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A Review on the Synergistic Approaches for Heavy Metals Bioremediation: Harnessing the Power of Plant-Microbe Interactions

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ABSTRACT: *Heavy metals contamination is a serious threat to all life forms. Long term exposure of heavy metals can lead to different life-threatening medical conditions including cancers of different body parts. Phytoremediation and bioremediation offer a potential eco-friendly solution to such problems. Different microbes can interact with heavy metals in a variety of ways such as biotransformation, oxidation/reduction, and biosorption. Phytoremediation of the heavy metals using plants mostly involves rhizofilteration, phytoextraction, phytovolatization, and Phytostabilization. A synergistic approach using both plants and microbes has proven much more efficient as compared to the individual applications of microbes or plants. This article aims to highlight the synergistic methods used in bioremediation, emphasizing the potent collaboration between bacteria and plants for environmental cleaning, along with the discussion of the importance of site-specific variables and potential constraints. While identifying the necessity for all-encompassing solutions, this review places emphasis on the combination of methodologies as a multifarious rehabilitation approach. This discussion offers insightful suggestions for scholars, scientists and decision-makers about the sustainable recovery of heavy metal-contaminated environments using a comprehensive strategy.*

Keywords: *Heavy metals, Phytoremediation, Bioremediation, Plant-Microbe Interaction*

INTRODUCTION

Life as well as ecosystems are at stake because of the environmental contamination, mainly caused by industrial processes. Bioremediation is a potential approach in the search for long-term pollution mitigation options. The general goal of bioremediation technology is to use biological processes to remove contaminants such as pesticides, heavy metals, radionuclides, organic waste, and others from contaminated sites or industrial discharges. It may frequently be completed on site, is well accepted by the people, and requires very little in the way of technology. The kind of pollutants, the amount of time, and the technique all affect the outcome of the remediation processes (Saha et al., 2021).

High concentrations of various contaminants are present in industrial effluent that is often dumped into water bodies. The toxicity of these toxicants not only harm the human health, but other life forms as well. It also disrupts the ecosystems and thus affects the overall quality of the environment (Choudhary et al., 2017). Because heavy metals are persistent and cannot be biodegraded in wastewater treatment, their toxicity especially at high concentrations has become a major concern worldwide. Lead, uranium, selenium, zinc, arsenic, cadmium, chromium, mercury, copper, and nickel are some of the most important contaminants in this regard. Typically, mining activities, ore refinement, sludge disposal, paints, alloys, batteries, fly ash from incinerators, radioactive material processing, metal plating, or the production of electrical equipment, insecticides, or preservatives generate these hazardous compounds (Junaid et al., 2017). Toxic metals are not just released by industry; occasionally, natural processes also release heavy metals into the environment. Climate change, volcanic eruptions, leaching and erosion are some of the natural processes that can release heavy metals into the environment (Wuana and Okieimen, 2011). These processes increase the human exposure to such contaminants as they persist in the environment. While small concentrations of these elements are essential for optimal health, larger concentrations are hazardous both in the short and long-term exposure. Depending on the heavy metal and the individual exposure, heavy metals can have teratogenic, nephrotoxic, fetotoxic, and neurotoxic consequences (Leong and Chang, 2020).

Bioremediation methods are often categorized as *in situ* i.e., at the contaminated location, or *ex situ* which refers to removing a pollutant from its original location and treating it somewhere else. *In situ* bioremediation is an economical and ecologically acceptable way to treat polluted soil and groundwater. It needs the right climate, vital nutrients, a healthy population of microbes, and enough time for natural breakdown. Because the rates at which microorganisms break down / transform the toxicants vary, process optimization requires constant observation and modification (Sharma, 2020). *Ex situ* bioremediation is the process of transferring contaminated materials somewhere else for treatment. This is frequently done when *in situ* treatments are not feasible. Methods like bioreactors and biopiles provide better environmental control and can handle vast amounts of polluted medium (Azubuike et al., 2016).

