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# Diazotrophs-Assisted Phytoremediation of Pesticides: A Novel Approach

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**Abstract:** Pesticides have hazardous effects on environment, human health, crop productivity, plant growth and metabolism and they can remain in the atmosphere for longer periods of time due to their persistent nature. Remediation of pesticides is absolutely crucial. Phytoremediation is a newly developed technique utilized in several approaches for the purpose of remediation. Research is being conducted to improve this plant-based technology's efficacy. In this way, symbiotic and rhizospheric microbes are essential to the bioremediation of pesticides. A summary of the data from the ongoing research was used to determine that helping diazotrophs can improve the effectiveness of phytoremediation. Diazotrophic bacteria are essential for pathogen resistance, phytohormone production, nutrient uptake efficiency, biological nitrogen fixation, and degradation of pesticides. They also reduce the need for chemical inputs in sustainable agriculture by enhancing plant health. Diazotrophic bacteria are well known for their biological nitrogen fixation (BNF), biosynthesis of auxins like Indole-3-Acetic acid (IAA), ability to release phosphate from biologically inert forms in the environment for plant uptake. Degradation of pesticides by microbes happens in three phases. First stage reduces the toxicity of pesticides and involves oxidation-reduction mechanisms. Second phase includes conjugation of amino acids and in the last stage secondary conjugation occurs that completely metabolizes the substances. This review's objective is to demonstrate how diazotrophs may support pesticide phytoremediation in polluted soils. An effective combination of diazotroph and phytoremediation technology is suggested by the innovative current review of literature.

**Keyword:** Bioaccumulation; Diazotrophs; Pesticides; Phytoremediation; Plant Microbe Interaction.

## 1. Introduction

The rise in industrialization and use of agrochemicals have resulted in a release of harmful substances into the environment [1]. Farming chemicals are a broad category that includes different chemicals used in agriculture as well as related activities. These substances or mixtures include growth-promoting agents, feed additives, veterinary drugs, pesticides, fertilizers and other chemicals [2-3] those agrochemicals have been substantially responsible for the acceleration of the green revolution in the majority of wealthy countries where agricultural outputs have increased dramatically [4].

The green revolution, which was fueled by an increasing population's need for food, increased agricultural output per unit area after the industrial revolution. But this rise also brought about a rise in the use of synthetic pesticides in agriculture. Worldwide the extensive application of inorganic fertilizers in agriculture has resulted in severe health issues and permanent environmental harm ([5]. These toxic compounds often have impacts, such as being highly toxic accumulating in the environment and causing harm to human health due to their man made and mutagenic properties [6].

The purity of agricultural landscapes is seriously threatened by farming operations as well as agro-aids including synthetic fertilizers, livestock composts, bug-killing agents, and waste-processing residues. Using plastic ground covers and watering farms are two major contributors to this issue. Microbiology assisted phytoremediation is a potential in-situ remediation technique that has attracted a lot of interest lately for its ability to clear up contaminated soil. In addition to their capacity to biodegrade pollutants and improve their removal, a number of helpful bacteria and the endophytes also promote intrinsic vegetation growth-promoting characteristics. These include the synthesis of siderophores, nutrients, and the chelator [7]

The term "phytoremediation" describes the process by which plants biologically clean the air, water, and soil. The symbiotic relationship that plants have with microbes aids in the restoration of the environment, particularly when it comes to removing organic and heavy metal contamination. Because of its affordability, simplicity of use, and environmental friendliness, phytoremediation is well-liked by the general people [8-9].

Pesticides, a type of chemicals, are not only linked to farming land, but also on lawns, train tracks, and other communal areas [10]. Pesticides use for crop protection is anticipated to rise due to the increasing global population and the demand for higher food production. Pesticides can boost agricultural yields, but via the food chain can pose a threat to mammals due to the harmful effects of pesticides [11-13] in many parts of the world pesticides are designated as carcinogenic as they pose a serious threat to the surrounding environment [14]. Thus, overuse of these substances for the past fifty years has presented significant health concerns to humans [15-16]. There are a lot of reports about pesticide residues found in cereals [17], milk [18], vegetables [19], and fish [20].

