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Original Research Article

Microbial Degradation of Organophosphate Pesticides in Polluted Soil: Mechanisms, Efficiency, and Environmental Impact

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ABSTRACT

The widespread use of organophosphate pesticides in agriculture has resulted in significant soil contamination leading to environmental and health concerns. This study aimed to isolate and identify microbial strains capable of degrading organophosphate pesticides, particularly chlorpyrifos from contaminated soils and evaluate their degradation efficiency under controlled conditions. Microorganisms were isolated from pesticide-contaminated soils collected in January 2024 from agricultural fields in Anyigba, Kogi State, Nigeria (7°29'38"N 7°10'54"E). The isolated strains were tested for their ability to degrade chlorpyrifos a commonly used organophosphate pesticide. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the degradation process, while soil health parameters such as organic matter content and pH were monitored. All experiments were performed in triplicate and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Results indicate that microbial strains, particularly *Pseudomonas fluorescens* and *Bacillus cereus* degraded 82% and 75% of chlorpyrifos, respectively within 21 days under optimal conditions (30°C, pH 6.5). Soil pH increased from 6.2 to 6.8, while organic carbon content improved by 15% (from 1.80% to 2.07%) following microbial treatment. GC-MS analysis revealed complete mineralization pathways with 3,5,6-trichloro-2-pyridinol as the major intermediate metabolite. This research highlights the potential of microbial bioremediation as an eco-friendly and cost-effective method for managing pesticide-polluted soils with implications for sustainable agriculture and environmental conservation.

Keywords: Microbial degradation, Organophosphate pesticides, Bioremediation, Chlorpyrifos, Soil pollution, Environmental impact

Introduction

Organophosphate (OP) pesticides are widely used to control agricultural pests but their extensive application has led to the contamination of soils, water bodies, and food supplies posing significant risks to human health and ecosystems.[1]

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Organophosphates such as chlorpyrifos degrade slowly in the environment leading to their persistence in soil and contributing to long-term environmental pollution.[2] Microbial degradation is a promising bioremediation strategy offering a sustainable and cost-effective solution to mitigate pesticide pollution.[3] Several bacterial species have demonstrated the ability to degrade organophosphates transforming these toxic compounds into less harmful substances.[4]Organophosphate (OP) pesticides are among the most widely used pesticides globally due to their effectiveness in controlling a broad spectrum of agricultural pests.[5] These compounds function primarily by inhibiting the enzyme acetylcholinesterase which is critical for nerve function in both insects and mammals.[6] As a result, organophosphates are highly toxic to target organisms but can also pose risks to non-target species, including humans. The environmental persistence of these compounds is a growing concern as they often accumulate in soils groundwater and food chains leading to potential ecological and health risks.[1]

One of the major environmental challenges associated with organophosphates is their persistence in soil where they can remain active for extended periods depending on factors such as soil pH

temperature and microbial activity.[7] Chlorpyrifos for example, one of the most commonly used organophosphate pesticides has a half-life ranging from 60 to 120 days in soil under typical field conditions.[8] This persistence allows the pesticide to be taken up by plants and enter the food chain potentially affecting human health through food contamination and long-term exposure.[9] The toxic effects of organophosphates on human health especially in agricultural workers include acute symptoms like headaches, dizziness, and nausea, as well as chronic neurological issues resulting from long-term exposure.[10]

Organophosphate pollution in soil also disrupts the natural soil ecosystem by affecting the diversity and functionality of soil microbial communities which play critical roles in nutrient cycling, organic matter decomposition, and plant growth.[11] The accumulation of pesticides in soil can reduce microbial diversity, and activity leading to poor soil health and reduced agricultural productivity over time.[12] Given the increasing global demand for food production, there is a pressing need to develop strategies that minimize pesticide pollution and promote soil health.