One effective approach is to utilize the beneficial interactions between microbes and plants, utilizing the distinct advantages of each application. The goal of plant-microbe synergy is to increase the effectiveness of environmental cleaning. As phytoremediators, plants are very effective because they can draw many toxic compounds, including heavy metals, out of soil or water. Simultaneously, microbes that live in the rhizosphere of plants or create symbiotic relationships with plant roots provide their biochemical and metabolic skills to aid in the breakdown, immobilization, transformation, or uptake of the pollutants. This integrated bioremediation strategy's interactions between bacteria and plants result not only in greater pollutant remediation, but also better plant health. In addition to cleaning up polluted areas, the goal of these cooperative initiatives is to advance environmentally sound behaviours that support ecosystem restoration and balance. The framework for investigating the many aspects of plant-microbe interactions in bioremediation—

which include phytoremediation, rhizoremediation, myco-remediation, phyto-stabilization, and bioaugmentation—is discussed in this review.

1. BIOREMEDIATION

Microorganisms or their enzymes are used in bioremediation to eliminate pollutants from a contaminated location. The primary microorganisms utilized in the bioremediation process to clean up polluted soil and water are bacteria and fungus (Strong and Burgess, 2008). Methane, phenol, and toluene are examples of substrates that may be used to stimulate bioremediation activity through microorganisms. Other methods include adding nutrients (nitrogen and phosphorus) and electron acceptors (e.g., oxygen) (Romantschuk et al., 2023).

This decade has seen a rise in interest in the use of bacterial, fungal, and algal biomass as an absorbent medium for the removal of heavy metals. Because it may act as a bio trap for heavy metals, renewable biomass from a variety of microorganisms may prove to be an environmentally beneficial alternative to physicochemical remediation techniques. Biotrap is any living thing or part of a living thing that can connect with a poisonous metal and change its shape, allowing the metal to be removed and recovered from contaminated soil or water or turning it harmless (Crusberg and Mark, 2000). Microbial adsorbents have emerged as an eco-friendly and efficient material choice (Valls and De Lorenzo, 2002). Reclamation of contaminated places greatly depends on how microorganisms react to harmful heavy metals (Congeevaram et al., 2007). For growth, microorganisms need the ideal conditions of temperature, nutrition, and oxygen. Heavy metals have a variety of effects on the physiology of microorganisms, but many of them manage to resist these pressures. To survive in environments where metals are scarce, bacteria have developed a few strategies. These processes allow them to mobilize, immobilize, or change metals, making them inert enough to withstand the toxicity of heavy metal ions (Nies, 1999). These mechanisms include chelation (metals form complex with the metal-binding proteins, e.g., metallothionein's), extrusion (metals are pushed out of the cell through chromosomal/plasmid-mediated events), and exclusion (the metal ions are kept away from the target sites or other cell components), methylation-demethylation, and biotransformation (conversion to less hazardous ones) (Umrania, 2006). These mechanisms enable metabolic activity of bacteria in environments polluted by metals. These processes may be inducible or constitutive. Bacteria most likely pick up their resistance to heavy metals by natural changes on plasmids and transposons or gene transfer. For example, the *czc* system is responsible for resistance against cadmium, zinc, and cobalt in Gram-negative bacteria (such *Ralstonia eutropha*). These metals are exported via the cation-proton antiporter (CzcABC), which is encoded by the *czc* genes (Nies and Silver, 1995). Many fungi and bacteria have been employed for the removal of heavy metals, each with a unique mechanism, as explained below.

1.1. Microbial Mechanisms Involved in Bioremediation of Heavy Metals

Many microorganisms interact with heavy metals in the environment and find applications in bioremediation. The following are the most common microbial mechanisms involved in this process (Fig. 1).

