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MICROBIAL-ASSISTED PHYTOSTABILIZATION OF HEAVY METAL CONTAMINATED SOIL

Fatima Bibi[1], Pakeeza Eman[*2], Muhammad Arham[3]

[1, *2,3]*Department of Environmental Science, University of Peshawar, Peshawar, Pakistan*

[*2]Pakeezaeman04@gmail.com, [3]marham6732@yahoo.com

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Corresponding Author: *
Pakeeza Eman

Abstract

Heavy metal contamination resulting from industrial activities, mining, agriculture, and urbanization poses serious risks to ecosystems and human health. Toxic metals such as cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), and nickel (Ni) persist in soils, accumulate in food chains, and disrupt ecological balance. Traditional remediation approaches, including soil excavation and chemical treatments, are often expensive, environmentally unsustainable, and can degrade soil quality.Phytoremediation, especially phytostabilization, offers a sustainable alternative by employing plants and their associated rhizobacteria to immobilize metals in the rhizosphere, thereby reducing metal mobility, limiting groundwater contamination, and minimizing transfer through the food chain. Metal-tolerant rhizobacteria enhance this process by producing siderophores, exopolysaccharides, biofilms, and phytohormones, which aid in metal immobilization, improve plant tolerance, and maintain soil fertility. They also suppress pathogens and enhance nutrient uptake, supporting plant growth in contaminated soils.Despite these benefits, challenges such as low biomass production, heterogeneous soil conditions, and uncertainties regarding long-term effectiveness restrict large-scale application. Future research should aim to optimize plant–microbe interactions and clarify their mechanisms under varied environmental conditions. Rhizobacteria-assisted phytostabilization thus represents an eco-friendly and sustainable strategy for mitigating heavy metal pollution while promoting soil restoration and enhancing agricultural productivity.

INTRODUCTION

Heavy metal contamination is a global environmental problem resulting from human activities, including mining, industrial discharges, agriculture, and improper waste disposal. Metals, along with lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg), are particularly toxic, continually present in the environment, and may accumulate in plant life, animals, and human beings, leading to excessive health and ecological issues. (Abdu et al., 2017; Goyal et al., 2020). Traditional strategies for mitigating heavy metal contamination, which include soil excavation, washing, or chemical immobilization, often show high prices and might cause further ecological degradation (Basta & McGowen, 2004; Haldar & Ghosh, 2020).

Phytoremediation, especially phytostabilization, is a promising bioremediation technique that utilizes plants and their related soil microbes to reduce the mobility and bioavailability of poisonous metals in contaminated soils (Riaz et al., 2022). In phytostabilization, plant roots act as anchors, reducing soil erosion, while metal-tolerant rhizobacteria enhance the plants ability to tolerate and immobilize heavy metals, therefore stopping their uptake via flora and next access into the food chain. (Haldar & Ghosh, 2020)

1.1 Metal contamination

Metal contamination is defined as the excessive presence of metallic qualities containing elements with atomic masses greater than 20. Cr, Hg, Zn, Cd, and Pb are all well studied as the utmost environmental hazards (Ashraf et al., 2017; Mehmood et al., 2017). Multiple recent developments (like urbanization, the industrialization of society, the population boom, agricultural production, the production of food, and so on) have been responsible for a rise in Heavy metal contamination worldwide (Mehmood et al., 2020). The soil contains a substantial amount of heavy metals, and water has continuously eroded their aspect, making them more poisonous, unsuitable, and dangerous to living organisms.

Furthermore, heavy metals penetrate the food cycle through contaminated soil and water, making the consumption of food potentially hazardous to both human and animal health. As a result, Heavy metal pollution is being acknowledged as a serious global public health risk (Hembrom et al., 2020). According to recent statistics, more than 10 million locations are theoretically contaminated, with more than half of them having high levels of metal contamination universally. The location of all these heavy metal contamination places revealed that the continent of America (USA) had the most polluted locations, with over 100,000 sites classified as contaminated, and more than 70% of these locations having increased metals pollution. Europe follows the United States (80,000 locations) and Australia (50,000 locations) in terms of polluted sites, with metal contaminated sites accounting for thirty-seven percent and 60 percent, notably (He et al., 2015).

Metal-impacted areas are particularly prevalent in industrialized nations due to increased industrial, mining, and agricultural activity. Several other topographic features have a crucial role in the increased land contamination in those areas. Metal-rich rocks are also organic Metal- reservoirs of harmful metals that occur in metal-contaminated areas.

Heavy metal pollution is a worldwide issue that necessitates cooperation among academics, policymakers, and governments at regional, provincial, national, and international levels.

1.2 Toxicity of heavy metals

Heavy metal-induced soil pollution in agriculture is becoming a serious environmental issue due to its negative natural impacts. These hazardous pollutants are referred to as soil poisons due to their widespread availability and severe, persistent impact on plants cultivated in polluted soils. Every different harmful impact on plants is different (Rahman & Singh, 2019).

