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RHIZOSPHERE MICROBES AS BIOSORBENTS FOR HEAVY METAL REMOVAL

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ABSTRACT Intense industrial activity in the twentieth century, particularly in developing nations, has resulted in significant environmental pollution, resulting in a vast number and variety of polluted sites that pose a threat to the surrounding ecology. Metal pollution has become one of the most serious environmental problems today as a result of human activities such as metal mining and smelting, electroplating, gas exhaust, energy and fuel production, fertilizer, sewage and pesticide application, municipal waste generation, and so on (Kabata-Pendias and Pendias, 2000). Most plants are affected by an excessive buildup of heavy metals. Heavy metal ions are disproportionately absorbed by roots and translocate to shoots when present at high levels in the environment, resulting in decreased metabolism and growth. In recent years, phytoremediation has gained a lot of market acceptance. Phytoremediation technology appears to be a potential cleanup solution for a wide range of metal-contaminated locations, albeit it does have limitations, according to preliminary study. The rhizosphere is a biologically active zone of the soil around plant roots that contains soil-borne microorganisms including bacteria and fungi. Plant–microbe interactions in the rhizosphere can be advantageous to the plant, the microbes or to neither of them.

KEYWORDS : Rhizosphere, microorganisms, heavy metal pollution

INTRODUCTION

In India, the application of industrial and city effluents to land as an alternate way of treatment and disposal has become popular in recent years, with soil residence durations of thousands of years. Heavy metals, which can stay in the soil for thousands of years, pose a number of health risks to higher organisms. They're also known to stunt plant development, reduce ground cover, and harm soil microbes (McGrath *et al*, 2001). Metals are naturally occurring elements in soil, with a number of heavy metals serving as micronutrients for plants. However, since the beginning of the industrial revolution, toxic metal pollution of the biosphere has increased dramatically. Kapahi and Sachdeva (2019) stated that heavy metal contamination of soils and streams has resulted from rapid industrialization and anthropogenic activities such as uncontrolled use of agrochemicals, fossil fuel burning, and sewage sludge dumping. Heavy metals are non-biodegradable and have a long shelf life. As a result, remediation is needed to prevent heavy metal leaching or mobilization into the environment, as well as to make heavy metal extraction easier. The preservation of soil quality and remediation solutions for managing soils contaminated with trace metals, metalloids, or organic contaminants are gaining in popularity. Heavy metals are accumulated in soils as a result of air input, mineral fertilizer or compost use, and sewage sludge discharge.

Contamination of water and soil with heavy metals is a major environmental and human health concern. Furthermore, high metal concentrations in contaminated soils reduce soil microbial activity and fertility, resulting in yield losses (McGrath *et al.,* 1995). At reality, heavy metal stress can make nodulation and nitrogenase activity very sensitive, yet heavy metal tolerant rhizobial strains have been found in contaminated areas that are capable of symbiotic nitrogen fixation. The legume–rhizobia symbiosis is well known for its ability to detoxify heavy metals and enhance the condition of contaminated soils (Checcucci *et al.,* 2017). From heavy metal polluted soils, the most typically documented fungi are *Ascomycota* and *Basidiomycota* (Narendrula-Kotha and Nkongolo, 2017).

Many kinds of microorganisms that inhabit this zone are not cultivable in the laboratory, which is one of the biggest challenges that plant biologists and microbiologists have when researching these interactions. Recent advances in molecular biology tools have offered some information on the microbial diversity of the rhizosphere.

A=Amoeba consuming bacteria; BL=Energy limited bacteria; BU=Non-energy limited bacteria; RC=Root derived carbon; SR=Sloughed root hair cells; F=Fungal hyphae; N=Nematode worm
Source:https://en.wikipedia.org/wiki/Rhizosphere