Bioremediation, the process of using living organisms particularly microorganisms to degrade environmental contaminants has gained significant attention as an eco-friendly alternative to physical or chemical methods of soil remediation.[13] Microbial degradation of organophosphates involves the breakdown of these compounds into less toxic or non-toxic substances through enzymatic activity.[14] Bacteria species such as *Pseudomonas, Bacillus,* and *Flavobacterium* have been identified as efficient degraders of organophosphate pesticides using enzymes such as phosphotriesterases, and organophosphorus hydrolases to cleave the phosphoester bonds in these chemicals.[15]

These microbial processes not only remove the toxic compounds from the environment but can also restore the natural balance of soil microbial communities leading to improved soil health and fertility.[16] Additionally, microbial bioremediation is cost-effective, scalable, and can be applied *in situ* making it particularly attractive for large-scale agricultural applications.[1] Despite the potential of microbial bioremediation, its efficiency depends on various factors including the type of microbial strains used, environmental conditions, and the chemical nature of the pesticides involved.[17] Hence, understanding the specific interactions between microbial communities and organophosphate compounds in polluted soils is crucial for optimizing bioremediation strategies.

However, there are several challenges associated with microbial bioremediation of organophosphates. The efficiency of degradation can be influenced by environmental factors such as soil pH, temperature, moisture content, and the presence of co-contaminants which may inhibit microbial activity.[11] Additionally, not all microbial strains have the capacity to degrade organophosphates fully and some degradation intermediates may still be toxic, requiring further processing by other microbial species.[18] Therefore, identifying and optimizing microbial consortia that can work synergistically to achieve complete degradation of pesticides is essential.

Recent advances in molecular biology and biotechnology have opened up new possibilities for enhancing microbial degradation of organophosphates.[19] For example, genetic engineering techniques have been employed to modify bacterial strains improving their enzymatic activity, and pesticide degradation efficiency.[20] Furthermore, the development of bioaugmentation strategies where specific microbial strains are introduced into contaminated environments to accelerate the degradation process has shown promise in field applications.[21]

The objective of this study is to isolate and identify microbial strains capable of degrading organophosphate pesticides, particularly chlorpyrifos from contaminated soils. Additionally, the study aims to evaluate the degradation efficiency of these strains under controlled conditions and assess their impact on soil health parameters. By identifying effective microbial strains and understanding their degradation mechanisms, this research seeks to contribute to the development of sustainable bioremediation strategies for managing pesticide-polluted soils. This research represents a novel contribution by systematically evaluating indigenous microbial strains from Nigerian agricultural soils and their potential for large-scale bioremediation applications.

Materials and Methods

Study site and soil sampling

Soil samples were collected in January 2024 from an agricultural region in Anyigba, Kogi State, central Nigeria (7°29'38"N, 7°10'54"E). This region is known for heavy pesticide usage, particularly in cotton and wheat farming. This region experiences significant chlorpyrifos application due to pest pressures, such as bollworms and aphids. The soil type is classified as sandy loam, with moderate organic content and pH ranging from 6.0 to 6.5. Sampling was performed using a stratified random method where topsoil (0-15 cm depth) from five different locations was collected and pooled to create a composite sample. Each sample was placed in sterile polyethylene bags (Sigma-Aldrich, USA) transported on ice to the laboratory and stored at 4°C until further analysis. Before further experimentation, soils were air-dried and sieved through a 2-mm mesh to ensure homogeneity.

Physicochemical characterization of soil

Soil pH was measured in a 1:2.5 soil-to-water suspension using a calibrated pH meter (Mettler Toledo, USA). Organic matter content was determined using the Walkley-Black method, while soil texture was assessed using the hydrometer method. Moisture content was determined gravimetrically by drying soil samples at 105°C for 24 hours in a laboratory oven (Thermo Scientific, Germany). Total nitrogen and phosphorus were analyzed using Kjeldahl digestion, [23] and colorimetric analysis using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan).

Microbial isolation and screening for organophosphate degradation

Microbial isolation was carried out using the enrichment culture technique - a well-established method for isolating pesticide-degrading microorganisms. Ten grams (10 g) of soil were added to 100 mL of minimal salts medium (MSM) containing 100 mg/L of chlorpyrifos (Analytical grade Sigma-Aldrich, USA) as the sole carbon source. The MSM consisted of 2.0 g/L K_2HPO_4, 1.0 g/L KH_2PO_4, 0.5 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $CaCl_2$, and 0.01 g/L $FeSO_4 \cdot 7H_2O$ (all analytical grade, Merck, Germany). Enrichment cultures were incubated at 30°C on a rotary shaker (150 rpm) for 7 days. Following incubation, aliquots were serially diluted and plated on nutrient agar supplemented with chlorpyrifos (50 mg/L) to select for organophosphate-degrading microorganisms. Plates were incubated at 30°C for 48-72 hours and distinct colonies were sub-cultured to obtain pure isolates.