Plant photosynthesis, nutrient, and water uptake decrease as they are exposed to high hazardous quantities of cadmium metal on a continuous basis. Furthermore, plants that grow in cadmium-contaminated soil develop chlorosis, growth inhibition, and eventually die (Gil-Díaz et al., 2016). Wastewater sludge, fertilizers, urban waste, carbon emissions, and human activities collectively contribute to the accumulation of excessive zinc in soil. Zinc is an essential element for all creatures that live, but Cd is minor and may be harmful to larger plants and other organisms. Zinc accumulation in contaminated soils can lead to phytotoxicity. Significant amounts of Zn in soil impede various plant metabolic functions, resulting in slower plant development and aging. Zinc contamination in plants has an impact throughout the root and shoot systems. Furthermore, zinc pollution in early leaves causes chlorosis, which might extend to different plant components (Yaashikaa et al., 2022).

1.2.1 Copper

Copper is regarded as an essential plant micronutrient, playing a significant role in ATP production and CO uptake. Advances in extraction and manufacturing processes prompted large amounts of copper being deposited into the environment. Anthropogenic activities like copper smelting and mining help to increase deposition. Mining activities generate a large quantity of rocks and tailings that are deposited on the surface. An excess of copper in the soil promotes the stress of soil, which harms plants. That inhibits plant growth and causes chlorosis. Plants subject to increased copper concentrations produce ROS and oxidative pressure (Wang et al., 2017). Atmospheric pressure interrupts metabolic processes and destroys macromolecules. Massive deposition of mercury in arable land has led to mercury contamination in our food supply chain (Ling et al., 2010). Increased soil copper levels are primarily due to the combustion of fossil fuels, sewage sludge applications, and manure usage. The ecological risks posed by copper are managed through quality models and environmental guidelines. Plants show species-specific differences in copper uptake and transport, and high copper levels can reduce water potential and transpiration rates (Htwe et al., 2020; Su et al., 2019).

1.2.2 Mercury

Mercury is considered an interesting metal as it exists in various forms. When applied into the soil, it becomes solidifies as it is absorbed by clay particles, natural compounds, and sulfides. A substantial amount of mercury inhibits mitochondrial mobility and promotes oxidative stress by activating ROS. This disrupts the lipid layer of membrane and cell degradation in plants (Mondal et al., 2015).

1.2.3 Chromium

Chromium, a heavy metal, pollutes the environment, particularly soil, silt, and groundwater. The tanning industry consumes a significant amount of water, most of which is discharged as effluent containing high levels of chromium. Excess chromium negatively affects plant growth by causing chlorosis in leaves, disrupting nutrient uptake, damaging root tips, and inhibiting overall development (Ertani et al., 2017). Additionally, chromium exposure alters germination, impairs the growth of roots, stems, and leaves, and reduces dry matter production and yield efficiency (Singh et al., 2013). Chromium also interferes with plant metabolism by either inhibiting enzymatic activity or diminishing the capacity to generate reactive oxygen species (Wakeel et al., 2020).

1.2.4 Lead

Lead deposition in soil is frequently connected to sources such as municipal trash, industrial effluents from the paper and pulp industries, paints, mining, and petroleum products. Elevated lead levels have a deleterious impact on plant shape, growth, and photosynthetic processes (Cenkci et al., 2010). Excess lead disturbs enzyme functioning, produces water imbalances, changes membrane permeability, and impairs mineral absorption. It also increases oxidative stress by producing reactive oxygen species (ROS) (Afaj et al., 2017; Yaashikaa et al., 2022). Arsenic, which competes with phosphate for uptake via plant root transporters, has been shown to cause tolerance in various plant species (Armendariz et al., 2016; Yaashikaa et al., 2022).

1.2.5 Nickel

Nickel, a naturally occurring transition metal, exists in low concentrations in soil but can accumulate due to human activities such as mining, smelting, sewage discharge, phosphate fertilizer usage, coal combustion, and pesticide application. Excess nickel leads to physiological changes in plants, including necrosis and chlorosis, which adversely affect growth and development (Ahmad & Ashraf, 2011; Sreekanth et al., 2013).

1.3 Remediation measures

Heavy metals cannot be eliminated biologically (there is no "degradation," or change in the nuclear structure of the element), but rather are changed from one oxidation state or organic complex to another (Hasanuzzaman & Fujita, 2012). It is more difficult to remediate heavy metal contamination in soil. Metal treatment in soil is a complex undertaking, and numerous solutions have been devised to ensure optimal metal contamination cleanup. For example, Garbage dumps, soil drilling, electro reclamation, acid rinse, and thermal processing are all useful yet difficult to implement. These methods have considerable drawbacks, such as high prices, land degradation, harm to soil texture and characteristics, danger to both macro- and micro flora, poor efficacy, and non-sustainability (Haldar & Ghosh, 2020).

Phytostabilization, a form of phytoremediation, is a successful approach for treating heavy metal-contaminated soils. Unlike other remediation procedures such as Garbage dumps, soil drilling, electro reclamation, acid rinse, which are typically costly, environmentally destructive, and unsustainable (Haldar & Ghosh, 2020), phytostabilization uses plants and their associated rhizobacteria to stabilize heavy metals in soil. This method limits the mobility and bioavailability of certain metals, lowering their potential to contaminate groundwater or enter the food chain.

