International Journal of Frontiers in
Biology and Pharmacy Research
Journal homepage: https://frontiersrj.com/journals/ijfbpr/
ISSN: 2783-0454 (Online)

(REVIEW ARTICLE)

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Rhizo-nanoremediation: Overview on practices: advances and perspective for the treatment of contaminants

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International Journal of Frontiers in Biology and Pharmacy Research, 2022, 02(02), 016–028

Publication history: Received on 01 April 2022; revised on 10 May 2022; accepted on 12 May 2022

Article DOI: https://doi.org/10.53294/ijfbpr.2022.2.2.0037

Abstract

Industrial development has brought new processes, operations and advance materials for the production that generate waste and effluents. The present treatments are not effective and efficient for the treatment of contaminants to comply with the standards. Bioremediation technology has emerged out with the latest current practices and advances in the treatment viz. Rhizo-remediation, nano-rhizoremediation, bioaugmentation, metagenomics approaches as well as nano-technology involving innovative techniques and nanomaterials for application in nano-rhizosphere, nano-phytoremediation, and nano-rhizoremediation. The present paper highlights an overview of bioremediation practices and current new perspective for remediation of contaminants for environmental protection.

Keywords: Bioremediation; Nanotechnology; Pesticides; Rhizo-remediation; Bioaugmentation

1. Introduction

The effects of the industries have been evident all over the world since the Industrial Revolution in the 1700s and 1800s. As the demand for raw commodities, low-cost labour, and markets grew, the world's face altered. In their thirst for greatness, industrialized countries devastated others. Human lives were not taken into account or spared. The property was not well-maintained, and neither was the environment. Everything was taken advantage of and mistreated. Wars were waged for dominance, and some sections of the world are still striving to rise from the ashes in the twenty-first century.

Industrial development, urbanization, and organic food production have all expanded as a result of rising population needs; environmental pollutants are the main concern of environmentalists as a result of industrialization. Due to pollutants present in treated waste/effluents and/or partially treated or untreated waste discharged into the soil water ecosystem, industrialization has exacerbated the problem of environmental pollution. Bioremediation has emerged as a viable option.

Microorganisms are used in bioremediation technology to remediate contaminants utilizing low-tech and low-cost procedures. It is dependent on the existence of specific microbes as well as a mix of favorable natural circumstances (Adams *et al.* 2015). Because it leverages natural microbial activity mediated by distinct consortia of microbial strains, this increasingly inventive technique is particularly promising for treating waste chemicals and media with the ability

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to breakdown components. It could be any method that uses microbes, fungi, green plants, or their enzymes to restore the natural environment after it has been contaminated (El Amrani *et al.* 2015; Rathore *et al.* 2014).

Rhizoremediation is a type of bioremediation that uses plants to help the indigenous soil microbial community degrade pesticides (Yu *et al.* 2003; Singh *et al.* 2004; Sun *et al.* 2004; Korade and Fulekar, 2009). Heavy metals (Gaur and Adholeya, 2004) and other organic hydrocarbons (Jordahl *et al*. 1997; Nakamura *et al.* 2004; Chaudhry *et al.* 2005; Biryukova *et al.* 2007) decontamination has also been reported by rhizosphere remediation, i.e., enhanced contaminant removal in the rhizosphere zone compared to bulk soil. *Pennisetum pedicellatum*, for example, has a fibrous root that allows it to flourish in chlorpyrifos-contaminated soil while also forming microbial interactions in the rhizosphere (Dubey and Fulekar, 2011).

Nanotechnology is a new science that can be used to clean up pollutants in the environment, such as pesticides, metal contaminants, and organics. Green technologies for the production of diverse nanoparticles have emerged as a significant field of nanotechnology in recent years, generating significant interest in the chemical, electrical, and biological sciences. Treatment and remediation, sensing detection, and pollution prevention are three types of potential environmental benefits that nanotechnology might provide.

Nanotechnology aids phytoremediation productivity by allowing nanoparticles to be used to remediate soils, water contaminated with heavy metals, and natural and inorganic pollutants. Nanoparticles can be used in a blend with phytoremediation in compound-based bioremediation. This constraint could be overcome by combining nanotechnology and biotechnology: complicated natural mixes would be polluted into fewer complex mixes by nano-encapsulated chemicals, which would then be quickly corrupted by the collaborative efforts of bacteria and plants (Pillai and Kottekottil, 2016).

2. Pesticides

Pesticides are defined by the United Nations' Food and Agriculture Organization (FAO) as "any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs," or "any substance or mixture of substances intended for preventing, destroying, or controlling substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or avoiding premature fruit fall are included in this category. Also used as a pre or post-harvest treatment for crops to prevent them against deterioration during storage and transportation (International Code of Conduct on the Distribution and Use of Pesticides, 2002).

Algicides, Avicides, Bactericides, Fungicides, Herbicides, Insecticides, Miticides, Molluscicides, Nematicides, Rodenticides, Slimicides, and Virucides are some of the numerous types of pesticides. Access to, use of, and accumulation of these compounds in the soil–water ecosystem may result in bioaccumulation in living beings, vegetation, or sediments (Gani and Kazmi, 2017).

When a new pesticide is put into the soil-water ecosystem, it will follow the same path that many other pesticides have already established. These pesticides can be contaminated through physical, chemical, or biological means, bind and stay in the soil environment, and are picked up by the root zone - plant and accumulated. The most often used pesticides in agriculture and other industries were hexachlorocyclohexane, endosulfan, atrazine, lindane, and dichlorodiphenyltrichloroethane (DDT). Dichlorodiphenyltrichloroethane and hexachlorocyclohexane covered about 67 percent of all insecticides.