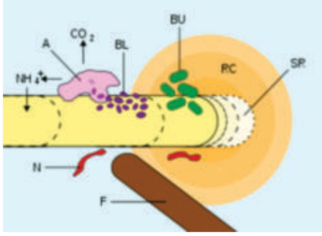


Fig. 1a illustration of the rhizosphere

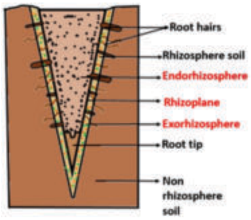


Fig 1b

Source: https://biologyreader.com/rhizosphere.html

Root exudation is a critical step for C transfer into the soil in the rhizosphere (Fig.1and 2), regulating the role of soil microbial communities in organic matter decomposition and nutrient cycling (Ostle, 2003; Butler, 2003). Although this connection is complex, root exudates have been proven to boost the bulk and activity of soil microorganisms and animals present in the rhizosphere. Soil microbes rely on plant carbon and, in turn, decompose soil organic matter to deliver nitrogen (N), phosphorus (P), and other minerals to plants. Plants in the rhizosphere appear to choose for taxonomic and functional groups, according to available reports. By contributing carbon and energy to soil organisms, root development and turnover have a direct impact on biogeochemical cycling. Plant-microbe interactions are also critical in C sequestration, but we don't have a good grasp of how rhizodeposition, root turnover, and microbial activity interact. Uncertainty in such estimates hinders ecosystems from properly quantifying net primary productivity and belowground carbon allocation (Grayston *et. al*. 1998 & 2001).

Technologies for Remediation

Heavy metals cannot be eliminated biologically (no "degradation," or alteration of the element's nuclear structure), but can only be converted from one oxidation state or organic complex to another (Alkorta and Garbisu, 2001). Heavy metal pollution in soils is more difficult to remove. Because of their high cost, low efficiency, large destruction of soil structure and fertility, and high dependence on the contaminants of concern, soil properties, site conditions, and so on, methods used for their remediation such as excavation and land fill, thermal treatment, acid leaching, and electroreclamation have not been suitable for practical applications. As a result, phytoremediation solutions for heavy metal-contaminated soils are required (Chaney *et al*., 2000).

Plant-beneficial rhizosphere microbe interactions can boost biomass output and plant tolerance to heavy metals, making microorganisms an important component of phytoremediation technology (Glick, 2003). When heavy metal concentrations surpass the maximum allowed level, heavy metals are generally hazardous to most plants' metabolism and growth. The rhizosphere is a complex and dynamic milieu in which microorganisms develop distinct communities in conjunction with roots that have significant potential for plant growth promotion (Belimov *et al.*, 2005) and toxic chemical detoxification (de Souza *et al.*, 1999). Although many soil bacteria are heavy metal tolerant and play important roles in heavy metal mobilization and immobilization (Gadd, 1990), few studies of the rhizospheric bacteria of metal accumulating and hyperaccumulating plants and their involvement in heavy metal tolerance and uptake by the plants have been conducted (Belimov *et al.*, 2004). Plant growth-promoting rhizobacteria (PGPR), both free-living and symbiotic, can promote plant nutrition and development, plant competitiveness, and plant responses to external stress factors such as drought and osmotic stress (Dell'Amico *et al.*, 2005). Rhizobacteria increase plant biomass and nutrients by solubilizing phosphate and other mineral complexes, nitrogen fixation, the creation of siderophores for acquiring trace metals, and the release of phytohormones for improved root growth and reducing the effects of harmful species. Many research have been undertaken to assess the role of PGPR in phytoremediation effectiveness action on metal contaminated soils (Khan, 2007); these bacteria stimulate plant growth by continuously supplying nutrients and hormones through their metabolic activities.

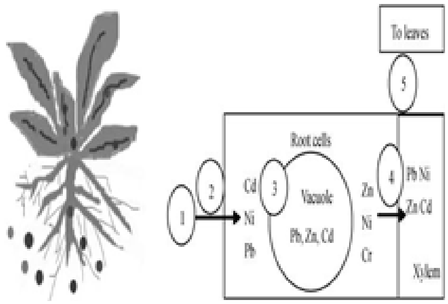
Advantages and disadvantages of phytoremediation

Metal-contaminated soils are known for being difficult to clean up. Soil excavation and either landfilling or soil washing, followed by physical or chemical separation of contaminants, are the current technologies. Soil remediation costs vary widely, depending on the chemicals of concern, soil qualities, and site conditions. Traditional engineering approaches for cleaning metal-contaminated soils can be prohibitively expensive. Because of the high expense, less expensive cleanup technologies are required. Phytoremediation is proving to be a cost-effective option. Several studies have shown that the cost of metal phytoextraction is a fraction of the cost of traditional engineering approaches. Phytoremediation also avoids major landscape disruption and preserves the ecology because it remediates the soil *in situ*. Despite these benefits, phytoextraction's applicability is limited by a number of drawbacks and limits.