Metal-tolerant plants and their interactions with the rhizosphere are essential to the phytostabilization process. Plants with large biomass, resistance to polluted conditions, and the ability to store metals in roots or immobilize them in the rhizosphere make excellent candidates. (Mehmood et al., 2017) Rhizobacteria play an important role in improving phytostabilization effectiveness by promoting heavy metal detoxification and immobilization via biological, chemical, and physical mechanisms. These bacteria help to make heavy metals less toxic and more accessible for plant absorption, which is critical for successful phytostabilization (Haldar & Ghosh, 2020). Using phytostabilization as a remediation strategy has various advantages: it is an in-situ, low-cost, sustainable, and environmentally friendly solution that increases soil fertility, biodiversity conservation, and nutrient cycling. However, knowing the functional relationships between plants, rhizobacteria, and heavy metals at different pollution levels is critical for optimizing this process.

This approach, which combines phytostabilization and rhizobacteria-assisted approaches, not only immobilizes heavy metals but also reduces the negative effects on soil structure and macro- and microflora. (Haldar & Ghosh, 2020). Mechanisms underlying heavy metal tolerance and accumulation in plants, offering light on the possibility of rhizobacteria-assisted phytostabilization for successful heavy metal remediation.

1.4. Phytoremediation technologies for addressing contaminated sites

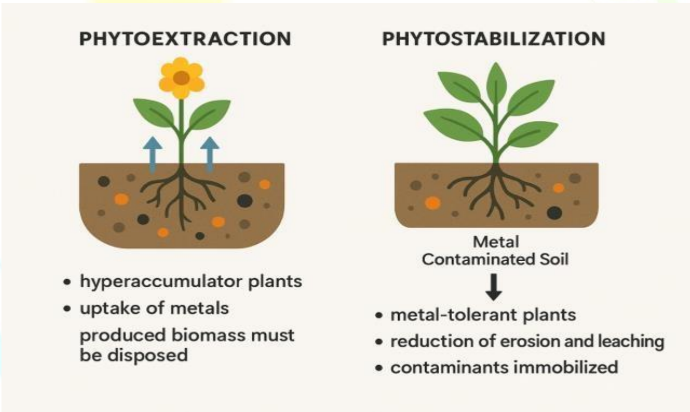


Figure 1. Comparison between phytoextraction and phytostabilization

Phytoremediation is an efficient, cost-effective, and environmentally friendly method of pollutant removal with little ecological impact (CRISTALDI et al., 2020). It is congruent with agricultural processes, offering an aesthetically pleasing solution that is frequently approved by the public at many sites and locales. Compared to prior approaches, phytoremediation uses solar energy. which reduces energy consumption and maintenance costs (Gaurav et al., 2020). This plant-based strategy is mainly self-sustaining, relying on natural geochemical processes and requiring few external inputs. Economically, it is a feasible alternative to conventional remediation methods. (Mehmood et al., 2017). Notably, phytoremediation allows for the recovery of precious metals via plant uptake and accumulation. Metals that have been held in harvestable plant components can be retrieved using a method called Phyto mining. This strategy often requires specific plant species with deep roots and strong rates of metal transfer from roots to shoots (Guerrero-Zúñiga et al., 2020). Phytoremediation comprises a variety of metal reclamation processes such as phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization (Abdullah et al., 2020).

1.4.1 Phytoextraction

Phytoextraction utilizes hyperaccumulator plants, which can tolerate and concentrate high amounts of metals. Ideal hyperaccumulators have deep roots, grow rapidly, and produce a substantial amount of biomass. However, many hyperaccumulators grow slowly, produce little biomass, and are unsuitable for a wide range of soils and temperatures. Furthermore, most metal-contaminated sites have soils that are degraded and contain multiple pollutants. Plants that can accumulate a variety of metals are ideal for phyto extraction. One issue with this approach is the safe disposal of metal- enriched plant residues (Abdullah et al., 2020).

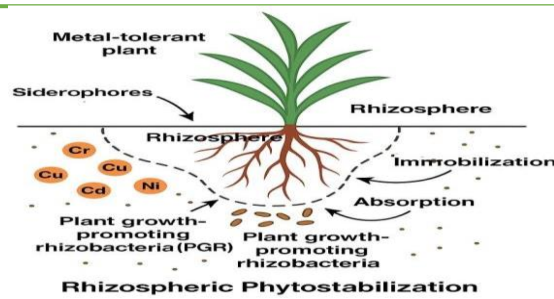
1.4.2 Phytostabilization

Phytostabilization is the process of stabilizing polluted soils by employing metal-tolerant plants to reduce erosion, airborne dispersal, and leaching of contaminants. Unlike phytoextraction, this strategy emphasizes limiting metal intake to reduce the potential of contaminants entering the wildlife food chain (Abdullah et al., 2020; Adesodun et al., 2010). Phyto stabilization is often the most cost-effective treatment for large-scale metal-contaminated sites, especially those with heavy pollution. By selecting suitable phytoremediation techniques, these plant-based technologies provide long-term and practical solutions for controlling contaminated sites.