3. Rhizosphere Environment

Plant roots attract and accumulate certain microorganisms in the rhizosphere environment, which refers to the rhizosphere microbial diversity. Many microbial communities play an important role in plant function by regulating their physiology and development. It's critical to understand which microorganisms are present in the rhizosphere microbiome and what they're doing in order to improve plant growth and health. Despite the fact that scientific studies have highlighted the functional capacities of microbial communities in the Rhizosphere, the precise mechanisms underpinning the impact of rhizosphere microbiome assembly are still being explored.

The rhizosphere, also known as the microbial storehouse, is a soil environment surrounded by plant roots where the biological and chemical characteristics of the soil are influenced by the roots. The root system acts as an anchor for

water and nutrient intake. Plant roots emit chemicals that act as a chemical attractant for a diverse range of microbial populations (Kang *et al.* 2010). This compound's composition is determined by the physiological state and species of plants and microbes.

Table 1 Different Plant Species recruit different specific microbial communities

S. No.	Plant Species	Specific Microbes	Root exudates	References
1	*Musa paradisiaca*	*Bacillus subtilis. N11*	Fumaric acid	Zhang *et al.* 2014
2	*Arabidopsis thaliana*	*Proteobacteria*	Triterpenes	Huang *et al.* 2019
3	*Zea mays*	*Pseudomonas putida*	Benzoxazinoids	Neal *et al.* 2012
4	*Citrullus vulgaris*	*Paenibacillus polymyxa*	Malic acid and Citric acid	Ling *et al.* 2011
5	*Sequoiadendron gigantea*	*Betaproteobacteria, Gammaproteobacteria and Actinobacteria*	Organic carbons	Eilers *et al.* 2010
6	*Glycine max*	*Bradyrhizobium japonicum*	Isoflavones	Morris *et al.* 1998
7	*Daucus carota*	*Arbuscular mycorrhizal*	Glycogen	Bago *et al.* 2003
8	*Medicago sativa*	*Rhizobium meliloti*	Luteolin	Peters *et al.* 1986
9	*Alopecurus pratensis*	*Azospirillum*	Polysaccharide	Steenhoudt *et al.* 2000

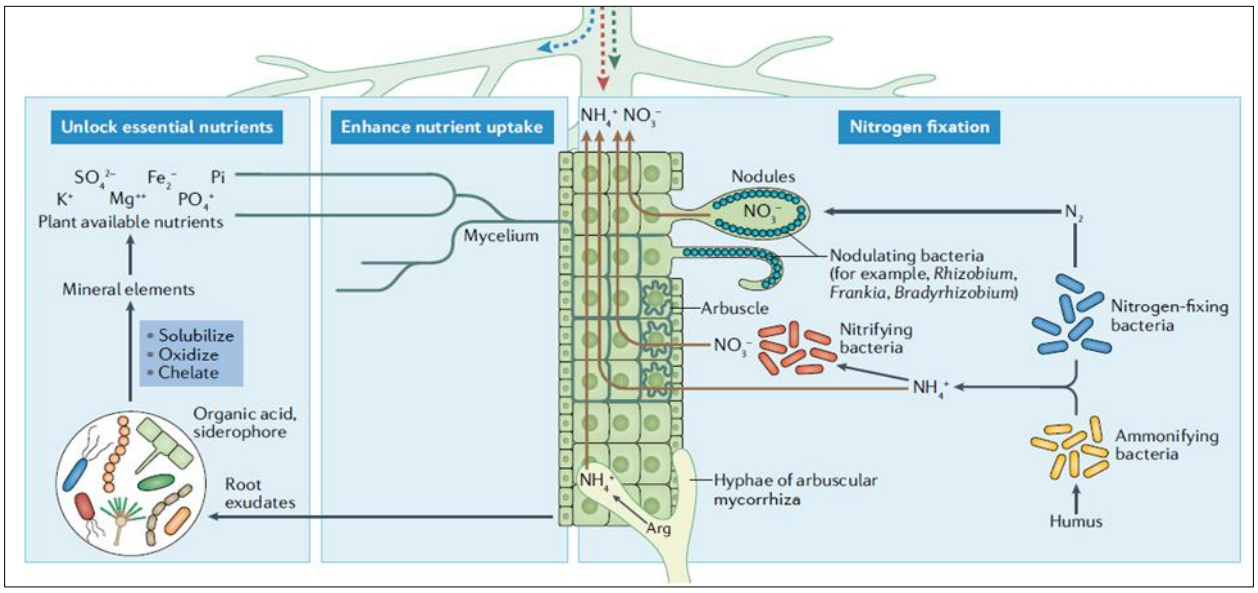

Figure 1 Rhizosphere Environment: Plant associated microbiomes (Trivedi P *et al.* 2020*)*

Rhizosphere is made up of three parts: a. Rhizosphere (soil), b. Rhizoplane, and c. Root itself. Rhizosphere is a root surface that firmly binds soil particles and is inhabited by microorganisms; a rhizosphere is a root surface that strongly binds soil particles and is colonized by microorganisms. The bacterial population in the rhizosphere is 10 to 100 times larger than in the bulk soil, making rhizosphere bioremediation beneficial for pesticide cleanup. In order for bacteria in the root zone to have a beneficial effect, they must compete with other rhizosphere microbes for nutrients released by the root. The contact between the plant and the rhizosphere is necessary for the extraction of water and nutrients from the soil, and it is beneficial to the plant and soil-borne microbes (Barea *et al.* 2005).