The term diazotroph can be defined as the group of microorganisms which have the capability of fixing atmospheric nitrogen with the help of plant roots. In particular, microbial cells and root structures of plants undergo anatomical and physiological changes brought about by the symbiotic rhizobacteria, resulting in specialized formations known as nodules [21]. In different habitats, nitrogen fixing bacteria helps plants in fixing nitrogen from the soil. In order to ensure soil sustainability, these nutritional needs are either entirely or partially met. Nitrogen can be supplied to plants by free-living, associative and symbiotic nitrogen fixing microbes that turn nitrogen from the atmosphere into ammonia. This process also increases plant production. Nitrogen fixation promotes soil health and enhances microbial diversity, these microbes have different mechanisms to fix atmospheric nitrogen which is beneficial to the long term sustainability of soil and plant health [22].

The process of nitrogen fixation requires a large energy input to generate ammonium from N2. The process of nitrogen fixation in biology fixes nitrogen by using ATP and the enzyme nitrogenase. In addition to the various metal cofactors, nitrogenase has two proteins: Fe and Mo-Fe. In environments, soil diazotrophs have the role of fixing nitrogen from the atmosphere into physiologically accessible ammonium [23].

## 2. Role of Diazotrophs in the Remediation of Pesticides

While pesticides are crucial for protecting crops from harmful pests and boosting agricultural output, their frequent use poses a significant threat. These chemicals seep into the soil and contaminate the food chain, causing harm to ecosystems and potentially endangering human and animal health [24] beyond agriculture, these pesticides find application in maintaining and enhancing non-agricultural spaces like public parks and athletic fields [25]. The reach of these chemicals extends far beyond agriculture. They are surprisingly common in unexpected places, like pet shampoos, certain building materials, and even on boat bottoms, to fight unwanted organisms [26].

Due to the overuse of chemicals like weed killers, pest killers, and other bad practices, the helpful tiny organisms living in the soil have been thrown out of balance. This imbalance doesn't just hurt the soil, it can also cause dangerous illnesses to spread in people and animals. While we use pesticides heavily in modern farming to grow more food, these chemicals build up in the soil, harming the environment and the delicate balance of life within it. They linger in the soil, pollute water sources, and travel through the food chain. This harms people, animals, and the whole environment. Some tiny organisms are tough enough to handle these chemicals and might even help clean things up. So those helpful nitrogen-fixing bacteria, diazotrophs, can also break down harmful pesticide toxins. This helps keep the environment cleaner [27].

Diazotrophs, microbes crucial for turning unusable nitrogen from the air into a form plants can use, are essential for agriculture [28]. Diazotrophs are microscopic allies that turn unusable air nitrogen into nutrients plants can absorb, fueling their growth and health. They also contribute to overall soil well-being [29]. Some studies have reported diazotrophs involvement in regulating pathogens and degrading toxic compounds diazotrophs like pseudomonas arthrobacter nostoc acinetobacter and flavobacterium have shown pesticide degrading properties as part of their xenobiotic degradation mechanisms [30].

These inorganic pesticides wreak destruction on soil health by inadvertently wiping out beneficial microbial communities alongside the targeted pests. Therefore, we need a cost-effective and environmentally friendly solution to remediate contaminated sites without creating new problems [31]. Contaminated soil can be addressed through a multi-pronged approach, utilizing physical methods, chemical treatments, and biological strategies. These techniques have proven effective in eliminating pollutants [32]. Physical methods like incineration, filtration, and adsorption can be employed to remove contaminants. Chemical treatments often utilize free radicals, while biological approaches harness the power of microbes to break down pollutants [33]. While physical and chemical methods offer options for contaminant removal, they come with drawbacks. Some treatments generate harmful secondary pollution. Additionally, these approaches can be expensive and time-consuming, requiring specialized equipment and expertise for proper implementation [34]. As a result, biological treatment, also known as bioremediation or biodegradation, has gained significant traction. This eco-friendly approach harnesses the power of microbes to break down pollutants, offering a natural and sustainable solution to soil contamination [35]. While some microbes develop resistance to pesticides, bioremediation remains a cost-effective and safe method for creating a non-toxic environment. These resilient microbes, armed with potent enzymes, can effectively degrade targeted pollutants [36]. These microbes act like tiny recyclers, breaking down the pesticides into harmless byproducts. These byproducts become a valuable source of energy and nutrients for the microbes themselves, creating a closed-loop system that avoids generating any additional pollution [37].