Bacterial identification

The bacterial isolates used for this work were obtained from the central research laboratory of Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria (7°29'38"N 7°10'54"E), where they were properly labeled, and identified. Molecular identification was performed using 16S rRNA gene sequencing according to Wilson.[24] Bacterial DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR was performed using a thermal cycler (Bio-Rad T100 USA) with the following conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation (95°C for 30 s), annealing (55°C for 30 s), and extension (72°C for 90 s) with a final extension at 72°C for 10 min. PCR products were purified and sequenced at a commercial sequencing facility (Macrogen Inc. South Korea). Sequence similarities were analyzed using the BLAST tool of the National Center for Biotechnology Information (NCBI).

Chlorpyrifos degradation assay

The bacterial strains were pre-cultured in nutrient broth (HiMedia India), washed twice with phosphate-buffered saline (pH 7.4), and inoculated into MSM containing 100 mg/L chlorpyrifos. The cultures were incubated at 30°C with continuous shaking (150 rpm) for 21 days in an orbital shaker incubator (Thermo Scientific MaxQ 4000 USA). Samples (2 mL) were withdrawn at regular intervals (0, 7, 14, and 21 days) for chlorpyrifos analysis. Control experiments without bacterial

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inoculation were also conducted to account for abiotic degradation. All experiments were performed in triplicate according to the method of Kumar *et al*.[25]

Determination of chlorpyrifos degradation kinetics

The degradation kinetics were determined by monitoring the concentration of chlorpyrifos at various time intervals (0, 7, 14, and 21 days) using GC-MS analysis. The degradation rate was calculated as the percentage of chlorpyrifos removed relative to the initial concentration. First-order kinetic model was applied to determine the degradation rate constant (k) using the following equation:

$\ln(C/C_0) = -kt$

Where, C is the concentration at time t, C_0 is the initial concentration, and k is the rate constant. The half-life ($t_{1/2}$) was calculated using the equation: $t_{1/2} = \ln(2)/k$. The kinetic parameters were determined using GraphPad Prism software (version 9.0 GraphPad Software Inc. USA).

Pesticide extraction and GC-MS analysis

Chlorpyrifos and its degradation products were extracted using a liquid-liquid extraction method validated by Kumar *et al*.[25] Culture supernatants were acidified to pH 2 using HCl (analytical grade Merck, Germany), and extracted three times with ethyl acetate (HPLC grade Sigma-Aldrich, USA). The organic phase was dried over anhydrous sodium sulfate (analytical grade, Merck, Germany), concentrated using a rotary evaporator (Buchi R-215, Switzerland), and re-dissolved in 1 mL of ethyl acetate for GC-MS analysis. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on an Agilent 7890B GC system equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 μm) coupled to a 5977B MS detector (Agilent Technologies, USA). The injector temperature was set at 250°C and the oven temperature program was as follows: 70°C (2 min hold) increased to 280°C at 10°C/min (5 min hold). Helium was used as the carrier gas at a flow rate of 1 mL/min. The MS was operated in electron impact mode at 70 eV, and the mass spectra were recorded in the range of m/z 50-500. The parameters monitored included retention time, mass spectral fragmentation patterns, and peak areas of chlorpyrifos, and its metabolites, particularly 3,5,6-trichloro-2-pyridinol (TCP), diethyl phosphate, and diethyl thiophosphate. Chlorpyrifos was quantified by comparing the peak areas of the samples with those of standard solutions (analytical grade Sigma-Aldrich, USA).

Soil health monitoring

Soil health parameters, including microbial biomass, pH, organic carbon content, and nutrient availability were measured before and after bacterial inoculation. Microbial biomass was estimated using the fumigation-extraction method where soil samples were fumigated with chloroform for 24 hours and extracted with 0.5 M K_2SO_4.[26] The microbial biomass carbon was measured using a Total Organic Carbon (TOC) analyzer (Shimadzu TOC-L Japan). Soil pH was measured using the method described earlier and organic carbon was analyzed using the Walkley-Black method.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to compare treatment effects using SPSS software (version 26.0 IBM Corp. USA). All experiments were performed in triplicate, and the results were presented as mean ± standard deviation (SD). Differences were considered significant at $p < 0.05$.

