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PLANT-MICROBE INTERACTION BY MEANS OF RHIZOREMEDIATION

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ABSTRACT

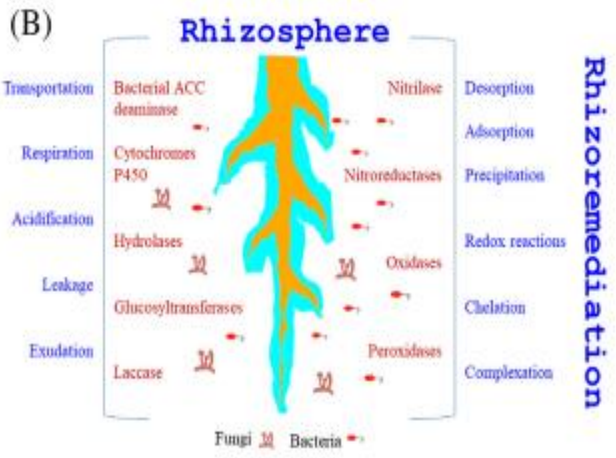
The use of bioremediation techniques came up as a result of the hunt for substitutes for excavation and incineration to clear polluted environments. Here we are going to focus on two approaches, phytoremediation and rhizoremediation resulting in beneficial plant- microbe interaction. In phytoremediation we use plants to extract, sequester, or detoxify pollutants. It is environmental friendly, visually attractive, and the structure of the soil is highly maintained. Pollutants which can be the target for phytoremediation are of two types: elemental pollutants and organic pollutants. During phytoremediation, plant enzymes establish the degradation of pollutants; whereas, the microbial population (indigenous) performs the degradation during natural attenuation or bioaugmentation. In many cases, contribution of microbes present in the rhizosphere of plants (used during phytoremediation or of plants which are emerging as natural vegetation on a contaminated site) is found to the degradation of pollutants, which is termed as rhizoremediation. Combination of bioaugmentation and phytoremediation results in rhizoremediation, which can be able to solve the problems related to both of the individual techniques. Consortium of bacteria, in which each bacterium with different parts of the catabolic degradation route, involved in the degradation of a certain pollutant is more efficient than single strain with complete pathway. Microscopy, microscopy combined with the use of marked strains, or strains equipped with reporter genes can be used to study the patterns of microbial plant root colonization. Studies on the selection of suitable rhizosphere bacteria or communities which can sustain and proliferate on the root system of a plant and thus suitable for rhizoremediation or phytoremediation is the future thrust related to this field.

INTRODUCTION:

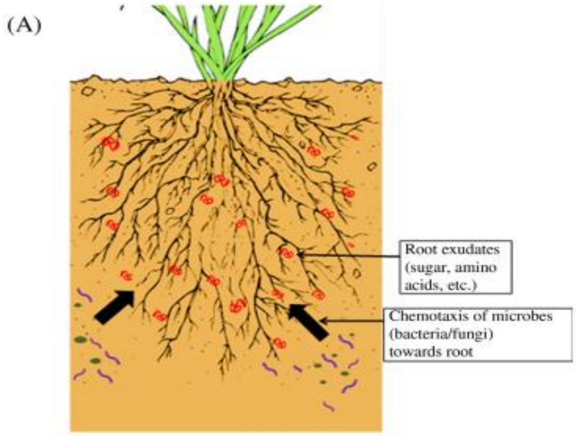
The steadily rising pollution levels have given rise to significant environmental worries. Rapid technological development, industrialisation, widespread use of pesticides and fertilisers, etc., have all added to the pollution load. Hydrocarbons, heavy metals, dioxins, chlorinated chemicals, and other contaminants are some of these pollutants (Zhuang et al., 2007). This issue has been addressed using a variety of conventional physicochemical strategies, such as composting and land formation. All of these are intrusive, time-consuming, produce more harmful waste, and even cause the production of greenhouse gases. Biological remediation of these contaminants is thus a practical solution. Rhizoremediation is one such bio restorative method that has gained attention. Numerous microbes have been the subject of extensive research over the past few years to determine their potential as bio therapeutic agents. One such method uses rhizobial microflora, primarily rhizobacteria, and is called rhizoremediation. It is a method that is popular right now, is eco-friendly, even affordable, and has promising future potential. Rhizoremediation is a method of in-situ cleanup that uses microorganisms for the biodegradation of different toxins in the root zone, including organic pollutants. Microorganisms can flourish at the expense of plant roots thanks to the rich niche provided by plant roots. Exudates act as biocatalysts to eliminate the contaminants, and bacteria in turn do the same. The damaging contaminants such as pesticides, herbicides, and polycyclic aromatic hydrocarbons (PAHs) are changed into substances that can be broken down, whereas heavier metals like zinc, copper, lead, and tin,cadmium and other elements undergo oxidation state change or organic complex transformations. Several mechanisms are used, including the production of bio-surfactants, which are amphiphilic molecules that form spherical or lamellar micelles and solubilize hydrophobic contaminants in their cores and enhance their bacterial degradation to simple harmless compounds; the production of metal chelating siderophores for the acquisition of heavy metals; increased humification; the production of biofilm and acid; etc. Temperature, pH, soil conditions, the type of pollution, local microflora, and other chemical, physical, and biological elements all have an impact on the process. Interactions between plants and bacteria are crucial to the process and are characterised by the following: bacterial colonisation of the roots, maintenance of the catabolic activity, and impact of the external environment on the relationship. Rhizoremediation has a bright future as a technology, especially with technological developments and greater understanding of sequencing methods.