Microbial degradation of pesticides relies on enzymatic mechanisms within the microbes (Kumar et al., 2018). The first act of microbial enzymes is to bind to toxins, rendering them inactive. Next, these enzymes catalyze various reactions that break down the toxins into smaller molecules [38]. Microbial enzymes break down toxins through a multi-step process. In phase 1, oxidation, reduction, and hydrolysis reactions make the toxin water-soluble and less harmful. Phase 2 involves conjugation, where sugars or amino acids are attached to the toxin, further increasing water solubility and reducing toxicity. Finally, phase 3 utilizes secondary conjugation to completely break down the toxin into harmless end products like water and CO2, which can be used by microbes as a food source (Ortiz-Hernández, Sánchez-Salinas, Dantán-González, & Castrejón-Godínez, 2013). Several factors influence how effectively microflora can deplete pesticides. These include soil pH, temperature, condition, moisture levels, and oxygen availability (Qin et al., 2019). Microbial degradation requires energy, fueled by ATP molecules obtained from breaking down the pollutants themselves. A variety of enzyme classes are crucial for this process, including hydrolases, esterases, oxidoreductases, transferases, halogenases, dehalogenases, and oxygenase [39]. Microbes can dismantle pesticides through two main strategies: extracellular and intracellular enzymes. Extracellular enzymes are like external tools that break down pesticides directly in the surrounding environment. This process is efficient and leads to mineralization, converting the toxin into harmless components. Intracellular degradation is a slower process. Here, microbes first adsorb the pesticide onto their cell walls. If the pesticide can penetrate the cell membrane, it encounters internal enzymes that break it down through a series of rapid reactions [40].

Scientists typically isolate individual bacterial colonies from a soil sample using streaking and spreading techniques [41]. Once isolated, the chosen bacterial colony's growth will be monitored in the presence of varying pesticide concentrations. These different concentrations will serve as potential food sources for the bacteria. Following these initial [42] growth tests, researchers will employ a series of confirmatory assays to delve deeper into the microbe's behavior with the pesticide. These techniques aim to elucidate the specific enzymatic mechanisms responsible for the degradation of the targeted pesticides [43]. Initial tests rely on basic characteristics to distinguish between bacterial colonies. These characteristics include morphology (shape, size, and color), cell count, Gram staining behavior, and even basic biochemical and physiological assays. These tests are conducted with the help of microscopes and other tools [44].

**Table 1.** Basic Fundamentals of Diazotrophic Bacteria

| Diazotrophic Bacteria | Pesticide/ Chemical degraded by bacteria | Percentage | Reference |
|---|---|---|---|
| Azotobacter salinestris | pendimethalin | 100% | Chennappa et., 2018 |
| Bacillus subtilis | diazinon | 3.79 - 58.52% | Nasrollahi et al., 2021 |
| Staphylococcus | pyrene | 56.% | Sun et al., 2014 |
| Sphingomonas strain HJY | chlorpyrifos | 75% | Feng et al., 2017 |
| Klebsiella pneumoniae strain BCH1 | imidacloprid | 78% | Phugare et al., 2013 |
| Bacillus weihenstephanensis | imidacloprid | 46 - 78% | Shetti et al., 2014 |
| Pseudoxanthomonas sp. AAP-7 | acetamiprid | 80% | Wang et al., 2013 |
| Ochrobactrum sp. Strain D-12 | acetamiprid | 300 mg·L−1 | Wang et al., 2013 |
| Ensifer adhaerens | Thiamethoxam | 87% | Zhou et al., 2013 |
| Ensifer meliloti | thiacloprid | 86.8% | Ge et al., 2014 |