This procedure is known as bioremediation, botanical bioremediation, or green remediation because plants make soil pollutants harmless. Once established, plant-based remediation technology can operate with little maintenance, but it is not always the best solution to a contaminated situation. The application of phytoremediation is limited by the meteorological and geologic parameters of the cleaning site, as well as temperature, height, soil type, and agricultural equipment accessibility. On the one hand, phytoremediation is significantly less destructive to the environment, has a high likelihood of public acceptability, eliminates heavy traffic excavation, and has the capacity to treat a wide spectrum of dangerous contaminants. However, it takes longer than other methods, contaminants can accumulate in fuelwood, contaminants gathered in leaves can be discharged back into the environment during litterfall, and vegetation establishment may be limited by environmental toxicity extremes. Many of the restrictions of phytoremediation can be summarized by the fact that the metals must be bioavailable to the plant and its root system. Following careful study of these variables, as well as the substantially cheaper cost of phytoremediation, it appears that it may be employed in much larger-scale cleanup operations than other approaches.

Phytoremediation assisted by soil rhizobacteria

The use of plants to extract, sequester, and/or detoxify pollutants through physical, chemical, and biological processes has been reported to be an effective, in situ, non-intrusive, low-cost, aesthetically pleasing, ecologically benign, and socially accepted technology for remediating polluted soils (Alkorta and Garbisu, 2001). It also aids in the prevention of landscape destruction and increases the activity and diversity of soil microorganisms in order to preserve healthy ecosystems, making it a more appealing alternative to existing heavy metal contamination approaches than traditional ways. The mechanism of metal uptake and buildup in plants is depicted in Figure 2. (Melo, *et al.*, 2011).



Source: (Reza Amirnia,2013)

Fig.2 Metal transfer in plants

Metal transfer in plants 1: A metal iron is sorbed at root surface; 2: Bioavailable metal moves across cellular membrane into root cells; 3: A fraction of the metal absorbed into roots is immobilized in the vacuole; 4: Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem); 5: Metal is translocated from the root to aerial tissues (stems and leaves).

Heavy metal phytoremediation can take numerous forms, including phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization. Phytoextraction alludes to processes in which plants are utilized to focus metals from the soil into the roots and shoots of the plant; rhizofiltration is the utilization of plant roots to retain, concentrate or encourage metals from effluents; and phytostabilization is the utilization of plants to lessen the versatility of weighty metals through assimilation and precipitation by plants, in this manner diminishing their bioavailability; phytovolatilization is the take-up and deliver into the environment of unpredictable materials like mercury-or arsenic-containing compounds. According to Pires *et al.* (2017), the bacterial population in heavy metal contaminated locations is dominated by *Firmicutes, Proteobacteria*, and *Actinobacteria,* with *Bacillus, Pseudomonas*, and *Arthrobacter* as the most common taxa. Rhizobia are significant plant growth-promoting (PGP) bacteria that can be found in the rhizosphere.

Plants suitable for phytoremediation

The ideal plant for phytoextraction should be able to grow quickly, produce a lot of biomass, and tolerate and accumulate high metal concentrations in the shoots. The Brassicaceae family is home to the majority of well-known heavy metal accumulators (Kumar *et al.*, 1995). Although hyperaccumulator plants have a high metal accumulating capability, most of them grow slowly and generate only a little quantity of biomass when the available metal concentration in the polluted soil is very high. Use species like Indian mustard, sunflower, sun hemp, and others that have a lesser metal accumulating capacity but faster growth rates as an alternative. The usage of rhizobacteria in conjunction with plants is expected to result in high phytoremediation efficacy (Abou-Shanab *et al.,* 2003). As a result, the ability of rhizobacteria to increase phytoremediation of soil heavy metal pollution, as well as the exact mechanism by which they do so, has recently gained some attention (Whiting *et al.,* 2001). Burd *et al* (1998) found that adding K to a nickel-contaminated soil enhanced both the number of Indian mustard seeds that germinated and the achievable plant growth by 50 % to 100 %. A linked plant growth-promoting rhizobacteria with phytoremediation of Se and Hg inbuilt wetlands was examined in a recent study. Rhizobacteria in wetland plant tissues increased the highest levels of Se and Hg accumulation.