1.5 Basic concepts of rhizospheric phytostabilization

Rhizospheric phytostabilization is a biological strategy in which plants interact with their rhizosphere —the active soil zone surrounding roots —to immobilize, absorb, and stabilize heavy metals. This important zone promotes nutrient uptake, water absorption, and a varied microbial community that aids in metal detoxification and stabilization. In this environment, complicated interactions between plants and bacteria result in specialized systems for metal pollution control (Glick, 2012). Plant growth-promoting rhizobacteria (PGPR) play a crucial role in this process, as they reduce the bioavailability and mobility of harmful metals. These bacteria, recognized for their pollution resistance and geochemical usefulness, improve metal stabilization by producing siderophores, modulating soil pH, and altering metal phosphate solubility (Rajkumar et al., 2012). Such effects reduce heavy metal uptake by plants, lowering the chance of them entering the food chain (Khan, 2020). Furthermore, rhizobacteria influence root development and exudation patterns, thereby modifying the rhizosphere environment to mitigate metal toxicity. *Pseudomonas* and *Azotobacter* are known to create siderophores in response to metals such as Zn, Cr, and Cd, which improves metal chelation and reduces toxicity. (Ma et al., 2011). According to research, plants such as *Brassica juncea*, *Alyssum murale*, and *Thlaspi caerulescens* stabilize metals like Zn, Ni, and Pb more effectively when rhizobacteria are present (Srivastava, 2020). Heavy metal stress also alters rhizospheric microbial populations, reducing total diversity while encouraging metal-tolerant species that contribute to stability. *Bacillus megaterium*, for example, produces more hydroxamic acid under heavy metal stress, which aids in the stabilization of metals such as Cr, Cu, and Al (Gupta et al., 2024).

This phytoremediation technology is particularly effective for treating polluted soils, as it focuses on immobilizing metals to prevent them from seeping into groundwater or spreading through erosion. Plants with broad root systems are especially effective because they improve soil stability and immobilize pollutants in the rhizosphere. Unlike phytoextraction, which aims to remove toxins, phytostabilization focuses on in situ containment, retaining metals in a less hazardous and stable state within the soil (Ali et al., 2013).



Metal-tolerant plants, or metallophytes, play an important part in this process by absorbing Cd at the root-soil interface. They reduce Cd absorption in the roots, limit its transfer to the shoots, and so reduce Cd availability in the soil (Raza et al., 2020). As a result, the distribution of Cd through air and water is minimized, and the chance of it entering the food chain is significantly lowered (Lin et al., 2022). Ryegrass (*Lolium multiflorum*) is a popular ecological reconstruction plant due to its fast germination and growth rates, well- developed root system, and high tolerance to Cd stress up to 400 mg/kg in soil (Ke et al., 2021). Recent research has revealed ryegrass as a good candidate for Cd phytostabilization (Li et al., 2022). Water lot and Hechelski (2019) found that the extractable Cd from low-molecular- weight organic acids in ryegrass-planted soil was less than 0.5% (43.7 mg Cd/kg). (Ke et al., 2021) discovered that when exposed to 50 mg Cd/L, perennial ryegrass germinated at a rate greater than 80%. Furthermore, in soil contaminated with 30 mg Cd/kg, the Cd content in roots was nearly six times greater than in shoots. Jia *et al.* (2019) discovered that annual ryegrass had a translocation factor (TF) of less than one, with values of 0.38 mg and 0.31 mg in soil containing 0.8 mg and 4 mg Cd/kg, accordingly. Although phytostabilization has considerable promise, its usage is limited due to poor plant development, Cd toxicity, and low biomass production (Ke et al., 2021).

1.6 Scope of Rhizobacteria in Phytostabilization

Rhizobacteria play a crucial role in phytostabilization processes by enhancing plant growth, improving soil stability, and facilitating the immobilization of heavy metals in contaminated environments. As root colonizers, these bacteria survive in a variety of environmental stressors, promoting plant growth and forming multifunctional relationships with microbial flora. Such interactions allow rhizobacteria to perform plant protection, growth promotion, and development functions (Abou-Shanab et al., 2019; Manoj et al., 2020).

Plant Growth-Promoting Rhizobacteria (PGPR) has unique functional characteristics that improve their phytostabilization ability. These include the ability to act as biofertilizers (increase soil fertility), Phyto stimulators (produce phytohormones that encourage plant growth), and metal solubilizers (change metal speciation to improve bioavailability) (Manoj et al., 2020). Some bacterial strains produce lytic enzymes and metabolic chemicals that operate as biopesticides, protecting plants against infections and illnesses.

Rhizobacteria contributes to phytostabilization through both specific and non-specific methods. Specific processes include encouraging cell division, improving metabolic stability, increasing food availability, and activating root networks. Non-specific processes include lowering metal toxicity, inhibiting infections, and increasing disease resistance. Several factors influence the effectiveness of rhizobacteria-assisted phytostabilization, particularly bacterial activity, bacterial community structure, metal content and accessibility, plant development, and the plant's natural tolerance to metal toxicity (Liu et al., 2020; Manoj et al., 2020).