3.1 Mycorrhiza – Rhizosphere Bioremediation

Mycorrhiza is a mutualistic relationship between fungus and plant roots. The vesicular-arbuscular form of mycorrhizal association is the most frequent, producing fungal structures such as vesicles and arbuscules in the cortical region of the roots (Korade and Fulekar, 2009). Around 80% of terrestrial plant species have a symbiotic relationship with vesicular-arbuscular mycorrhiza (VAM). By modulating host root colonization, VAM increases alterations in the host root exudation pattern. Pesticide contamination, as well as a lack of beneficial soil microbiota, are common in highly polluted areas. Mycorrhiza – Rhizosphere bioremediation is a well-known method for removing pesticide pollutants (hazardous organic and inorganic compounds) from soil and water ecosystems.

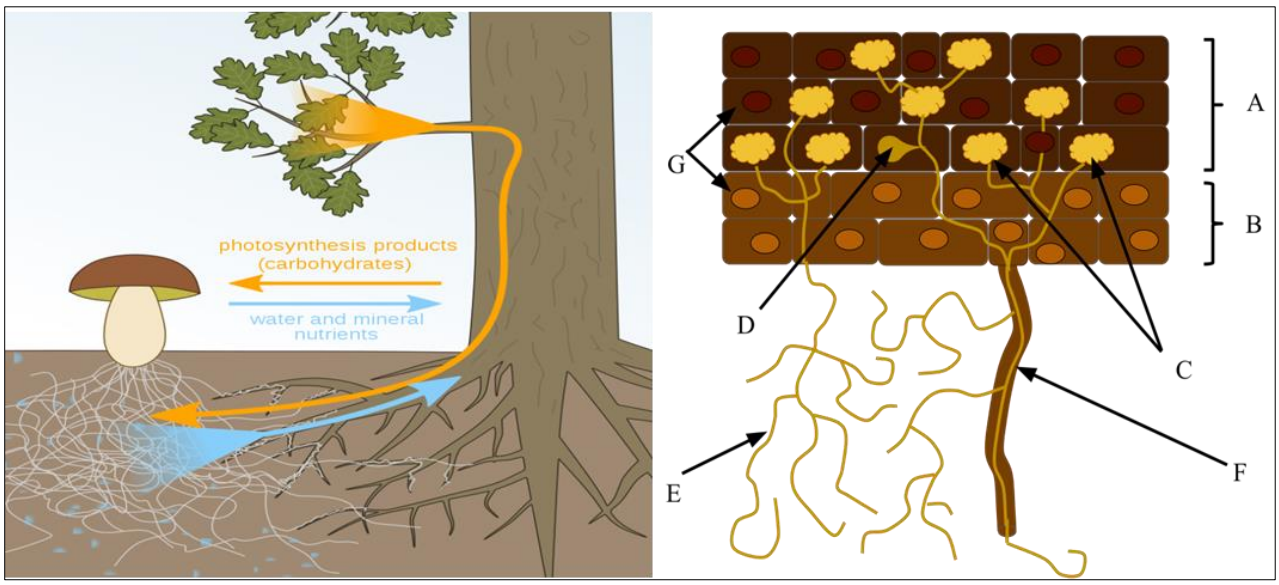

Figure legends: E=surface area of the root, A=root cortex, B=root epidermis, C=arbuscle, D=vesicle, F=root hair, G=nuclei

Figure 2 Mutualistic association of Mycorrhiza and Plant. A. Mycorrhiza between *Quercus robur* and *Boletus reticulatus* (mutualism), B. Symbiotic association between plant roots and fungi (https://en.wikipedia.org/wiki/Mycorrhiza)

Rhizosphere Bioremediation (RB) is a natural process that involves xenobiotic bioremediation. Natural rhizosphere repair occurs with a minimum concentration to the extent of tolerated limits under that environmental state, with environmental variances depending on the elements in play. However, rhizo-bioremediation is a slow process in nature that can be accelerated by using Plant Growth Promoting Factors (PGPFs), maintaining nutrients, maintaining ecological balance, bio-augmenting with microbial culture, and using nano-catalysts as a nutrient. Recent literature also supports the use of nano-catalysts as a nutrient for Rhizo-bioremediation (RB). Rhizo-bioremediation is plant-assisted bioremediation that has many effects on the bioremediation process due to a symbiotic relationship between bacteria, fungus, and actinomycetes in the rhizosphere's root zone (Korade and Fulekar, 2008). Because of the remediation mechanism supplied by microorganisms, the rate of degradation of pollutants – pesticides are higher in Rhizo-bioremediation (Brandt *et al.* 2006; Euliss *et al.* 2008; Sathishkumar *et al.* 2008). According to the literature, the breakdown of pollutants such as pesticides is faster when compared to plants or bacteria alone (Gurska *et al.* 2009).

3.2 Pesticide degradation in the Rhizosphere – A detailed mechanism:

Plant-secreted compounds in the root zone may aid pesticide breakdown, making them useful in the rehabilitation of polluted soils. The rhizosphere is the soil zone around the root where the root system influences microorganisms, generating a dynamic root-soil contact (Kuiper *et al.* 2004); (Pilon-Smits, 2005); (Barea *et al.* 2005).