Rhizospheric Interactions

The interactions between soil, heavy metals, microbes, and plants determine the potential for phytoremediation. As shown graphically in Fig.3, a range of factors influence these complex relationships, including plant and rhizobacteria traits and activity, climatic circumstances, soil parameters, and so on.

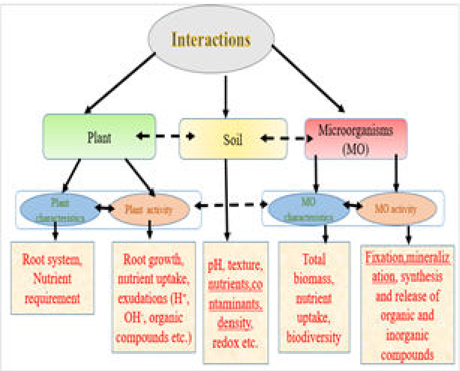


Fig. 3 Plant – soil –microbial interactions in the rhizosphere

A. Plant-bacteria interactions

Microbes have the ability to lower the activity of various metals or convert active forms of metals to inactive forms. The microorganisms utilized in bioremediation are chosen based on the availability of energy and carbon sources, as well as environmental factors such as temperature, oxygen, moisture, and the presence of harmful pollutants. Plant roots interact with a variety of microorganisms, and these interactions are important factors of the level of phytoremediation. Both the micro-partner (plant-associated bacteria) and the host plant can have an impact on the functioning of associative plant-bacterial symbiosis in heavy-metal-polluted soil. Soil microbes perform important roles in plant nutrient recycling, soil structure preservation, toxic chemical detoxification, insect control, and plant growth. Plant roots can provide root exudate, as well as increase ion solubility.

Although there is growing evidence that rhizosphere bacteria play a role in metal extraction, the mechanisms of these plant-microbe interactions are yet unknown. Due to the active mobilization of roots and microorganisms, the rhizosphere of heavy metal accumulating plants provides a niche for adapted metal resistant microbes, and heavy metal mobility is higher in the rhizosphere of metal accumulators than in bulk soil (Idris *et al*, 2004; Lodewyckx *et al*, 2002 and Lasat *et al*, 1996).

Recent advancements in molecular techniques and genomics have created an intriguing opportunity to reshape the connection between plants and the bacteria that live in their rhizosphere. Microbial ecologists have developed a number of approaches that allow them to directly link a specific metabolic activity to phylogenetically identifiable units in natural habitats (Gray and Head, 2001; Dahllof, 2002 and Wagner, 2004). Due to rapid advances in genomics, complete genome sequences of hundreds of microorganisms, predominantly bacteria, are now available (Delong, 2002). Several ecological studies, other than those involving plant–microbe interactions, have effectively used a combination of novel molecular methods and genomics to link microbial phylogeny with function.

B. Heavy metal-bacteria interactions

Rhizobacteria have been demonstrated to have numerous characteristics that can affect heavy metal bioavailability (Lasat, 2002) by releasing chelating compounds, acidifying the microenvironment, and altering redox potential shifts. Adsorption, precipitation, ion exchange, and chelation / complexation are four distinct mechanisms that interact with metals and microbes.

C. Plant-bacteria-soil interactions

Soil factors, which affect contaminant bioavailability, root exudate composition, and nutrient levels, influence the specificity of the plant-bacteria interaction. Furthermore, the metabolic requirements for heavy metals remediation may define the type of plant-bacteria interaction, such as selective or nonspecific.

Effect of rhizobacteria on phytoremediation

Plant growth promoting rhizobacteria (PGPR) are microorganisms that live in the rhizosphere and are tightly connected with roots . Plant growth-promoting rhizobacteria are a varied group of free-living soil bacteria that can help host plants grow and develop in heavy metal-contaminated soils by reducing heavy metal toxicity (Belimov *et al.*, 2005). In the presence of heavy metals such as nickel, lead, and zinc, some plant growth-promoting bacteria can greatly improve plant growth (Burd *et al*, 2000), helping plants to develop longer roots and become more established during the early stages of growth (Glick *et al*, 1998). Once the seedling is established, the bacterium can assist the plant in obtaining enough iron for optimal growth. The buildup of potentially hazardous trace elements into plant tissues is aided by bacteria in the rhizosphere. The siderophores are another key metabolite generated by plant growth-promoting rhizobacteria that helps to reduce heavy metal toxicity by increasing the plant's iron supply (Ma *et al*, 2009). Heavy metals in soils may enhance bacterial siderophores production.