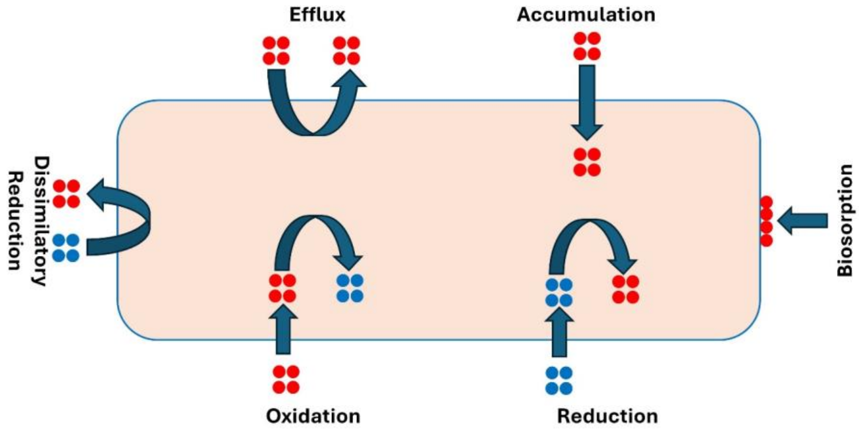

Fig. 1. Most common modes of microbial interactions with heavy metals

a) **Biosorption:** Many functional groups are present on the cell surface of microorganisms, such as carboxyl, amino, and phosphate groups. Many bacteria are also known to produce extracellular polymeric substances (EPS). In the case of fungi, they also contain chitin as a main constituent of their cell walls. These and other functional groups found on cell walls of microbes can bond with heavy metal ions, causing a buildup of metals on the surface of the cells. This interaction leads to biosorption and not only helps in reducing the bioavailability of the heavy metals but can also help to remove them (Wu et al., 2021).

b) **Bioaccumulation**
Many bacteria have been reported to accumulate heavy metals in their cells. This is possible due to several channels that exist in the membranes of the microorganisms through which the heavy metals are transported inside, followed by their interaction and complexation with different proteins (Igiri et al., 2018). Microorganisms such as fungi can also accumulate heavy metals in their mycelia. It includes the sequestration of metals in intracellular compartments and the creation of metal complexes. Heavy metals can be absorbed by algae into their cells. The metals can be attached to certain cellular elements or kept in vacuoles of cells (Chugh, M et al., 2022).

c) **Bio-mineralization:** Bacteria use this process to immobilize heavy metals. Some bacteria can change reduce the solubility of metals, rendering them less hazardous, and may even lead to their precipitation (Kapahi and Sachdeva, 2019).

d) **Oxidation / Reduction:** Some bacteria have the capacity to oxidize or reduce heavy metals, thus rendering them less toxic and less soluble too. This is also a detoxification strategy in case of many heavy metals (Pande et al., 2022). Many microbial species are known to oxidize or reduce different heavy metals (Silver 2011; Silver and Phung, 2005).

Many bacterial species have been reported to interact with heavy metals including *Pseudomonas*, *Bacillus*, *Aeromonas*, *Shewanella*, and many other bacterial species. Sulfate reducing bacteria such as *Desulfovibrio* species can cause the precipitation of metal sulfides, and thus can aid in the bioremediation of metals. Applications for bioremediation using bacteria include the removal of polluted sediments, wastewater, and soils. Applying them in in-situ or ex-situ treatment techniques is contingent upon the environmental circumstances. (Kapahi and Sachdeva, 2019). Fungi are used for cleaning up soils, sediments, and water bodies polluted with metals. They have capacity to form mutualistic connections with plants and prove advantageous in augmenting plant well-being and absorption of metals (Joshi et al., 2011). Examples include *Aspergillus*, *Trichoderma*, and many other fungal species. Microalgae and macroalgae alike microorganisms are more important when there is need to eliminate heavy metals from aquatic environments. Wastewater treatment frequently uses algae-based bioremediation, particularly to remove heavy metals. Before being released, tainted water can be treated in artificial wetlands with algal mats (Ankit et al., 2022). Examples include *Chlorella*, *Spirogyra*, *Ulva* (Sea Lettuce), and many other algal species.

1.2. Microbial Strategies Used in Bioremediation

The most important microbial strategies used in bioremediation of heavy metals include biosorption, bioaugmentation, bioaccumulation, biomineralization, microbial transformation, and rhizoremediation.

a) Bioaugmentation

It can be used for increasing microbial populations' ability to withstand and immobilize heavy metals in polluted settings. Microorganisms that can bind metals, either natural wild type or genetically modified, are introduced at the site of the contamination. This leads to higher number of these microorganisms at these sites which enhance the efficiency of bioremediation. The microorganisms then remediate the heavy metals by the processes as mentioned above.