Rhizobacteria regulate HM bioavailability, which is often limited by their chemical speciation with inorganic and organic elements. In the rhizosphere, these bacteria release compounds such as biosurfactants, siderophores, and extracellular polysaccharides (EPS), which change metal speciation via oxidation, reduction, acidification, chelation, immobilization, mineralization, and precipitation. These mechanisms improve phytostabilization efficiency by reducing hazardous metal mobility and accessibility in soil, hence minimizing environmental concerns (Abdullah et al., 2020; Abou-Shanab et al., 2019; Mehmood et al., 2017). (Mousavi et al., 2018) found that siderophore-producing bacterial strains promote plant development while immobilizing metals such as Pb and Zn. Similarly, Ali *et al.* (Adesodun et al., 2010) and (Mishra et al., 2016) found that siderophore-producing PGPR can stabilize Zn, Pb, and Fe in polluted soils, making them less bioavailable and lowering their environmental impact. These findings highlight the ability ofrhizobacteria to boost phytostabilization effectiveness via metal immobilization and plant-microbe interactions.

1.6.1 Microbe-assisted phytostabilization

Rhizobacteria reduce metal bioavailability in contaminated environments through various mechanisms, including precipitation, alkalization, biological transformation, biosorption, and absorption of water via transparent exterior capsules composed of EPS and ionic functional assemblies on bacterial cell surfaces. These functional groups include sulfonate, amine, sulfhydryl, hydroxyl, carboxyl, and amide, which contribute to metal retention and limit bioavailability to plants and microbes (Suyal et al., 2024).

(Sinha & Mukherjee, 2008) suggested that the anionic microbial cell walls help Hordeum vulgare resist cadmium poisoning. (Pratush et al., 2018) suggested that rhizobacteria reduce metal mobility through bioaccumulation and biosorption. During phytostabilization, plant roots can coat or fix metals, preventing them from moving through the soil and eventually entering the food chain. This technique also helps restore plant communities on land degraded by metal toxicity, reducing wind erosion and metal dissemination once plant species have developed tolerance to the toxic metals.

Microorganisms, including bacteria, contribute to metal stabilization through sequestration, transformation, precipitation, and accumulation. For example, they can alter the oxidation state of metals such as iron (Fe), manganese (Mn), mercury (Hg), and selenium (Se), converting them into less toxic forms through oxidation or reduction processes. (Krithika & Balachandar, 2016) studied the microbial conversion of hexavalent chromium Cr (VI), defining four phases: Cr (VI) adsorption of bacterial membrane functional groups, transport using phosphate/sulphate transporters, lowering in the cytosolic solution, and immobilization of Cr (III). (Srivastava & Thakur, 2012) discovered that Serratia sp. aids in the reduction of Cr (VI) to Cr (III) by processes such as nonexchange, consolidation, combined precipitation, and immobilization. (Majumder et al., 2013) Showed that oxidizing microorganisms such as *Geobacillus* sp. and Bacillus sp. could transform As (III) to less toxic As (V), whereas Gregorio *et al.* (2005) discovered that the species *Stenotrophomonas maltophilia* could decrease and immobilize Se in soil. These findings demonstrate that rhizobacteria play a crucial role in reducing metal bioavailability and mitigating environmental risks.

1.7 Capability of rhizobacteria in phytostabilization

1.7.1 Plant development

Elevated metal concentrations in soil are harmful to plants, especially hyperaccumulators, which can only tolerate metals to a certain extent. Metal stress frequently causes iron deficiency, chloroplast damage, and reduced chlorophyll production, which results in chlorosis. Rhizobacteria, on the other hand, can create siderophores, which are iron-binding molecules that increase iron availability to plants under such stress situations. Inoculating plants with siderophore- producing rhizobacteria can help to prevent iron deficiency in metal-contaminated soils. These bacteria promote plant development, root elongation, and early establishment in soils contaminated with metals, including Zn, Ni, and Pb (Becerra-Castro et al., 2018).

Rhizobacteria continues to boost nutrient uptake and iron availability after plants have established themselves on contaminated land. For example, *Pseudomonas* from paint industry effluents can reduce chromium (Cr) uptake and promote Triticum aestivum germination in soils contaminated with potassium bichromate. Furthermore, certain rhizobacteria can induce the buildup of harmful metals in diverse plant tissues. Under regulated conditions, rhizobacteria can collect up to eighty percent of selenium (Se) from Saltmarsh bulrush roots and 60% from shoots. Furthermore, rhizobacteria influences metal accessibility by altering metal speciation and solubility in the rhizosphere, thereby creating a more favorable environment for plant metal uptake. Rhizosphere acidification, which is caused by bacterial activity, can increase the bioavailability of metals like mercury (Hg), allowing plants like *Thlaspi caerulescens* to absorb them more effectively (Seneviratne et al., 2017). Bacteria also convert toxic metals like Cr (VI) to less damaging forms like Cr (III), which plays an important role in phytostabilization. (Zulfiqar et al., 2023).

1.7.2 Suppression of plant pathogens

Rhizobacteria help plants resist a variety of diseases, including those caused by fungi, bacteria, viruses, and pests such as nematodes and insects. They accomplish this through mechanisms such as competition for space and nutrition, the creation of antibiotics (e.g., pyocyanin, pyrrolnitrin), and the release of siderophores (e.g., pseudobactin), which limit iron availability for pathogens (Brereton *et al.* 2020). Rhizobacteria produce lytic enzymes, including chitinases and β-1,3-glucanases, that break down fungal cell walls and break down pathogen-produced poisons. These defense mechanisms help plants stay healthy and develop in polluted surroundings (Wu et al., 2001).