RHIZOREMEDIATION:

A nutrient-rich region or zone that surrounds a plant's roots is called the rhizosphere. It is abundant in microorganisms that are mostly known as plant growth-promoting organisms, which either directly or indirectly promote plant development. These rhizobial microbes exhibit extensive interactions with plants and are also involved in the removal of harmful pollutants such as pesticides like atrazine, heavy metals like cadmium and mercury, poly-aromatic



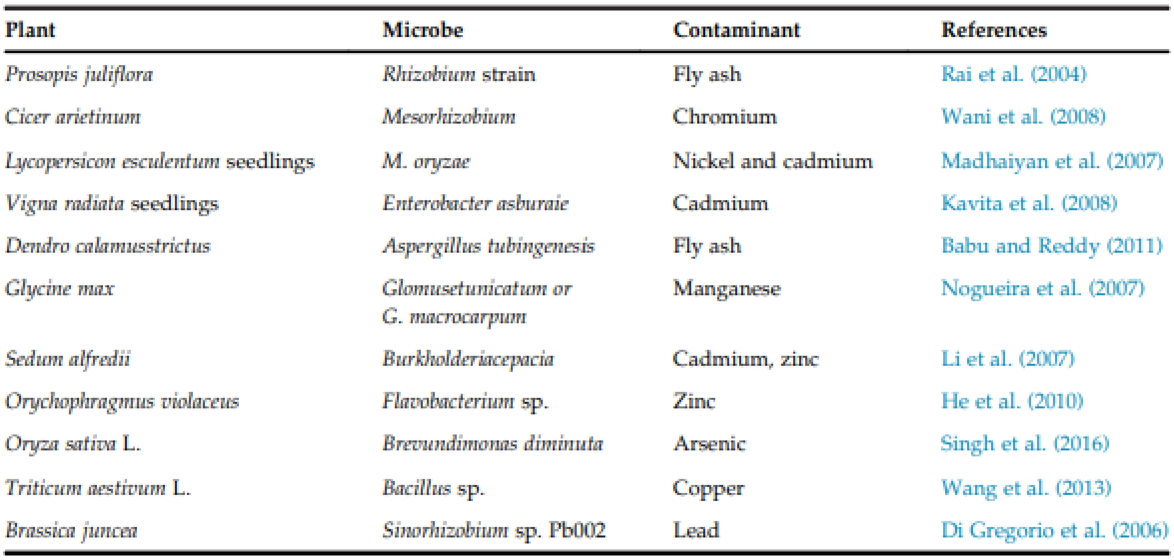
hydrocarbons, polychlorinated biphenyls, and halogenated compounds from the soil (Zhuang et al., 2007).