In the rhizosphere zone, three components have been identified: The rhizosphere is made up of soil, the rhizoplane is made up of the root surface, and the root tissue is made up of endophytes (Barea *et al.* 2005). Different microbial diversity in root-associated soil compared to bulk soil is responsible for enhanced metabolic activity in the rhizosphere environment (Barea *et al.* 2005); (Kuiper *et al.* 2004); (Pilon-Smits, 2005); (Salt *et al.* 1998).

Rhizospheric bacteria have densities that are two to four orders of magnitude higher than those found in adjacent bulk soils, and they have a wider range of metabolic capabilities, including the capacity to breakdown a variety of pesticides (Pilon-Smits, 2005); (Salt *et al.* 1998). As a result, in comparison to non-vegetated soils, vegetated soils show a faster rate of biodegradation of organic contaminants. For a number of chemicals, the effects of the rhizosphere on xenobiotic biotransformation have been examined, but the processes by which particular plants accelerate biodegradation are still unknown. Plant tolerance to phytotoxic chemicals in soil may be linked to their ability to generate microorganisms that will detoxify these xenobiotics in the soil environment. Phytoremediation research has primarily concentrated on densely rooted, fast-growing grasses and plants with fine root systems, such as *Brassica sp.* Mulberry (*Morus alba L.*) and poplar (*Populus deltoides*) trees have been utilized to phytoremediate chlorophenols and chlorinated solvents like trichloroethylene (TCE) (Stomp *et al.* 1993). Many anthropogenic compounds are structurally similar to salicylic acid, flavonoids, and monoterpenes in that they are tiny, mobile molecules that are susceptible to cellular absorption and may interact through signal transduction pathways to trigger the production of certain degradative enzymes.

4. Rhizo-bioremediation (RB)

Phytoremediation and bioremediation are combined in rhizo-bioremediation. Korade and Fulekar, (2009) looked at the rhizosphere bioremediation of pesticides in particular. The potential of ryegrass in a pot culture experiment was used to explore the rhizosphere bioremediation of pesticides, specifically Chlorpyrifos, in mycorrhizal soil. Chlorpyrifos bioremediation in the rhizosphere was investigated at various concentrations, and degradation was recorded on a daily basis for 15 days over a two-and-a-half-month period. The potential bacterium *Pseudomonas nitroreducens* was examined utilizing a laboratory scale up technique for Chlorpyrifos and the rhizosphere consortium survived at a high concentration. In a pot culture procedure, the discovered potential organism was used as a culture and bioaugmented the rhizosphere bioremediation of Chlorpyrifos. Chlorpyrifos bioaugmentation rhizosphere bioremediation was shown to be more successful than the compound's rhizosphere bioremediation.

In a similar work (Dubey and Fulekar, 2012), the potential of *Pennisetum pedicellatum* was explored in a pot culture experiment for rhizosphere bioremediation of Chlorpyrifos. After rhizosphere bioremediation was examined for identifying possible organisms, the microbial consortium persisted at higher concentrations. In a laboratory bioreactor, the *Stenotrophomonas maltophilia* MHF ENV20 strain was used to bioremediate Chlorpyrifos. For the mineralization of pesticides, rhizosphere bioremediation has proven to be a viable and efficient approach. Furthermore, an Ecological Remediation Unit was devised and created, in which fibrous-rooted grasses were cultivated on mycorrhizal soil for rhizosphere breakdown of organic compounds and phytoextraction of heavy metals. For the decontamination of hazardous material, rhizosphere bioremediation systems have been demonstrated in the field. Rhizosphere bioremediation would be a low-cost green technique for cleaning up the environment.

In a separate study, Dubey and Fulekar (2013) looked into the increased breakdown of cypermethrin, a pyrethroid pesticide. For periodic evaluation in the pot culture trials, cypermethrin was added to the soil mixture (10, 25, 50, 75, and 100 mg/kg). Cypermethrin degradation was observed to be greater in rhizospheric soil than in bulk soil. *Pennisetum pedicellatum* and *Stenotrophomonas maltophilia* MHF ENV 22 were identified as promising rhizospheric isolates.

In non-rhizosphere soil, rhizosphere soil, inoculated non-rhizosphere soil, and inoculated rhizosphere soil, simazine (SIM) degradation, microbial biomass carbon, plate counts of heterotrophic bacteria, and most probable number (MPN) of SIM degraders were all measured by Liao et al, 2008. The half-lives of SIM in the four treated soils were measured to be 73.0, 52.9, 16.9, and 7.8 days, respectively, at a concentration of 20 mg SIM/kg, and the kinetic data showed first-order kinetics. The final results showed that rhizosphere soils, particularly injected rhizosphere soil, had greater SIM degradation rates.

The mineralization of [U-14C]2,4-dichlorophenoxyacetic acid (2,4-D) in rhizosphere soil was studied by Shaw and Burns, (2004). Herbicide treatment data was gathered from 0 to 116 days after *Lolium perenne* and *Trifolium pratense* were planted. The rates of mineralization, the quantity of 2,4-D degraders, and the diversity of genes producing 2,4-D/-ketoglutarate dioxygenase (tfdA) were also looked into. The influence of the rhizosphere on [14C]2,4-D mineralization (50 g g1) was shown to be species and age dependent. For 25 and 60 days, there were substantial (P 0.05) decreases in the lag phase and increases in the maximum mineralization rate when compared to nonplanted soil. There were few 2,4-D degraders in both planted and nonplanted soil. The increased mineralization in *T. pratense* rhizosphere soil was

not attributable to rhizodeposits enriching 2,4-D degrading bacteria, and an alternative mechanism in which one or more rhizodeposit components trigger the 2,4-D pathway was suggested.