1.7.3 High surface-area-to-volume ratio

Rhizobacteria have a high surface-area-to-volume ratio, which improves their contact with metals and thus their ability as biochelators. Bacteria, for example, can stimulate root hair growth in plants such as Indian mustard, leading to increased selenium accumulation. However, root hair enhancement alone does not entirely account for metal buildup. Plants can sometimes accumulate high metal concentrations without the help of microbes. Studies on arsenic (As) and chromium (Cr)-contaminated soils have shown that bacteria alone do not necessarily influence metal uptake in plants, implying that other variables may also play a role in metal buildup (Becerra-Castro et al., 2018).

1.8 Rhizosphere interactions in phytostabilization

Phytostabilization, a crucial step in phytoremediation, is highly dependent on interactions among plants, microorganisms, and soil components, particularly in soils contaminated with metals. These interactions are influenced by various parameters, including soil conditions, plant health, and pollution levels, which collectively determine the efficacy of phytostabilization mechanisms. Plant-microbe interactions, in particular, play a crucial role in enhancing the stability of heavy metals (HMs) in the soil by stimulating microbial growth in the rhizosphere and promoting plant development under stressful conditions, such as metal contamination (Gul et al., 2021).

1.8.1 Plant-Microbial Relationships improve heavy metal phytostabilization

Plants use phytostabilization to immobilize hazardous metals in the root zone, preventing them from moving to the water table or other sections of the ecosystem. Rhizobacteria in the root zone have a substantial impact on metal immobilization and stabilization, as they modify the bioavailability of metals. These microorganisms can increase the solubility of metals that would otherwise be insoluble in water and unavailable to plants. Plant root exudates, which contain organic acids such as citric and oxalic acids, have been found to bind heavy metals and aid their stabilization in the rhizosphere. These exudates also promote microbial development, which can aid in the immobilization of heavy metals via adsorption, precipitation, and complexation (Ma et al., 2016).

Organic root exudates function as natural chelators, sequestering metals such as cadmium (Cd), copper (Cu), and lead (Pb), thereby stabilizing them in the soil rather than facilitating their uptake by plant tissues. For example, research has shown that plants such as *Echinochloa crus-galli* produce organic acids that improve the ability to immobilize Cu, Pb, and Cd in the rhizosphere, inhibiting their transfer to the plant's aerial parts (Mehmood et al., 2017). Furthermore, root exudates have a dual purpose in allelopathy and detoxification. They promote microbial growth in the root zone, increasing the bioavailability of metals for immobilization and helping to stabilize pollutants in the soil matrix (Abou-Shanab et al., 2003).

To optimize phytostabilization, it is crucial to select suitable plant species based on soil characteristics and the specific contaminants present. Inoculating the rhizosphere with bacterial strains that promote metal immobilization can also improve phytostabilization results. The microbial community in the rhizosphere varies according to plant species, soil conditions, and contaminants, necessitating careful study when selecting the appropriate plant-microbe combinations. (Iqbal et al., 2023).

Advanced research into plant-bacteria interactions in metal-contaminated soils is ongoing, with the goal of enhancing metal tolerance and optimizing the geochemical processes crucial for successful phytostabilization. Despite tremendous advances, further research is needed to thoroughly understand the mechanisms governing plant- microbe interactions in phytostabilization and optimize these processes for large-scale applications in soil remediation (Rosario & Dev, 2024).

1.8.2 Plant-Bacteria interaction in phytostabilization

Plant-bacteria interactions are crucial in the phytostabilization process, as plants and their associated rhizobacteria play a significant role in soil stabilization and pollution reduction, particularly in areas contaminated with heavy metals. These microbial partners enhance soil structure, increase nutrient cycling, detoxify toxic compounds, and mitigate pests and diseases. Rhizobacteria enhance the overall phytoremediation process through these interactions, particularly in stressful environmental conditions such as metal toxicity, nutritional imbalances, and insect pressure. (Abou-Shanab et al., 2019; Ma et al., 2016). Rhizobacteria help to stabilize heavy metals in the soil by lowering their bioavailability to plants, preventing these metals from moving into plant tissues. In this symbiotic relationship, plants produce root exudates, such as organic acids, which increase the solubility and availability of metals in the soil, allowing bacteria to absorb them more effectively for immobilization. In turn, the bacteria support the reduction of harmful chemicals in the soil, thereby improving overall soil health and stability. These microbial communities also break down organic pollutants, which helps to detoxify the soil environment. Plant-bacteria interaction in phytostabilization is thus an important mechanism for lowering metal toxicity and stabilizing contaminated soils, contributing to environmental sustainability and ecosystem health (Sharma et al., 2023).