Rhizoremediation is the process of removing the dangerous substances and wastes by means of rhizomicroflora. A particular branch of phytoremediation is rhizoremediation. Its economical nature, convenience with few or no adverse effects have made it a developing technology. It is a clean and green phenomenon (Corgie et al., 2003). Despite the fact that it can be time-consuming, final results are outstanding as contaminant is either entirely destroyed or transformed into a safe form. It works for both large and small locations with minimal to moderate levels of pollution. Ex-situ or non-rhizospheric microbes can also be used to assist clean up polluted places, although they have some limitations compared to rhizospheric microflora. Lack of nutrients in the soil, soil surface characteristics, toxicity or reduced bioavailability of pollutants, competition with the native microflora, failure to express necessary catabolic activities, etc. are some of the drawbacks (Amora-Lacazano et al., 2010). Additionally, there are more microorganisms in the rhizosphere than in the overall soil. The plant benefits from increased pollution breakdown in the rhizosphere, which leads to better plant growth on contaminated soil. There are two parameters that must be met for a rhizoremedial technique to be effective. In order to increase the catalytic potential, the microorganisms must first be able to multiply in the root system. Second, active catabolic routes for pollution removal are required (Segura et al., 2009).

COLONIZATION IN ROOTS: Rhizospheric microbial communities can either come from bacterized seeds or be recruited from the soil reservoir as plants grow on contaminated soil. Chemotactic movement draws microorganisms to the root exudates (the secretion of plant roots), and they then disperse into the soil as the roots emerge and grow. The process of root colonisation and identification of genes that are activated during rhizosphere colonisation are being studied using in-vivo expression technology (IVET), transcriptomics, and mutants defective in motility. Rhizosphere colonization depends not only on the interactions between the plants and the microbes but also on the interactions with other rhizospheric microorganisms and the environment.

ROLE OF PLANTS IN RHIZOREMEDIATION: Plants play a fundamental role in the process because their roots can be thought of as a substitute for tilling the soil because they can spread the root-associated microorganisms through the soil and penetrate layers that are typically inaccessible to bacteria or other microbes. Plant roots can also incorporate nutrients, bring oxygen, and create better redox conditions, all of which help to stimulate and activate the rhizosphere microflora.The majority of plants also coexist harmoniously with mycorrhiza, which serve as a web-like extension of the root system and increase the absorptive area of plants. In addition, plants provide an efficient clean-up technology compared to conventional techniques: They pump up pollutants by photosynthesis with water stream making it much cheaper. This in-situ economical technology produces little to no waste.



Plant	Microbe	Contaminant	References
Prosopis juliflora	*Rhizobium* strain	Fly ash	Rai et al. (2004)
Cicer arietinum	*Mesorhizobium*	Chromium	Wani et al. (2008)
Lycopersicon esculentum seedlings	*M. oryzae*	Nickel and cadmium	Madhaiyan et al. (2007)
Vigna radiata seedlings	*Enterobacter asburaie*	Cadmium	Kavita et al. (2008)
Dendro calamusstrictus	*Aspergillus tubingenesis*	Fly ash	Babu and Reddy (2011)
Glycine max	*Glomusetunicatum or G. macrocarpum*	Manganese	Nogueira et al. (2007)
Sedum alfredii	*Burkholderiacepacia*	Cadmium, zinc	Li et al. (2007)
Orychophragmus violaceus	*Flavobacterium* sp.	Zinc	He et al. (2010)
Oryza sativa L.	*Brevundimonas diminuta*	Arsenic	Singh et al. (2016)
Triticum aestivum L.	*Bacillus* sp.	Copper	Wang et al. (2013)
Brassica juncea	*Sinorhizobium* sp. Pb002	Lead	Di Gregorio et al. (2006)

GENE EXPRESSION IN RHIZOREMEDIATION: A vital component of the rhizoremediation strategy is choosing the right plant to promote contaminant degradation, but the choice of the bio-degradative microflora is of paramount importance. Sugars, organic acids, fatty acids, secondary metabolites, nucleotides, and other substances released by plant roots are referred to as root-exudates. These substances are crucial in forming and determining the microbial population of the rhizosphere. These aid in the selection of the microorganisms that react quickly to the exudate smorgasbord. These secretions either directly or indirectly aid in the identification and control of the relevant bio-degradative microorganisms.

COMMUNICATION IN RHIZOREMEDIATION: Numerous signals are transmitted and received by both plants and microorganisms, and these signals are necessary for quorum sensing, mycorrhization, the recruitment of catalytic potential, and the detection of microbes. This relationship is significantly shaped by a number of biotic and abiotic parameters, such as the plant's growth stage, soil type, pollutant type, season, temperature, and the density of other microorganisms etc. For e.g., Catabolic alkane monooxygenase genes were found to be more common in rhizospheric microbial communities than in the overall hydrocarbon-contaminated soil, according to research by Siciliano et al. (Wu et al., 2006).