**3. Pesticides: Environmental Effects & Microbial Solutions**

India's growing population relies on widespread pesticide use to safeguard crops and secure food supplies. However, this essential practice comes at a significant ecological cost. Pesticides not only target pests but also contaminate soil, water, air, and land. This persistent pollution harms both terrestrial and aquatic animals, posing long-term threats to ecological [45]. Pesticide use harms soil health, leading to infertility, acidification, nitrate loss, tougher weeds, and vanishing wildlife [46]. Pesticides introduce a diverse mix of pollutants with unique ways of harming living things. This makes developing universal cleanup methods difficult. The ecological consequences of pesticide buildup are a tangled web, causing long-term problems that might not be immediately obvious, ultimately disrupting the food chain [47]. Pesticides act like nerve poisons, interfering with brain and body signals. This buildup disrupts chemicals needed for muscle control, leading to seizures, paralysis, and even death. Some pesticides, like organophosphates, can also damage genes and cause cancer [48].

Soil and water abound with diverse microbes that act as nature's cleanup crew for harmful pesticides. These resourceful bacteria can utilize a vast array of these chemicals as fuel, breaking them down into simple minerals that plants can readily absorb. Even persistent pollutants are no match for these adaptable microbes, as they can develop entirely new genes to tackle the challenge. This talent for breaking down pollutants isn't limited to soil microbes. Diverse communities of bacteria in freshwater, saltwater, and even wastewater can also tackle a wide range of organic compounds. Compared to harsh chemical methods, using these microbes for bioremediation is a much gentler and more effective way to clean up pesticides. Thanks to recent advances in omics research, we're gaining a deeper understanding of how these microbial clean-up crews work in different environments, from lakes and rivers to oceans [49]. To truly harness the power of microbes for a sustainable future, we need better ways to measure their activity at specific sites. This will allow us to develop more advanced strategies that take advantage of the natural cleanup abilities of these tiny organisms [50]. Pesticide use and cleanup are two sides of the same coin in protecting our environment. Understanding the harm pesticides cause and employing effective bioremediation is crucial for preserving ecosystems and achieving sustainability. By acknowledging these risks and harnessing the power of microbes, we can safeguard biodiversity, ensure healthy ecosystems, and leave a cleaner, healthier planet for future generations.

**4. Role of Microorganisms in Remediation of Pesticide**

The issue of natural contamination by pesticides goes past the region where it is utilized. The rural pesticides that are widely applied to the land surface travel significant distances and can move down until arriving at the water table at visible fixations, arriving at amphibian conditions at altogether longer distances. In this manner, the intention of pesticides is frequently doubtful, they can spoil different regions that are far off from where they were initially utilized [51]. Pesticides pose chronic threats to human life as a result of prolonged exposure, as well as a high risk of ecosystem contamination. Pesticides can cause hormonal disturbance, reduced intelligence, and contraceptive abnormalities in people. Accordingly, the humiliation of persevering pesticides is extremely fundamental for the climate [52].

Prior strategies or advancements that were utilized to eliminate pesticides from climate were landfills, reusing, pyrolysis, and so forth, yet these arrestingly affect the climate by arranging poisonous intermediates [53].

In the case of pesticides, all of these methods have proven to be costly and difficult. One promising treatment strategy to eliminate or debase poisonous parts from climate is bioremediation. Bioremediation is the premeditated course of poisonous expulsion from climate. This is a promising option for physio-synthetic strategies for remediation since it is functional and can specifically acquire the destruction of harmful parts [54]. The utilization of microorganisms for the corruption and detoxification of various harmful pesticides ended up being a productive device to sterilize the dirtied destinations in the overall climate. Biodegradation of pesticides in soil and water is the primary method of pesticide breakdown and detoxification in many soils. Because, in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates, biological decontamination methods are preferred to conventional methods [55].