1.8.3 Rhizosphere-Plant-Microbe relationships

Soil environment is crucial for plant-metal-bacterial relationships. Changes in soil physical and chemical characteristics influence bacterial community structure, metallic bioavailability, and the growth of plants. Furthermore, plant-bacteria associations (specific or nonspecific regarding metal removal) may be influenced via various needs for metabolism. In addition to metal poisoning, various other characteristics (like water availability, nutrient levels, soil texture, and atmospheric conditions) have been demonstrated to have a significant impact on these interactions (Rai et al., 2020).

1.9 Rhizobacterial mechanisms affecting heavy metal uptake

Rhizobacteria, notably plant growth-promoting rhizobacteria (PGPR), play a crucial role in phytostabilization, a process in which pollutants are immobilized in the soil through interactions between plants and microbes. These bacteria use a variety of biochemical and physiological mechanisms to increase plant stress tolerance and immobilize harmful chemicals in the rhizosphere (Sobariu et al., 2017).

1.9.1 Metal mobilization or immobilization

Some rhizobacteria emit organic acids (e.g., citric acid, oxalic acid) and chelating agents (e.g., siderophores) to solubilize heavy metals in the soil, making them accessible for plant uptake. Rhizobacteria secretions play a significant role in the phytostabilization mechanism, which is facilitated by nitrogen fixation and the production of siderophores that solubilize and replenish iron in the soil. The synthesis of phytohormones like auxins and cytokinins can promote plant growth and improve the solubility of essential minerals, particularly phosphorus. Rhizobacteria indirectly inhibit phytopathogens that are damaging to plant growth and development (Kloepper et al., 1989; Patten & Glick, 1996).

1.9.1.2 Metal immobilization through exopolysaccharide (EPS) production

Other rhizobacteria precipitate metals or sequesters them in the soil matrix, lowering their bioavailability and protecting plants from toxins. Rhizobacteria create exopolysaccharides, which are high-molecular-weight polymers that bind to metal ions in soil, lowering their bioavailability and toxicity. EPS captures heavy metals such as cadmium (Cd), lead (Pb), and arsenic (As) and stabilizes them in the soil matrix. Certain species of *Pseudomonas* and *Bacillus* are known to produce EPS, which efficiently immobilizes metals (Naseem et al., 2024).

1.9.3 Production of siderophores

Rhizobacteria create iron-chelating chemicals known as siderophores. They can bind heavy metals such as cadmium (Cd), lead (Pb), and zinc (Zn), limiting their availability to plants or minimizing their toxic effects. Rhizobacteria produce metal-chelating compounds known as siderophores. These bioactive molecules are engaged in capturing Fe^{3+}, which increases the bioavailability of immobilized soil iron (Jing et al., 2007), thereby benefiting plants cultivated in metal-pollutant (iron-deficient) soil. Microbial siderophores control the supply of iron in the plant rhizosphere. Major contributing elements include the concentration of various types of siderophores, exchange kinetics, and the availability of Fe-complexes to microbes and plants (Katiyar et al., 2016).

Phyto siderophores have a lesser affinity for iron than microbial siderophores.

1.9.4 ACC (1-aminocyclopropane-1-carboxylate) deaminase activity

Rhizobacteria produce the enzyme 1- aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces ethylene levels in plants. Reduced ethylene production reduces heavy metal stress, allowing plants to thrive in contaminated soils. *Bacillus subtilis* and

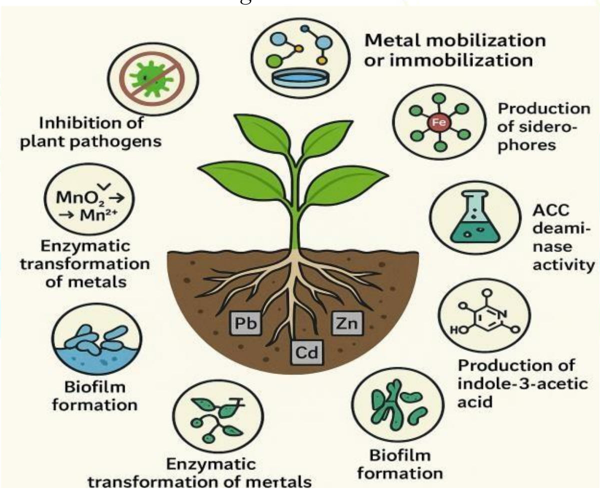


Figure 3. Rhizospheric phytostabilization of heavy metals via plant–microbe interactions

Pseudomonas putida have ACC deaminase activity, which helps plants resist cadmium and lead poisoning (Misra & Chauhan, 2020). The quantity of ACC is reduced and hydrolyzed by different PGPR containing the enzyme 1- aminocyclopropane-1-carboxylate (ACC) deaminase. ACC acts as an ethylene source of the plant hormone ethylene, reducing ethylene production in plants. Plants take ACC from roots or seeds, which are then cleaved by ACC deaminase into ammonia and α-ketobutyrate (Shahid et al., 2023). This ammonia is consumed as a nitrogen source by the bacteria, reducing ACC levels in the plants. To regulate the equilibrium between internal and external ACC levels, the plant needs to synthesize a significant amount of it. Lowering ACC levels in the plant reduces plant ethylene.