ENGINEERING IN RHIZOREMEDIATION: Rhizoremediation can be improved by molecularly manipulating plants and microorganisms. Engineered soil, in addition to engineered plants and microorganisms, aids in rhizoremediation by improving the soil, which promotes plant and microbial growth.

Engineered plants will have improved capacity for degradation and contaminant agglomeration. Similar to this, modified microorganisms are better able to degrade a larger variety of pollutants. Nutrient and waste amendment, such as that from plants, animals, and industry, is a part of soil engineering. Engineering can therefore enhance rhizoremediation in three different ways:

Depending on the characteristics of the soil, nutrient amendments (N, P, K, Ca, Zn, and Fe, etc.) can improve soil health. According to Kuzyakov et al. (2009) and Yu et al. (2013), adding biochar might also boost the soil's moisture and carbon content, which would improve the microbial community and promote plant growth. Fly ash, animal manure, and even plant waste can all help to improve the health of the soil. It has long been practised to improve the health of deteriorated soils through soil amelioration. Fly ash and press mud applications have been demonstrated to be effective at improving soil (Inam, 2007; Singh and Pandey, 2013). Compost, animal manure, and earthworms when added to press mud increased the population of nutrients and microorganisms, turning it into nutrient-rich soil (Prakash and Karmegam, 2010). Additionally, the addition of various nutrients can both increase and decrease the intake of heavy metals contaminants from soil (Follett et al., 1981).

Microbial engineering may affect the rhizosphere in two different ways. This implies enhanced nutritional availability as well as enhanced microbial breakdown of pollutants (Pii et al., 2015; Drogue et al., 2012). The rhizosphere's microbial fauna has a limited capacity to break down pollutants. However, the usage of genetically altered bacteria could improve microbial capacity. Through the inclusion of specific genes, genetically altered bacteria are better able to break down pollutants (Lorito et al., 1998). *Mesorhizobium haukii* was given the phytochelatin synthase gene that was obtained from *A. thaliana* (Sriprang et al., 2003), which increased the phytochelatin activity in *M. haukii*. Similar to this, the siderophore receptor was transferred into *Pseudomonas fluorescens* to improve its suitability for the rhizospheric environment (Raaijmakers et al., 1995).

Rhizoremediation involves increased pollutant uptake and degradation, In other words, speeding up the phytoremediation. The development of plants is possible through either traditional gene transfer or molecular gene breeding. Breeding in willow trees, *Salix* sp. produced *S. alba* and *S. viminalis*. It was observed that these two Salix species has improved Cd accumulating capacity along with Zn (Janssen et al., 2015). The mining of metals like Ni, Cd, Se, Co, and Cu has been proposed and implemented using hyperaccumulating plants (van der Ent et al., 2013). According to Johnson et al. (2011), plants have been genetically modified to better accumulate and stabilise pollutants. The use of genetically modified plants to remove toxins has been extensively studied (Eapen et al., 2007; Raskin, 1996). P450 cytochromes are used in plants to enhance their detoxification process. Plants that had cytochrome P450 2E1 added to them had a higher rate of halogenated hydrocarbon metabolism (Doty et al., 2000).