b) Biosorption

Heavy metals can be extracted by biosorption from polluted water sources, mining runoff, and industrial effluents. On their cell surfaces, microorganisms including bacteria, fungus, and algae have functional groups that bind to heavy metal ions. These groups consist of phosphate, amino, and carboxyl groups.

c) Rhizoremediation

Rhizoremediation can be used for soils and streams polluted with heavy metals. Plants exude root exudates, which facilitate the development of fungi and bacteria that interact with the metals in the rhizosphere. In the root zone, these microbes help to convert or immobilize heavy metals. Many plants such as willows (*Salix* spp.) and sunflowers (*Helianthus annuus*) are used in phytoextraction of heavy metals. Plant roots are home to mycorrhizal fungus, which improve the intake of nutrients and metals.

d) Biotransformation

Heavy metals can have their chemical forms changed via microbiological transformation to reduce their toxicity or facilitate their immobilization. Heavy metal ions are enzymatically changed by microorganisms into less hazardous or transportable forms. This might entail procedures like complexation, oxidation, or reduction. Insoluble metal sulfides can precipitate from the reduction of sulfate to sulfide by sulfate-reducing bacteria, such as *Desulfovibrio* species. Additionally, certain fungi aid in the transformation of heavy metals.

By improving microbial communities' capacity to deal with metals, by using biological entities for metal adsorption, by taking advantage of plant-microbe interactions for metal uptake, or enzymatically converting heavy metals into less hazardous forms, these microbial techniques are essential to the process of heavy metal bioremediation. The characteristics of the pollutants as well as the physiochemical properties of the polluted site determines which treatment is best (Emenike et al., 2018).

2. PHYTOREMEDIATION

Phytoremediation utilizes plants to bio-transform or remove the toxic compounds from soil or water. Lots of different pollutants such as heavy metals, pesticides, insecticides, hydrocarbons and others can be removed through this approach (Hong-Bo et al., 2010). Many plant species have been reported to not only withstand but also uptake large concentrations of heavy metals. Both wild type as well as transgenic plants have been used in phytoremediation (Sabreena et al., 2022). The interactions between plants and metals, environmental conditions, physiochemical properties of the site (water or soil) as well as the presence of other toxicants effect the performance of phytoremediation (Pandey and Bajpai, 2019). A lack of precise knowledge of these factors continues to impede the wide-scale use of this technology (Shen et al., 2022).

2.1. Strategies of Phytoremediation

The following are the most common processes employed by plants to reduce the toxicity of heavy metals (Fig. 2):

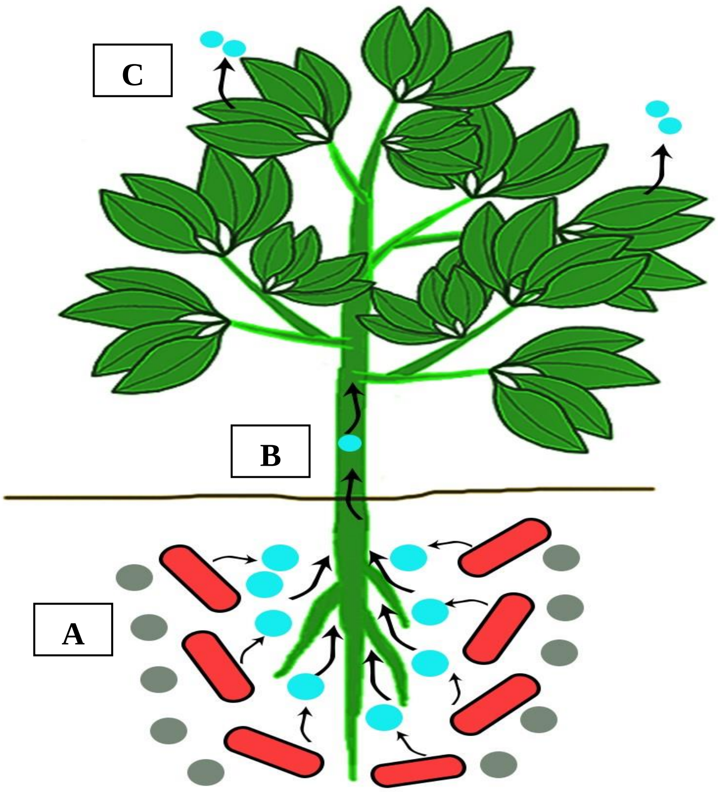

Fig. 2. Effects of microbial transformation of heavy metals on phytoremediation. **A:** microbes in the rhizosphere increase the mobility of the heavy metals through biotransformation, **B:** Plants efficiently uptake these heavy metals and store in their shoots and leaves i.e., phytoextraction, **C:** Plants are also able to release the heavy metals in the atmosphere via transpiration i.e., phytovolatilization