Sun *et al.* (2004) looked examined how aldicarb degraded in sterile, non-sterile, and plant-grown soils with the goal of accumulating the pesticide. Aldicarb had half-lives of 12.0 and 2.7 days in sterile and non-sterile soil, respectively, and degradation showed first-order kinetics, indicating that the soil microbiota was significant in pesticide degradation. In soil planted with mung bean, corn, and cowpea, aldicarb was shown to vanish, with half-lives of 1.4, 1.6, and 1.7 days, respectively. The increased elimination of pesticide - aldicarb in plant-grown soil was attributable to plant-promoted degradation in the rhizosphere, according to a comparison of sterile, non-sterile, and plant-grown soils.

Rhizosphere bioremediation of butachlor in non-rhizosphere, wheat rhizosphere, and inoculated rhizosphere soils was measured by Yu *et al*. (2003). The rate constants for butachlor degradation in non-rhizosphere, wheat rhizosphere, and inoculated rhizosphere soils were 0.0385, 0.0902, 0.1091 mg/kg, 0.0348, 0.0629, 0.2355 mg/kg, and 0.0299, 0.0386, 0.0642 mg/kg, respectively. Butachlor in the soil has half-lives of 18.0, 7.7, 6.3 days at 1 mg/kg, 19.9, 11.0, 2.9 days at 10 mg/kg, and 23.2, 18.0, 10.8 days at 100 mg/kg, according to calculations. The experimental results demonstrated that butachlor degradation can be considerably accelerated in the wheat rhizosphere, particularly in the rhizosphere injected bacterial population that can degrade butachlor.

In a containerized rhizosphere system, Pai *et al.* (2001) investigated the fungicide mefenoxam. *Zinnia angustifolia* in a bark/sand potting mix was employed in the rhizosphere system, which was compared to bulk potting mix. Prior to adding mefenoxam (20 g/g mix), the rhizosphere microbial community was allowed to expand for three weeks. The concentrations of mefenoxam and degradation products were determined using an HPLC system. After 30 days, the first degradation product was N-(2, 6-dimethylphenyl)-N-(methoxyacetyl)-DL-alanine. N-(2,6-dimethylphenyl)- acetamide, another compound, was also found in trace levels. During 30 days, the microbiome populations in the rhizosphere increased, which was connected with an increase in the breakdown of the parent chemical. Within 54 hours, *Pseudomonas fluorescens* and *Chrysobacterium indologenes* isolated from the rhizosphere system had entirely decomposed to free acid.

Furthermore, the rhizosphere's microbiome outnumbers the microbiome in the bulk soil. Increased contaminant - pesticide breakdown in the rhizosphere is favorable to the plant's growth on polluted soil (Oberai M. and Khanna V, 2018).

5. Bioaugmentation

5.1 Plant growth promoting rhizospheric (PGPRs) microorganisms:

PGPR maintains plant health in an environmentally favorable manner, and their interactions with plants are commercially exploited for long-term agricultural sustainability (Gonzelez *et al.* 2015). PGPR is involved in a variety of biotic activities in the soil ecosystem to ensure long-term crop production. PGPR colonize plant roots and promote plant growth through a variety of mechanisms, including nitrogen fixation (Glick, 2012), siderophores (Jahanian *et al.* 2012), phosphate solubilization (Ahemad and Khan, 2012), production of indole-3-acetic acid (IAA), hydrogen cyanate and ACC deaminase (Liu *et al.* 2016). Furthermore, a small number of PGPRs infer more particular plant growth-promoting pathways, such as heavy metal detoxification, salt tolerance, and biological control of phytopathogens and insects (Egamberdieva and Lugtenberg, 2014). The PGPR application's long-term viability is dependent on mutualistic interactions between the plant and the PGPR in the rhizosphere (Kuiper *et al.* 2004). Plant growth-promoting rhizospheric microbiomes capable of digesting harmful contaminants play a vital role in rhizoremediation.

5.2 Microbial bioaugmentation

The pot-culture experiment was used to explore the potential of ryegrass for rhizosphere bioremediation of chlorpyrifos in mycorrhizal soil in the research study (Korade and Fulekar, 2009). The chlorpyrifos concentration in the pot culture soil was degraded totally within 7 days, while the remainder of the modified concentrations (25-100mg/kg) declined rapidly as the incubation progressed to 28 days under the impact of the ryegrass micro-rhizosphere. The microorganism associated with the roots of the ryegrass rhizosphere is responsible for chlorpyrifos bioremediation in soil. As a result, the microorganisms that survived in rhizospheric soil spiked at the maximum dosage (100mg/kg) were evaluated and used to isolate chlorpyrifos degrading bacteria. *Pseudomonas nitroreductase* PS-2 was discovered as a possible degrader utilizing the 16S rDNA BLAST method. PS-2 was used as an inoculum in the experimental setup, which was similar to the previous one, to further accelerate chlorpyrifos biodegradation. The heterotrophic bacteria and fungus in the inoculated and non-inoculated rhizospheric soil were also counted. In bioaugmentation trials, the percent dissipation

of chlorpyrifos in inoculated rhizospheric soil was 100 percent, compared to 76.24, 50.36, and 90.80 percent in non-inoculated soil at initial concentrations of 25, 50, and 100 mg/kg at the 14th, 21st, and 28th-day intervals, respectively.