Microorganisms provide a cost-effective and innovative approach employing bioremediation techniques for the removal of pesticides in water because of their advantage linked to environmental safety, biodegradability, effectiveness, and target-specificity [56]. Microorganisms then again have broken up being effective in the remediation of ecological toxins. They are like plants in remediation, this is because of their simplicity of development, fast development period, and simple control. To ensure a long-term environment, it is therefore necessary to enhance the use of microbes as bioremediation agents. Microorganisms can change over poisonous components into water, carbon dioxide, and other less harmful mixtures, which are additionally corrupted by different organisms in a cycle alluded to as mineralization [57]. Bacteria, fungi, algae, and other organisms can be used in bioremediation. Organisms are pervasive in nature, and they use many substrates as carbon sources; subsequently, they are found in strange conditions where they can retain a large number of poisons [58]. Pests are controlled by microbial pesticides, which contain living organisms like bacteria, fungi, algae, and viruses. They stifle bugs either delivering poisonous metabolites that cause harm and illnesses or forestalling the foundation of different microorganisms [59].

**Table 2.** Genetically Engineering Diazotrophs

| Genetically engineered diazotrophs | Pesticides | References |
|---|---|---|
| Pseudomonas putida | cadusafos | Abo-Amer & Aly E (2012) |
| Burkholderia cenocepacia | methyl parathion | Fernández-López et al., 2017 |
| Sphingomonas paucimobilis | cadusafos and ethoprophos | Karpouzas et al., 2005 |
| Escherichia coli: | Methomyl | Kulkarni et al., 2018 |
| Bacillus amyloliquefaciens YP6 | hlorpyrifos, dichlorvos, dipterex, phoxim, and triazophos | Meng et al., 2019 |

## 5. Enhancing Microbial Remediation

Microbial remediation is a useful asset for the clean-up of contaminations, however it very well may be slow. To give microorganisms a lift, researchers utilize a few strategies. Biostimulation adds supplements and oxygen to the climate, giving local organisms the assets they need to flourish and separate contaminations. Bioaugmentation presents uniquely picked organisms that target explicit pollutants. Moreover, specialists are investigating hereditary designs to make organisms with upgraded venality capacities. By consolidating these strategies, researchers are attempting to make microbial remediation quicker and more successful in handling natural contamination [60]. Usable circumstances, for example, temperature, pH, water content, and kinds of contaminations, influence the variation, improvement, and job of a bacterial strain. Besides, during the debasement interaction, metabolites can frame and lead to extra-natural issues since they might be harder to eliminate than the first compound, and this should be viewed as a downside. Soil dregs cannot just give the circumstances to human existence and microbial action yet in addition further develop water quality and go about as a cradle against ozone-harming substances in the climate, and can advance microbial remediation [61]. However, with the massive increase in the discharge of industrial waste, the improper use of agricultural pesticides, the improper extraction of petroleum, and a variety of pollutants entering soil/sediments in a variety of ways, all of these issues can be addressed by increasing microbial remediation [62]. The advancement and culture of the utilitarian microbial resident area are vital to the remediation execution. By and large, toxin evacuation requires different microbial exercises. Besides, various microorganisms are expected for the expulsion of explicit toxins [63].

## 6. Phytodegradation

In this process, plants use and beat poisons inside plant tissues. It entails the deterioration of complex natural molecules to basic particles or incorporating them into plant tissues [64]. Some problems have been remedied by Phytodegradation. Natural impurities, like chlorinated solvents, and herbicides also, can address foreign substances in soil, dregs, or groundwater [65].

Phytodegradation, additionally called phytotransformation, is the breakdown of impurities taken up by plants through metabolic cycles inside the plant, or the process by which contaminants outside the plant are broken down by the impact of mixtures (like catalysts) created by the plants. Poisons are corrupted, integrated into the plant tissues, and utilized as supplements [66]. The concentration throws natural atoms that are debased into less complex particle pollutants in soils, silt, wastes, and groundwater mediums. Similar to phytoextraction and phytovolatilization, plant take-up happens when the toxins' solvency and hydrophobicity fall into a specific fine reach. Plant decomposition has been seen to remediate a few natural foreign substances, like chlorinated solvents, herbicides, and weapons, and it can address toxins in soil, residue, or groundwater [67-68].

