmental and physiological processes at minimum amount, approximately 1 μ M, that expressed in plants either t-RNA component or free base form. Cell division and controlling cell fate are key roles of cytokinins in plants. In addition, cytokinin's have a multi-dimensional effect on healthy plant growth, axillary shoot growth, development, nutrient metabolism regulation, leaf expansion, chlorophyll accumulation and aging delay. Bacteria that produce cytokinin have been identified and characterized in various cultures such as *Pseudomonas* species, *Agrobacterium* species, *Azospirillum*, *Rhizobium*, *Azotobacter*, *Paenibacillus polymyxa* and *Bacillus*. Investigations have reported that cytokinins supplement initiate cell division and increase cytokinin levels that have positive effect of rhizosphere microorganisms on plant growth and development. Plant growth promotion is very complicated phenomena and is a result of combination of many mechanisms. It is known that the uptake of nitrogen, solubility of nutrients, uptake of phosphorus and iron triggers the growth of plants. Nitrogen is one of the most important elements for plant growth and is found in biomolecules, proteins, and nucleic acids. Though, plants cannot absorb nitrogen directly from atmosphere and converted into usable forms through different processes such as a). Nitrogen to nitrogen oxide; b). then ammonia; c). Fixation of nitrogen by micro-organisms by complex enzyme systems activity. Thus, intensive use of plant-bound nitrogen-fixing bacteria act as biofertilizers is an alternate to inorganic nitrogen fertilizers. Various nitrogen-fixing bacteria such as *Azospirillum brasilense* Sp-245, *Bacillus fusiformis*, *Enterobacter* species, *Xanthobacter* species, *Azotobacter species*, *Bacillus species*, *Pseudomonas corrugate*, *Azotobacter chroococcum*, *Sphingomonas trueperi*, *Pseudomonas tolaasii*, *Pseudomonas fluorescens* and *Pseudomonas veronii* have been sequestered from different plant rhizospheres, that were reported to increase plant nitrogen content and seed yield.

Biosorption of Heavy Metals

Biosorption makes use of microbial biomass's ability to collect heavy metals from aqueous media, also known as biological ion exchange [97]. Many microorganisms from various groups such as fungi, bacteria, algae and yeasts, have binding ability many heavy metals. The main biosorption mechanisms include bio precipitation, adsorption, and ion exchange [98]. Techniques for immobilization have recently been discovered, however they are expensive and difficult to use. Recently biosorption considered as secure and cost-effective technique for eliminating heavy metals from various aqueous mediums [99]. Biosorption main advantages are the effi-

ciency for removing heavy metals from various media compared to conventional approaches. The presence is minimal as ppb of residual metal in the effluents. Bioadsorption can be employed at broad range of temperature, pH and pressure [100]. This technology is advantageous due to it can be developed from low-cost raw materials and generates minimal chemical sludge and simple to disposal. However, most difficult task of bioadsorption technology is identifying biomass that is abundant and affordable. Volesky et. al, reported different microbes with the ability to bind metals [101].

Several studies have shown the ability of activated sludge to accumulate heavy metals. Activated biomass collects primarily Copper and Zinc from the acid mine effluent. PH stabilization is key to accomplish maximum metal efficiency of sludge. Liu et al., reported aerobic granules as biosorbent to remove Cd from industrial wastewater. Cd uptake ranged from 43 to 566 mg g⁻¹, reliant on initial biomass and Cd + 2 concentration [100]. Walnuts, candles, peanut shells, and peanuts in their natural or modified form also from agricultural waste act as bio-sorbent. These materials were reported to absorb Ni (2), Pb (2), Cu (2), Zn (2) and Cd (2). In few cases, heat treatment use to increase the absorption efficiency of phosphoric acid and citric acid [102]. Coconut shell as biosorbent was reported to remove Cadmium in water at a concentration of 201000 mg/L [103]. Bio-sorbent had increase Cd (II) biosorption ability, yielding 285.7 mg g⁻¹ Cd (II). Coir pith was studies on biosorbent of Co (II), Cr (III) and Ni (II) in ionic solution. Biosorption capacity of Ni (II) was 15.9 mg/g while Cr (III) was 11.6 mg/g and for Co (II) was 12.8 mg g⁻¹. The peels of Orange have been proven as an inexpensive adsorbent for removing Ni (II) from electroplating effluents [104]. The optimum biosorption was achieved at 96% and at 50°C at 6 pH with a preliminary amount 50 mg/L. Potato peel biosorption from waste in aqueous for Ni (II) has also been studied. As the Ni (II) concentration increased from 20 to 120 mg/L, metal absorption increased from 0.07 to 0.20 mmol/g. Table-2 show the important waste that can be used as biosorbents.

Table 2: Heavy Metal Removal from Different Agricultural Waste Biosorbents [105]

Metals	Adsorbents
Pb+2, Zn+2, Fe+2, Ni+2	Waste tea leaves
Cr+3, Cd+2, Pb+2	Saw dust
Pb+2	Maize
Cd+2, Co+2, Cr+3, Pb+2	Sargassum natans
Cu+2, Cd+2, Ni+2, Pb+2	Peat material
Pb+2, Hg+2, Cd+2, Cu+2	Rice husk
Cr+2, Pb+2, Mn+2, Fe+2	Fly ash
Pb+2	Lemna minor
Ni+2	Cassia fistula
Cd+2	Cellulose xanthate

The advantage of fungal biomass is that it has a high percentage of cell wall material with strong metal binding potential. In the world of microbes, cCultures immersed in *Rhizopus nigricans* as a Pb (II) biosorbent in aqueous solution were demonstrated by kogej

et. al, reported biosorption *Phanerochaete chrysosporium* fungal biomass for Pb, Cu and Cd ions [106]. Cu, Pb and Cd uptake rates by *Phanerochaete chrysosporium* dry biomass were 26.6, 85.9 and 27.8 mg g⁻¹, respectively. It was observed that increase in absorption with an increase in pH 2-6. Biosorption activity was also observed using algal biomass. *Enteromorpha compressa* was used as a biosorbent to extract Zn (II) and Cd (II) from landfill leachate by *sahmurova* [107]. Optimal conditions include pH 4, exposure time 1 hour and temperature about 25°C. *Kaewsarn* used calcium-treated seaweed *Candina* sp. biosorbent for absorbing Cu (II) in aqueous solution. At pH 5.0, the greatest adsorption capacity was 0.8 mmol g⁻¹ solution [108].

Conclusion

The advancement in bioremediation is yet at trial at developmental stages. The new innovation system will analyze the potential improvements in-situ remediation, ex-situ remediation. The contaminants will influence the crop yield, profitability and humane health care. To date, swift and large-scale applicable methodologies for bioremediation are needed for further studies to elucidate the underlying mechanism and potential for the decontamination. Resource exploration, heavy metal concentrations, fractions of heavy metals, types, extent of soil contamination, bioavailability are significant territories of research. Heavy metal accumulating algae and plants are also very important for ecosystem health as well as humane health. Their natural potential for decontamination is not well known and need deep investigations for the evaluation of optimal one. Corresponding to environmental and ecological conditions, different types of microorganism have shown different types of treatments and responses. Refining one or more specific species and prioritizing it for research in academic projects may solve preliminary environmental problems for further Investigations. Physiological and ecological traits are of utmost importance for refining/selection of specie. Various strategies have evolved by microorganisms to cope with the heavy metal detoxification. Multiple mechanisms can be applicable to designing strategies for detoxification approaches of heavy metal. According to the Contaminant types, concentration, region, optimal technique can be refined and utilized for economic purposes.

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