5.3 Microbes as plant growth promoters

Rhizoremediation happens in the proximity of plant roots, and it has the potential to boost plant root growth as a positive result of rhizoremediation. The success of rhizoremediation is dependent on the plant root system's ability to proliferate. As a result, the PGPR linked to roots encourages plant development, which aids in stress tolerance and can be employed for phytoremediation. Bioaugmentation is a procedure in which beneficial microbial culture is added to the rhizosphere remediation in a laboratory experiment or in the field to improve the rhizosphere remediation.

Korade and Fulekar (2009) bioaugmentation study found that adding a microbial culture – consortium of identified potential microorganisms to the rhizosphere bioremediation results in the successful removal of harmful organic pollutants such as pesticides and heavy metal remediation. The bioaugmentation study used the remediated rhizosphere soil that had been amended with a greater quantity of Chlorpyrifos (100mg/kg) in the first batch of the experiment. The soil was chosen because it is thought to include microorganisms that have evolved to higher contamination concentrations and have the ability to breakdown chlorpyrifos. This rhizospheric soil's microbiological characterization was also completed. In the enrichment investigation, microorganisms that could use chlorpyrifos as a sole carbon source were isolated using nutrient culture medium. Mineral salt medium was used as the culture medium. From the minimal agar media plates containing chlorpyrifos as the sole source of carbon, colonies were chosen for further isolation and identification. A phenol: chloroform extraction technique was used to extract genomic DNA from isolated microorganisms. Universal primers were used to amplify the 16s rDNA (Weisburg *et al.* 1991). 2.5 liters of 10XPCR buffer containing 1.5 M MgCl2, 2.5 litres of 2mM dNTPs, 1.25 litres of 10pmol/l primers, 1.5 litres of total genomic DNA (30ng), 0.24 litres of Taq DNA polymerase, and 15.76 litres of nuclease-free glass distilled water were used in the PCR mixture. Amplification was carried out using a thermal cycler, with the following steps: step 1, 3 minutes of initial denaturation at 94°C; step 2, 35 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C; step 3, 10 minutes at 72°C. The forward and reverse internal primers were used to sequence the PCR result in both directions. To identify the bacteria and their closest neighbours, the sequence data were aligned and processed on the NCBI, BLAST database. Potential bacteria discovered during ryegrass rhizosphere bioremediation were exploited for chlorpyrifos bioaugmentation in a pot culture approach. During the rhizosphere bioremediation of chlorpyrifos in a laboratory scale-up process bioreactor, a promising bacterium was found from *Pennisetum pedicellatum*.

6. Rhizosphere to Metagenomics

Rhizosphere microbiome: Microbial communities in the rhizosphere of plants have a role in plant function, including rhizoremediation of organic pollutants such as pesticides. Rhizoremediation is based on the interaction of the plant and microbiome in the rhizosphere, where root exudation in the rhizosphere allows the rhizosphere microbiome to take in more nutrients. As a result, it is thought to be one of the most important aspects of microbial community activity in the rhizosphere, as it aids in the biodegradation of organic molecules. Based on new omics methods such as metagenomics, metatranscriptomics, metabolomics, and metaproteomics, current literature summaries the understanding of organic chemical breakdown in the rhizosphere in the process of plant – microbiome interaction. Rhizosphere metagenome analysis can be performed to investigate the microbiome's structure, function, and composition. Bulgarelli *et al.* (2012); Lundberg *et al.* (2012) established that the microbial communities in bulk soil and the rhizosphere are distinct using metagenome sequencing of bacterial 16S rDNA. Nazir A, (2016) used a metagenomics strategy to discover new functional metabolic pathways for aromatic chemical biodegradation by identifying functional targets and discovering diverse groups of dioxygenases from various bacteria genera. Metagenomics of complex microbial communities gives researchers direct access to the genetic information of a habitat's whole community (Siddhapura *et al.* 2010).

Ratna *et al.* (2019) conducted a metagenomics investigation on *Paspalum scrobiculatum* and discovered a complex relationship between microbial communities and plant development and survival. The metagenomics of *Paspalum scrobiculatum* rhizosphere revealed a varied community with functional capacities associated with supporting plant growth and development in nutrient-deficient situations. In the rhizobiome of *Paspalum scrobiculatum*, 65 taxonomically distinct groups were discovered. The most numerous bacteria were Actinobacteria, followed by Proteobacteria. The discovery that the multifunctional rhizobiome performs several cytosolic and metabolic functions, including carbon fixation, nitrogen, phosphorus, sulphur, iron, and aromatic compound metabolism, stress response, secondary metabolite synthesis, and virulence, disease, and defence, led to the discovery that the multifunctional rhizobiome performs several cytosolic and metabolic functions, including carbon fixation, nitrogen, phosphorus, sulphur, iron, and aromatic compound metabolism, stress response. The abundance of genes involved in N, P, S, Fe, aromatic chemical metabolism, phytohormone synthesis, and other important processes clearly justifies plant growth,

development, and survival in nutrient-deficient dry environments. The kodorhizobiome has metabolic ability to defend itself against biotic stressors, as seen by the dominance of actinobacteria, the known antibiotic generating community.

Min-Jung Kwak *et al.* (2018) found that tomato plants (*Solanum lycopersicum*) are resistant to the soil-borne pathogen *Ralstonia solanacearum* because plant-associated bacteria play a role in disease resistance. Taxonomical microorganisms were studied in a mesocosm experiment by transplanting Hawaii 7996-resistant and Moneymaker-susceptible tomato varieties. Comparative investigations of rhizosphere metagenomes from resistant and susceptible plants led to the discovery and assembly of a flavobacterial genome that was considerably more common in the resistant plant rhizosphere microbiome than in the susceptible plant microbiome. In addition, in pot studies, researchers discovered that a flavobacterium called TRM1 might decrease *R. solanacearum* disease growth in a sensitive plant. Native microbiota protects plants from microbial diseases, according to the findings.