## 7. Role of Plant-Microbe Interactions in the Remediation of Pesticides

Plants and microorganisms are not solitary individuals in that frame of mind against pesticide contamination. They collaborate for a strong bioremediation methodology; this plant-microorganism communication makes light of an urgent occupation in breaking or changing pesticides into innocuous or dormant mixtures. Some of the pesticide metabolites that have broken down are taken up by plants. They may likewise deliver intensifies that stimulate the development and movement of their microbial accomplices, making a helpful cycle [79]. The escalated farming works on utilizing synthetic added substances have expanded the pesticide intensification amount in the dirt eco-profile. Until this point, the utilization of pesticides is thought of as satisfactory for safeguarding plant crops from different pests. However, at that point, only 1% of the pesticide arrives at the nuisance, while the leftover holds in the dirt, and water environment, in this way entering the well-established pecking order and eventually influencing vegetation, fauna, and humankind [80-81]. For example, pesticide-debased soil influences horticulture biology by influencing the dirt parts viz., microflora, proteins, supplements, and so on. Organisms are pervasive in regular environments. The connection of organisms with plants has both advantageous and adverse consequences. Plants and microorganisms are straightforwardly subject to one another for endurance, development, and advancement. The growth and development of plants can be facilitated by microbes by performing a variety of metabolic functions. Indeed, even microorganisms are exceptionally valuable in the bioremediation of pesticides. Plants likewise face dangers as microbial microorganisms. Unfavorably microorganisms get the danger from the plants. The protection component of the plants is exact and opposes pathogenesis [83-85]. Plant microorganism communications direct the arsenic bioavailability alongside giving resistance to plants in arsenic-sullied conditions, Microorganisms confine the bioavailability of arsenic to plants, either by animating plant development or by colonizing in the rhizosphere to weaken arsenic focus by oxidizing arsenic to arsenate [86-87]. Certain rhizospheric bacterial networks are hereditarily changed to create explicit proteins that assume a part in corrupting poisonous contaminations. Hardly any examinations recommend that the overexpression of extracellular catalysts discharged by plants, parasites, or rhizospheric microorganisms can work on the corruption of explicit natural toxins in the dirt. Microbes benefit from acquiring nutrients from root exudates, whereas plants benefit from certain plant growth-promoting metabolites, nitrogen fixation, phosphate solubilization, auxin production, siderophore production, and inhibition or suppression of pathogens produced by microbes. In this manner, the plant-organism collaboration lays out the groundwork of the dirt supplement cycle as well as diminishes soil poisonousness by the evacuation of hurtful contaminations [90-92].

**8. Conclusion**

In the last few years, people have become in contact with a wide range of toxins due to the rapid advancement of technology. Chemicals such as pesticides are one category of them. Despite their great utility, pesticides can be dangerous for the ecosystem, biodiversity, and food security. Pesticide pollution is now a worldwide problem, and recently this issue has gained popularity among the stakeholders. Several studies suggest that environmental and public health issues have emerged as major challenges for these nations. Effective pesticide removal often requires a variety of strategies that include ecological as well as physicochemical processes. Among all, diazotrophs-assisted phytoremediation of pesticides is the best approach. Microbes degrade pesticides with the help of enzymes. This process involves three stages. Stage one reactions oxidize, decrease, and hydrolyze the hazardous chemical to make it soluble in water and relatively less poisonous. Conjugation, or the conversion of the pesticide molecule into an amino acid or sugar, is a stage two reaction. Also, it increases the toxin's water solubility and renders it more naturally innocuous. Secondary conjugation occurs in stage three reactions, which entirely dissolve the parent substance and produce byproducts like $CO_2$ and water that can provide microorganisms with sustenance. The molecular mechanisms underlying pesticide degradation are still poorly understood, necessitating more study and investigation. The main focus should be on choosing the right microorganisms for bioremediation and creating efficient, eco-friendly solutions to the problems posed by pesticide residues. By taking steps like this, we may contribute to sustainable agriculture and the preservation of environmental balance. Diazotrophs assisting in the phytoremediation mechanism recommend investigating this innovative strategy for upcoming remediation research.

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