a) Rhizofiltration

It is described as a mechanism by which plants adsorb onto, or absorb into, their roots the pollutants from contaminated water sources. Acid mine drainage, agricultural runoff, and industrial discharge can all be partially mitigated via rhizofiltration. It is feasible to remove heavy metals as well as radionuclides using this technique (Jabeen et al., 2009). Plant species that have a strong affinity for specific heavy metals are selected for hydroponic growing or artificial wetland creation. Mathematical formulas for metal adsorption, root development, and moisture uptake have been generated through various studies. Designing phytoremediation programs requires knowledge of heavy metal transport in soil, water, and root system (Verma et al., 2006). One of the benefits of rhizo-filtration is that species other than hyperaccumulators can also be employed, and it can be applied both *in situ* and *ex situ*. Studies have been done on the capacity of many plants, including sunflower, Indian mustard, tobacco, rye, spinach, and corn, to extract metals from wastewater (Ghosh and Singh, 2005).

b) Phytostabilisation

The process of developing a plant cover on the surface of a contaminated site in order to reduce off-site pollution by limiting the transport of pollutants inside the vadose zone by root accumulation or immobilization inside the rhizosphere. It also increases the organic content of the soil that also binds the heavy metals. Certain plant species like grasses or ferns are chosen because of their capacity to withstand heavy metals and store them in their root systems. Heavy metals are taken up and transported from the soil to the roots of plants by metal transporters. Its primary application is in the remediation of sludges, sediments, and soil (Mueller et al., 1999). This process relies heavily on roots' ability to decrease contaminant movement and soil bioavailability. Phytostabilization can occur by sorption, precipitation, complex action, or metal valence reduction. Plant roots play a primary role in this regards, as they reduce the water movement through soil matrix, thus preventing not only soil erosion but also the movement of the toxic heavy metals to other areas (Fig. 3). It also prevents leaching of the heavy metals (Berti and Cunningham, 2000).

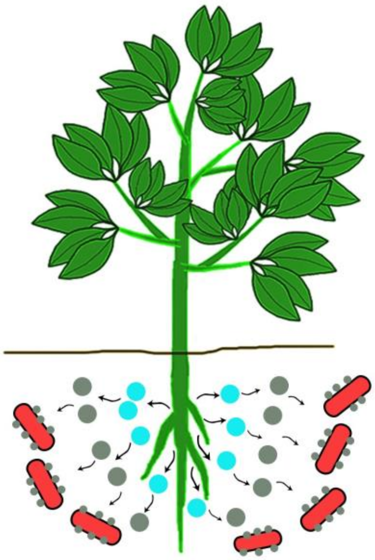

Fig. 3. Effects of Phytostabilization on microbial biosorption of heavy metals in the rhizosphere. Many plants can stabilize the heavy metals present in the soil through different means. These heavy metals are then efficiently adsorped / absorbed by the microorganisms present there, thus decreasing further their bioavailability

c) Phytovolatilization

Phytovolatilization is the conversion of heavy metals into volatile compounds through transpiration by plants. These volatile compounds are less toxic and are easily diluted by dispersion through the air. Many heavy metals such as mercury and selenium are volatilized by different plant species (Chandra et al., 2015). Volatilization may occur directly through stems and leaves, or indirectly by interactions between plant roots and the soil (Limmer and Burken, 2016). High rates of volatilization of heavy metals can be achieved by searching for new plant species with exceptional transpiration rates, as well as through genetic modification of already known plants (Guignardi and Schiavon, 2017). The main advantage of phytovolatilization is that, once the plantation is established, it seldom needs additional maintenance (Cristaldi et al., 2017).