The greatest driver of microbial biodiversity loss in Amazon soils, according to Dennis Goss-Souza *et al.* (2019) is the conversion of original forest to agriculture. In a long-term forest-to-agriculture conversion, metagenomics will be used to explore microbial patterns and roles in bulk soil and the rhizosphere of soybeans. The long-term forest-to-agriculture conversion resulted in microbial homogenization and loss of variety in both the bulk soil and the rhizosphere, owing to liming and fertilization in long-term no-till farming. Long-term no-till cropping resulted in a drop in *Acidobacteria*, *Actinobacteria*, and *Proteobacteria* abundances, according to the data. Regardless of the time after forest-to-agriculture conversion, - and -Proteobacteria abundances were higher in the rhizosphere than in bulk soil. The majority of the changes in functional potential occurred in bulk soil, with declines in activities related to potassium metabolism, pathogenicity, illness, and defence, and increases in functions related to nucleic acids metabolism. Except for those related to potassium metabolism, functions in the soybean rhizosphere remained steady. The soybean root system uses trade-offs to select microbial taxa in order to preserve functional resilience in the rhizosphere microbiome throughout time, according to the findings.

7. Nanotechnology

To combat environmental contamination, nanotechnology has transformed the science of bioremediation. Nanotechnological approaches include monitoring, treatment of contaminants, pollution control, pollution sensing, and remediation with nanocatalysts, which is the most important approach to pollution remediation. Nano-bioremediation is environmentally friendly, precise, cost-effective, ex-situ, and long-term green bioremediation method. Surface water, ground water, and industrial waste water contaminated by hazardous metal ions, radionuclides, organic and inorganic solutes, and reduced aromatic recalcitrant compounds from soil and air pollution are all treated by nano-bioremediation.

The desire for new technologies that can speed up the decontamination of hazardous sites while also lowering their costs is increasing. Recently, the utilization of nanomaterials, particularly iron nanoparticles, as a new approach of polluted site rehabilitation has gotten a lot of attention. Although various studies on nanomaterials have been conducted, little is known regarding their behaviors in soil pores, adsorption on mineral particles, and interactions with soil microbes. The use of nanomaterials in soil remediation, with an emphasis on their toxicity to soil microorganisms and the feasibility of integrating them with other remediation strategies, such as bioremediation. The global problem of contaminated sites, as well as the obligation and necessity to clean them up. The use of nanoparticles (NPs) for contaminated locations, including their applications in the field and the challenges of doing so. The effects of NPs on soil microorganisms, either negative (biocide) or positive (biostimulant), prompted the development of the Nanobioremediation (NBR) concept, which aims to combine two remediation strategies for effective, efficient, and eventually sustainable remediation. Depending on the soil conditions, the biogeochemistry of the contaminated site might have a significant impact on NP remediation and interactions with pollutants and microorganisms. However, ongoing research investigations aim to merge the use of nanotechnology with bioremediation sequentially or concurrently to improve the efficiency, efficiency, and sustainability of remediation processes.

Nano-bioremediation is a new breakthrough that combines nanoparticles and biological processes to improve measurement precision and expand biochemical applications in environmental research.

7.1 Nano-rhizosphere

The nano-rhizosphere is made up of micro-beads and nano-to-micro fibers of organic polymer that closely resemble the structure of the soil. The nanostructure in this work was made using electrospinning, nanotechnology that normally forms nanofibres, but also beads, by deposition under an electric field and onto a collector. Root exudates from crop plants were fed to microbial cultures in the form of a solution, an agar medium containing these chemicals, or the organic

nano framework itself, where the exudates had been loaded by mixing with polymer solution prior to the electrospinning process. Model microorganisms (*Actynomycetes*, *Pseudomonads*, or *Lactobacilli*) were previously isolated from the rhizosphere of several plants and utilized to reconstruct a proper rhizosphere ecology. The pure and mixed cultures were put to the test. Heavy metals were utilized as model soil contaminants to provide an environmental strain on either the formation of a new rhizosphere ecosystem.

7.2 Interaction mechanism - Nanoparticle with soil and microbiome

Due to the limited number of monitoring points and the short time periods of monitoring, comprehensive characterization of interactions of nanoparticles with soil particles and the local microbiota under field-scale circumstances is a difficult challenge. The oxidation-reduction potential of nZVI over time, dissolved oxygen (when applied in groundwater), pH, electrical conductivity, and iron concentration in the system (Fe0, Fe2+, Fe3+) are the main parameters evaluated throughout the administration of a remediation system (Saleh *et al.* 2007); (Cundy *et al.* 2008); (Tosco *et al.* 2012).

The microbiota in the soil varies depending on the peculiarities of each location, however it may include indigenous species capable of decomposing the pollutant in the soil. In these situations, the use of nZVI stabilized with organic polymers can also act as a growth stimulator, increasing harmful chemical biodegradation and improving site decontamination (Saleh *et al.* 2007).

Several scholars have looked into the interaction of nZVI with soil mineral components (Cundy *et al.* 2008); (Tosco *et al.* 2012). After the injection of nanoferro suspension, the co-formation of contaminants with Fe2+ or Fe3+ can chemically modify the nanoparticle surfaces in the ambient environment. These chemicals can have a direct impact on the local flora and can prevent inorganic contaminants like chromium(VI) and uranium from becoming immobilized for lengthy periods of time (IV).