1.9.5 Production of Indole-3-Acetic Acid (IAA)

IAA is a phytohormone that promotes root elongation and biomass development, which improves the plant's ability to stabilize soil. Improved root growth increases the plant's ability to immobilize pollutants in the root zone. Rhizobacteria, such as *Azospirillum* and *Bacillus* spp, promote root development by synthesizing IAA. Indole acetic acid (IAA) produced by PGPR may promote the growth of plant roots (Etesami et al., 2015). Rhizobacteria produce IAA at low levels, promoting primary root enlargement; however, high levels increase auxiliary and adventitious root development while suppressing main root development. Hence, beneficial rhizobacteria may enhance plant development by modifying the plant's hormonal balance. Similarly, an ethylene spike helps to interrupt seed inactivity, but excessive ethylene might break seed dormancy. Phytopathogen disease may increase ethylene levels. Plant growth-promoting bacteria (PGPB) produce ACC deaminase, which compensates for this.(del Carmen Orozco-Mosqueda et al., 2020). As a result, growth- promoting bacteria regulate the ethylene level in plants, minimizing the perceived toxicity of heavy metals to plants.

1.9.6 Biofilm formation

Rhizobacteria create biofilms on root surfaces, providing a barrier against hazardous pollutants. Biofilms improve root adhesion and form microenvironments that immobilize metals and minimize leaching. *Pseudomonas aeruginosa* biofilms can immobilize arsenic and lead (Li et al., 2024).

1.9.7 Enzymatic transformation of metals

Oxidize, or methylate, heavy metals, converting them into less dangerous or immobile forms. Arsenate reductase converts arsenate (As^{5+}) into arsenite (As^{3+}), which can then be immobilized. *Bacillus thuringiensis* converts chromium (Cr^{6+}) into less harmful Cr^{3+}. (Thacker & Madamwar, 2005) found that bacteria can transform hazardous heavy metals into forms that are easily absorbed by roots. For instance, in plants converting selenate (Se) in to organic Se enhance Se levels (Huang et al., 2005). The comparison between bulked soil and rhizosphere revealed that the proportional variations in organically bound Cu, Zn, and Pb with increase of +5%,
+23%, and +3% in the contaminated rhizosphere, and 0.8%,
-3%, and -2% in the non-contaminated rhizosphere. As a result, the infected rhizosphere contained significant levels of Cu, Zn, and Pb bound by organic matter. Soil associated bacteria affect metal accessibility by altering chemical characteristics such as pH, organic matter concentration, redox potential, and so on. This facilitates the leaching pollutants from soil. For instance, *Pseudomonasmaltophilia* strain has been demonstrated to convert mobility and hazardous Cr^{6+} become harmless and immobile Cr^{3+}, as well as to reduce the environmental mobility of other toxic ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+} (Henagamage et al., 2022).

1.9.8 Stimulation of transport protein

Bacteria rely on transition metals like manganese, zinc, and iron to survive and flourish in their environment and hosts. Cells, for example, may rigorously regulate zinc content within the cell since large levels of zinc are harmful
to cellular processes. Bacteria may also induce the sulphate transport protein, which transports selenate through the root plasma membrane (Ahemad & Kibret, 2014).

1.9.9 Inhibition of plant pathogens

PGPR eliminates plant pathogens in a variety of methods, including competition for resources and space, antibody production, and the manufacture to siderophores that limit the supply for iron to pathogen growth. (Brereton et al., 2020; Doyle, 2015). Important mechanisms include the synthesis of lytic enzymes like chitinases and β-1, 3-glucanases, which break down chitin and glucan in fungal cell walls (Wu et al., 2001).

1.10 Challenges and future directions

Regarding the great potential of metal-tolerant rhizobacteria for phytostabilization, numerous challenges exist. Phytostabilization depends on soil parameters like pH, temperature, moisture, and organic matter concentration. These factors can have an impact on the activity and efficacy of metal-tolerant rhizobacteria. Metal-contaminated soils might limit bacterial survival and activity due to excessive metal concentrations that impede growth or cause die-off. Phytostabilization efficiency depends on plant compatibility with rhizobacterial strains. Optimizing these plant-microbe interactions is still a significant topic of research. Phytostabilization may take years to stabilize metals in soil, making its long-term effectiveness unresolved Additionally, the ecological impact of adding non-native rhizobacteria must be carefully assessed.

Conclusion

Heavy metal contamination poses a significant threat to environmental and human health, necessitating effective and sustainable remediation strategies. Phytostabilization, particularly when enhanced by metal-tolerant rhizobacteria, offers a promising solution for immobilizing heavy metals in contaminated soils, reducing their mobility and bioavailability. This approach leverages the synergistic relationship between plants and rhizobacteria, which not only stabilizes metals but also improves soil health and promotes plant growth under stressful conditions. Despite its potential, challenges such as environmental variability, metal toxicity, and the need for optimized plant-microbe interactions must be addressed to ensure long-term effectiveness. Future research should focus on understanding the underlying mechanisms of plant-rhizobacteria interactions and developing tailored strategies for large-scale applications. By integrating phytostabilization with rhizobacteria-assisted techniques, we can achieve a sustainable and eco-friendly solution for mitigating heavy metal pollution, contributing to environmental restoration and public health protection.

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