d) Phytoextraction

Through their roots, plants draw toxins from the soil, water, or sediments and move them to the biomass above ground, where they build up in areas like shoots and other portions of the plant that may be harvested. This process is called as phytoextraction (Singh, N and Santal, AR, 2015). Plants that hyperaccumulate heavy metals (HMs) in above-ground portions and plants that produce a lot of above-ground biomass are used in phytoextraction as these two traits are crucial in defining the phytoextraction efficieny of plant species (Robinson et al., 1998). There are around 450-500 plants that are known to be hyperaccumulators (Chaudhary et al., 2018). Once the plants have been grown on contaminated areas, the metal-enriched biomass that grows above ground is removed and disposed. This ultimately removes the heavy metals from the polluted site. The transfer of the metals to the shoots is very important since it is far easier to collect the shoots as compared to collecting the roots. Phytoextraction is the best phytoremediation technique to remove heavy metals from contaminated soils. In addition, it is also the most financially achievable option of remediation. The capacity of plants to uptake and retain metals, as well as soil properties, heavy metal speciation, and heavy metal bioavailability all influence how successful phytoextraction might be (Yan et al., 2020).

3. BIOREMEDIATION OF HEAVY METALS THROUGH PLANT MICROBE INTERACTIONS

The key components needed to remediate industrial wastes like heavy metals, pesticides, and toxic chemical fertilizers are microorganisms (which include bacteria, yeast, fungi, and even archaeon) and plants. This is because they function as biological catalysts in a bioremediation system that is set up by appropriate elements to fix contaminated environments, meaning that appropriate plants can remove and restrain metals from the ground and microorganisms that can take up and transform heavy metals. Although heavy metals may look like hard-to-manage pollutants, bioremediation is a preferred technique to repair damaged soils; moreover, combining plants and microbes is a strategy to ensure a more thorough cleanup. Thus, using plants and the bacteria they are associated with to clean up heavy metal-contaminated settings is a growing field that has benefits for the environment, sustainability, and cost. A well-liked and effective remediation method involves mixing a variety of microbes and plants. Synergistically using many plant and microbe species can enhance remediation capacity overall and address a broader range of contaminants (Arantza et al., 2022).

3.1. Synergistic Effect of Microbial Biotransformation and Phytoextraction

A synergistic effect can be achieved by combining microbial biotransformation and phytoextraction. By complementing the capabilities of these two techniques, the effectiveness of metal bioremediation can be enhanced. The process involves the selection of hyperaccumulating plants in combination with metal resistant microorganisms in the rhizosphere. The metal-resistant microorganisms mediate the mobilization of the heavy metals in the rhizosphere, facilitating the uptake of the heavy metals by the plants. This leads to enhanced uptake of metals by the plants (Lebeau et al., 2011). However, selection of appropriate and compatible plants and microbial species as well as the optimization for optimal efficacy is very important. To combine both techniques, we first need to optimize metal-resistant bacteria living in the rhizosphere of hyper accumulator plants. Metals can be oxidized, reduced, and solubilized by these bacteria, increasing the metals' bioavailability in the rhizosphere. The rhizosphere is home to bacterial activity that alter soil properties and create a microenvironment that is favourable to the bacteria as well as the plants. Enhanced metal uptake environment is also influenced by pH, organic matter content, and nutrient availability changes. Metal release from soil matrices is also facilitated by bacterially generated organic acids and chelating agents. The transformed or bioavailable metals can be efficiently absorbed by hyperaccumulator plants through their roots. The xylem play role in transporting metals that are taken up by plant roots to tissues that are above ground. The plant's ability to remove metals is maintained or improved by metal-resistant microorganisms in the rhizosphere. Metal concentrations are decreased overall when harvested plants are removed from the site (Syranidou et al., 2016).

After correct selection of the plants, microorganisms that are resistant to metals and have active mechanisms of oxidizing / reducing the metals are selected. We can also use indigenous microorganisms which are already acclimated to the site or can go for bioaugmentation which is the addition of specific strains at the site. The rhizosphere can be inoculated with metal-resistant bacteria by techniques such as root dipping, soil amendment, or seed coating. However, inoculation methods must be optimized to make sure that the added bacteria are effectively colonized and established (Syranidou et al., 2016).