7.3 Nano-phytoremediation

To improve the degradation efficiency of chlorfenapyr-contaminated soil with *P. major*, boosting agents (SiO2, argal, and 1 percent ethanol) and nanoparticles (F–Fe0, Ag–Ip0, and Ag–Br0) were utilized. (1) uncontaminated soil with a *P. major* seedling; (2) chlorfenapyr-contaminated soil with no plants; (3) chlorfenapyr-contaminated soil with a *P. major* seedling; (4–6) chlorfenapyr-contaminated soil amended with enhancing agents (SiO2, argal, and 1 percent ethanol); (7–9) chlorfenapyr-contaminated soil amended with enhancing agents (SiO2, argal, and 1 percent ethanol). A chlorfenapyr (20 mg/kg soil) water-based solution was carefully poured to the pots, avoiding direct contact with the plant shoots. 2 ml of each nanoparticle was combined with 98 ml of water (2.0%) and applied to the soil to cover its surface (2 cm) and maintain the anaerobic condition in treatments 10–15, whereas treatments 2 and 3 were amended with water alone until flooding. Enhancing chemicals (750 mg/L SiO2, 0.37 percent argal, and 1 percent ethanol) were mixed with 100 ml water and amended in the soil in treatments 4–9. One seedling of *P. major* was provided to each pot for the treatments involving *P. major*. 400 g of air-dried sieved silty clay loam soil samples from Aboutwala, Minya al Qamh (Sharqia, Egypt), with 2.0 percent organic matter, 1.02 percent organic carbon, 42 percent clay, 45 percent silt, 13 percent sand, 7.9 pH, and 42.12 cmol/ kg cation exchange capacity, were transferred to individual plastic pots (plastic pots of 10 cm diameter were filled up to 2 cm below the rim with 400 g soil).To obtain 20 mg/kg of dry-weight soil, the soil was spiked with chlorfenapyr. The *P. major* plants were separated after four days of exposure, and the plant roots were washed with distilled water and wiped dry. The plants' individual roots and leaves were then separated. Chlorfenapyr levels were determined in 5 g of roots, 5 g of leaves, and 10 g of soil. *Plantago major's* integration of green nanotechnology, solubility-improving compounds, and phytoremediation plays a key part in the cleanup of chlorfenapyr-contaminated soil and water.

7.4 Nano – Rhizoremediation approach

Newer approaches, such as the use of metallic nanoparticles, have recently been used to improve classical rhizoremediation. The Biannual ECOtoxicology Meeting 2016 (BECOME) in Livorno focused on eco-friendly nanotechnology: state of the art future perspectives and eco-toxicological evaluation of nanoremediation applied to contaminated sediments and soils (Italy). The use of nanomaterials for environmental remediation is based on the simple concept of transferring the advantages of nanotechnology to macro dimensioned systems by going from nano-sized materials to nano-structural devices. Nanoparticles are not used directly in the remediation process, but instead serve as building blocks for a stable nano-structure system with increased micro and nano porosity (Corsi I. *et al.* 2018). This creates a novel type of sorbent with a large surface area that can remove organic and inorganic contaminants from contaminated soil, water, and air.

The utilization of metallic nano-particles such as zero valent nanoparticles for soil remediation (Rafael G. Lacalle *et al.* 2018) is extremely promising. Researchers present data on soil chemical (pseudo-total and CaCl2-extractable metal concentrations; petroleum hydrocarbon concentrations) and biological (microbial properties and phytotoxicity) properties after applying nZVI to soil simultaneously contaminated with Zn, Cu, Cd, and diesel, in the absence and presence of other remediation treatments such as the application of an organic amendment and the growth of *Brassica napus* plants (Rafael G. Lacalle *et al.* 2018). After one month, the soil was supplemented with the stated pollutants, and then nZVI were added to the soil, followed by seeds (*Brassica napus*). Plants of *B. napus* were let to develop for a month in the experimental setup. The application of nZVI had no influence on pollutant removal or soil metrics, according to the study. It also did not create an indirect toxic effect or root elongation in plants as a result of nZVI contact with soil organisms. This study shows that zero valent ion particles are effective in removing metal and hydrocarbon contamination from the soil without causing ecotoxicity. The potential and efficacy of nanotechnology in particular use of nanomaterial will require great efforts to be devoted to develop innovative and green, sustainable (nano) solution which own eco-safe features such as limited mobility in environmental media and no toxicological effect for humans and wildlife.

8. Conclusion

Rhizosphere bioremediation is a green technique that uses the interaction of bacteria with plant exudates to clean up soil contamination in the rhizosphere. In rhizo-remediation, the PGPR rhizospheric bacterium, which is capable of digesting harmful contaminants, plays an essential role. Furthermore, bioaugmentation of rhizo-remediation with a rhizosphere microbial consortium improves rhizoremediation. The uncultivated microbe and its involvement in rhizo-remediation were discovered using a metagenomic method. Recent research also shows that using nanomaterials like nZVI does not induce eco-toxicity and has no influence on microbial and plant growth, while simultaneously promoting rhizo-remediation. Rhizo-remediation using these bio-nano-based applications would be a viable method for removing toxins like as pesticides from the soil-water environment.

Compliance with ethical standards

Acknowledgments

The research was financially supported by Center of Research for Development, Parul University under Intramural Grant Order no. CR4D/DEC-19/13.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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