IMPROVEMENT IN RHIZOREMEDIATION: An intriguing method to increase removal efficiency is the selection of bacteria that can create biosurfactants in the rhizosphere of the plants. In this respect, Kuiper et al. identified bacteria producing biosurfactants in a PAHs-contaminated environment that aid in the solubilization of PAHs and subsequently the biodegradation of PAHs by microbes. A lot of biodegradative bacteria show positive chemotaxis towards the contaminants, which makes this trait interesting as well. Therefore, the combined effects of biosurfactant and chemotaxis can promote bacterial growth and microbial spread in contaminated soils, allowing for the cleaning of larger areas. The reduction of pollutant concentration in the vicinity of the roots caused by microbial degradation of contaminants in the rhizosphere benefits the plant, which can develop more successfully than in contaminated areas. It has been suggested that plants can choose particular genotypes to be present in their roots due to this reciprocal benefit. According to research done by Siciliano et al., the alkane monooxygenase gene was more common in rhizosphere and endophytic microbial communities than it was in bulk soil contaminated with hydrocarbons. The predominance of the xylene monoxygenase or naphthalene dioxygenase genes, on the other hand, was shown to be more prevalent in bulk soil microbial communities than those close or on the plant. This implies that if plants are having an impact on the rhizosphere, the pollutant is what determines the effect. Some researchers came to the similar conclusion that plant type affected the effect. This has given rise to the theory that choosing the ideal plant bacteria pair for each situation is connected to how effective rhizoremediation strategies are. According to an HPLC analysis conducted for a case study, rhizospheric *Pseudomonas* sp. of the *Calotropis* plant is an effective degrader of naphthalene (78.44%) and anthracene (63.53%). Thus, it can be inferred that *Pseudomonas* species with strong PGP characteristics, PAH degradation, and biocontrol activities against phytopathogenic fungi are found in the rhizosphere of *Calotropis* species. To prove their efficiency in real-world settings, more research is now being conducted. Researchers found that the rhizosphere bacteria *P. alcaligenes*, *P. stutzeri*, and *P. putida* chemotactically reacted to naphthalene, phenanthrene, and root exudates. Fascinatingly, anthracene and pyrene turned the microorganisms off. Competent bacteria may be drawn to the root zone, which may accelerate PAHs' breakdown in the rhizosphere and boost bioavailability. Enzyme induction may not happen during rhizodegradation of PAHs, according to research on *P. putida* phenanthrene-degrading activity after exposure to root extracts and exudates. Recombinant, root-colonizing bacteria (like *P. fluorescens*) that express degradative enzymes like orthomonooxygenase for the degradation of toluene and other organic pollutants were used in genetically modified plant microbial systems to improve rhizoremediation techniques. Numerous researchers have studied the plant growth promoting (PGP) activity of the rhizosphere of various plants, but there is no information about the rhizosphere community of a particular plant, its molecular characterization, or its use in sustainable agriculture, biofertilization, or ecorestoration. Rhizoremediation can be utilised to restore contaminated environments if the proper plant cultivar and rhizobacteria are selected, or if effective rhizobacterial strains are inoculated onto plant seeds. For efficient growth promotion and rhizoremediation, bacteria living in the rhizosphere of a suitable plant may be employed as a "bacterial injection system" in soils.

LIMITING FACTORS FOR SUCCESSFUL RHIZOREMEDIATION: A variety of physical, chemical, and biological variables can affect rhizoremediation. Temperature, pH, soil characteristics, microbial diversity, aeration, organic matter content, rate of exudation, age of the plant, nutrient availability, and the presence of pollutants may be some of these(Kala S 2014). Some more factors affecting rhizoremediation are:

1. Plant root exudates are among the most crucial elements in rhizoremediation because they draw a specific microbial community. The age of the plant impacts the type and quality of the exudates, which in turn affects the release of exudates from roots.
2. The pollutants' concentration and solubility.
3. A supply of

carbon and nitrogen for the microorganisms to use as energy.
4. The availability of nutrients, which is influenced by the degree of oxidation of the pollutants' constituent components.

5. The characteristics of the soil, such as its pH, temperature, humidity, and texture.

6. The number of microbes at the polluted site.
7. The kind and quantity of pollutants.

CONCLUSION AND FUTURE PROSPECTS: Contaminant cleanup via rhizoremediation is successful, economical, and environmentally beneficial. The rate at which pollutants degrade is accelerated by the chemical interactions between microorganisms and plant roots. Rhizoremediation has a faster rate of pollutant degradation than either phytoremediation or microorganisms by themselves. The rhizosphere is home to a wide variety of bacteria that can break down various pollutants. Through molecular biology, it is possible to modify microbes and plants to better accumulate or breakdown pollutants. Contaminants can only a certain extent be broken down by plants and microorganisms on their own. In order to comprehend the interaction between soil, roots, and microbes for the remediation of metal(loid)s and organic contaminants, rhizoremediation is a prospective research field. The interactions between soil, roots, and microbes in the remediation process should be thoroughly studied in order to better understand the mechanism of rhizoremediation of pollutants. These interactions include: (1) soil structure and its physicochemical conditions; (2) root structure and its morphology; and (3) microbial genetic diversity and the molecular mechanisms regarding pollutant remediation. With these factors in mind, there is potential to significantly improve the rhizoremediation process' ability to remove toxins. Rhizoremediation on a broad scale can be encouraged further by the use of microorganisms and soil improvers.