For example, Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with plant roots. We can combine it with hyper accumulator plants. In this synergistic approach, AMF enhances nutrient uptake by plants as well as improve metal uptake by sunflower shoots by increasing the mobilization of the metals in the rhizosphere. Moreover, it was also reported that AMF protects the roots of the plants whereas promotes metal uptake by the shoots. As a result of this, there occur enhanced metal uptake by sunflowers and improved nutrient cycling. However, effectiveness of this combination may vary depending on soil conditions and AMF species compatibility (Zhang et al., 2018).

Metal-resistant bacteria, such as *Cupriavidus metallidurans* can be combined with Willow trees, which are frequently employed in the phytoextraction of metals, such as nickel and copper. In this combination, metal-resistant bacteria improve metal solubilization and availability while willow trees accumulate metals. As a result, there will be increased copper and nickel removal from the soil, potential for improved soil quality. However, there may occur phytotoxicity in the presence of high metal concentrations, which can cause slow growth of willows (Manzoor et al., 2019).

3.2. Synergistic Effect of Microbial Biotransformation and Phytovolatilization

Microbial transformation and phytovolatilization are two different methods for removing heavy metals from polluted environments. The two techniques can have effective results when combined, speeding up the remediation process. Microbial transformation mostly includes oxidation / reduction of heavy metals, whereas phytovolatilization is a process in which plants absorb heavy metals, translocate them, and then release the volatile metals into the atmosphere (Ma et al., 2016).

Through microbial transformation, heavy metals get converted into more soluble and bioavailable forms. The mechanisms during transformation may involve oxidation, reduction or methylation. This helps enhance bioavailability of metals for plants which take these solubilized forms of heavy metals from soil through roots and transport it to upper parts of plants. Moreover, the microbial transformation also reduces the toxicity of the heavy metals. These metals are then volatilized into the environment through leaves by the process of phytovolatilization.

Mutualistic interactions, in which bacteria facilitate the uptake or transformation of metals and plants supply organic molecules to their microbial partners, can be fostered by symbiotic associations. Addition of specific microbial strains with improved metal transformation capacities can increase the effectiveness of metal removal overall (Sharma et al., 2023). For increased volatilization process, genetically modified plants with enhanced metal absorption capacities or altered metabolic pathways can also be considered.

The combination of phytovolatilization and microbial transformation provides a clear benefit in transforming pollutants into less harmful forms. But a thorough assessment of the environment is required since arguments may arise regarding volatile chemical emissions during phytovolatilization.

3.3. Synergistic Effect of Phytostabilization and Microbial Biosorption

It is feasible to work on the bioremediation of metals by combining Phytostabilization with biosorption, especially in circumstances when the two techniques function admirably together. Certain plants are employed in Phytostabilization to either immobilize or lessen the mobility of heavy metals in the soil. The selection of the plants for this purpose depends on their ability to grow in such polluted environments without up taking the metals in large concentrations. The plants used for such combination should also be able to promote the development of microbes involved in biosorption in the rhizosphere, the latter of which can be bioaugmented as well. Microbial biosorption involves the absorption and adsorption of heavy metals by microbes. The combination of Phytostabilization and microbial biosorption can enhance the bioremediation of heavy metals. The Phyto stabilized heavy metals can be biosorped by the microbial population in the rhizosphere in large amounts. The overall effectiveness on this synergistical approach depends on the physiochemical properties of the site as well as the climate conditions (Bingöl et al., 2017). Genetically modified plants microbes can also be considered for this synergistical approach.

CONCLUSION

Both plants and microorganisms are important for the remediation of heavy metals. Both phytoremediation and bioremediation involve different mechanisms to reduce the toxicity of the heavy metals. These plants and microbes - based mechanisms can be combined in a synergistical manner to increase the effectiveness of heavy metal remediation.

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N/A

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

CONTRIBUTION OF AUTHORS

Iqra Arshad and Hifza Iqbal contributed equally to drafting of the manuscript. Syeda Saira Iqbal and Muhammad Afzal assisted in the draft preparation. Yasir Rehman proposed the concept and provided a detailed outline, assisted in the draft preparation and edited the final draft.

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