Rhizospheric microorganism

In the context of plant growth, the microbial community structure of the rhizosphere population is critical. This is due to the fact that microbial populations frequently form some sort of cooperative relationship with the host plant system. Heavy metal contamination, for example, could result in the emergence of heavy-metal resistant rhizobacteria in the soil of industrialized areas (Aleem *et al.*, 2003). *Thlaspi caerulescens* and *Alyssum bertolonii* (Mengoni *et al.,* 2001) or *Alyssum murale* (Abou-Shanab *et al.*, 2003a) grown in soil contaminated with Zn and Ni or Ni, respectively, demonstrated that a substantial proportion of metal resistant bacteria persist in the rhizosphere. In a study conducted by Lin *et al*.(2121) *Trifolium repens L*. was able to successfully accumulate both anions and cations, and its planting compensated for the loss of soil enzyme activity induced by heavy metal. Ecologically beneficial rhizosphere and endosphere microorganisms were encouraged, and the micro-ecological properties of the rhizosphere were found to be strongly connected to phytoremediation.

Bioavailability of toxic heavy metals

By altering heavy metal speciation in the rhizosphere, soil rhizobacteria can also have a direct impact on metal solubility. In comparison to bulk soil, Cu, Zn, and Pb speciations changed significantly in the rhizosphere of Arbuscular Mycorrhiza (AM) infected and non-infected maize, indicating that mycorrhiza plays a role in metal speciation in the rhizosphere and has an impact on increasing host plant tolerance to excessive heavy metals in soil. The most significant change was in exchangeable Cu, which rose by 26% and 43% in non-infected and AM-infected rhizospheres, respectively, compared to bulk soil. Other speciations in the rhizosphere of AM and non-AM treatments were stable, with the exception of organic bound Cu in AM. The finding that copper and zinc levels were much lower in mycorrhiza-infected plants' roots and shoots than in non-infected plants could indicate that mycorrhiza effectively limited excessive copper and zinc absorption into the host plants (Huang et al., 2005).

CONCLUSIONS

Certain bacteria may be useful in the development of phytoremediation technologies in the future. Heavy metals can be removed from polluted soil by either improving the ability of plants to accumulate metals or increasing the amount of plant biomass. It may be possible to treat plants with plant growth-promoting rhizobacteria in extensively contaminated soil where the metal content exceeds the limit of plant tolerance, boosting plant biomass and metal bioavailability. However, much more research is required in areas where there is a dearth of understanding or information, such as:

1. There has been little research on the effects of microorganisms on metal accumulation in the rhizosphere of hyperaccumulator plants. In the rhizosphere, it's also difficult to pinpoint precise characteristics of microbial-plant and microorganism-soil interactions.
2. More research is needed to quantify the influence of rhizospheric processes generated by rhizobacteria on heavy metal phytoavailability.
3. Only a handful of research has been done to look into heavy metal speciation changes in the rhizosphere to see if these changes influenced heavy metal accumulation and distribution.
4. Rhizobacteria may play an essential role in plant metal uptake by sequestering metals from soil solutions. The uptake of cadmium by bacteria in plants is still a riddle.

It is necessary to increase research efforts into this new and environmentally benign "green" technology. Identifying remediating plants that are adapted to the local climate and soil conditions should be the focus of research. Because phytoremediation is a time-consuming process, biotechnological and traditional hybridization techniques should be used to create more efficient metal hyperaccumulator plant species with improved pollutant tolerance, root and shoot biomass, root architecture and morphology, pollutant uptake properties, and organic pollutant degradation capabilities.

In a nutshell, although the utilization of rhizobacteria in conjunction with plants may provide high effectiveness for phytoremediation, the microbial ecology in the rhizosphere is still unknown.

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