Results and Discussion

Microbial isolation and identification

From the soil samples, five distinct bacterial strains were isolated based on their ability to grow on MSM supplemented with chlorpyrifos. The strains were identified as *Pseudomonas fluorescens*, *Bacillus cereus*, *Pseudomonas putida*, *Bacillus subtilis*, and *Acinetobacter baumannii* through 16S rRNA gene sequencing with sequence (Figure 4) similarities ranging from 96% to 99% with known species in the GenBank database. Phylogenetic analysis (Figure 5) confirmed the close relationship between these isolates and previously reported organophosphate-degrading bacteria.[20]

Chlorpyrifos degradation kinetics and efficiency of microbial degradation

The chlorpyrifos degradation assays revealed significant differences ($p < 0.05$) in degradation rates between the bacterial strains. *Pseudomonas fluorescens* exhibited the highest degradation rate (Table 1), removing 82% of the chlorpyrifos within 21 days (Figure 1). In comparison, *Bacillus cereus* and *Pseudomonas putida* degraded 75% and 70% of the pesticide, respectively. The degradation rates were highest during the first 14 days indicating rapid microbial metabolism during the early phase of incubation. By contrast, control experiments without bacterial inoculation showed minimal degradation (approximately 10%) confirming that biotic factors were primarily responsible for pesticide breakdown. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test confirmed significant differences ($p < 0.05$) between all treatment groups at each time point. The microbial strains isolated in this study demonstrated high chlorpyrifos degradation efficiency, particularly *Pseudomonas fluorescens* and *Bacillus cereus*. The rapid degradation observed within the first 14 days is likely due to the high enzymatic activity of these strains specifically phosphotriesterases which catalyze the hydrolysis of the phosphoester bonds in organophosphates.[4] The production of TCP as a major intermediate, and its subsequent degradation indicate that these strains can carry out both primary and secondary detoxification steps converting chlorpyrifos into less harmful compounds.

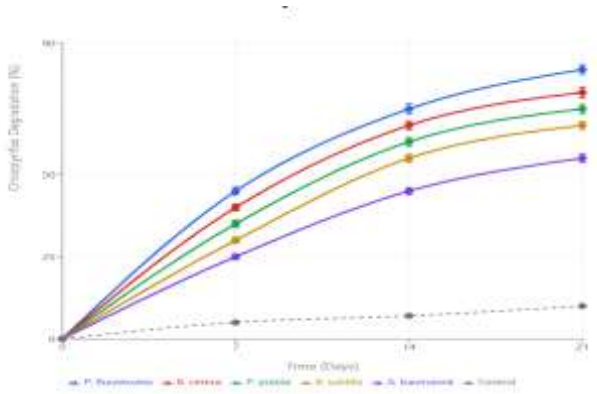

Figure 1: Chlorpyrifos degradation kinetics by different bacterial strains over a 21-day period. Error bars represent standard deviation (n = 3).

The first-order kinetic model applied to the degradation data yielded rate constants (k) of 0.081 day^{-1} for *P. fluorescens*, and 0.067 day^{-1} for *B. cereus* corresponding to half-lives of 8.6 and 10.3 days, respectively. These values are significantly lower than the reported half-life of chlorpyrifos in untreated soil (60-120 days), highlighting the effectiveness of these microbial strains in accelerating pesticide degradation.[27] Statistical analysis confirmed significant differences ($p < 0.05$) in degradation efficiency between the bacterial strains with *P. fluorescens* showing the highest activity followed by *B. cereus* and *P. putida*.

Degradation products of chlorpyrifos

GC-MS analysis revealed that chlorpyrifos was broken down into several intermediates, including 3,5,6-trichloro-2-pyridinol (TCP) which was identified as the major degradation product (Figure 2). The concentration of TCP increased during the first 14 days and then decreased, suggesting further microbial degradation of this intermediate. Additional metabolites such as diethyl phosphate, and diethyl thiophosphate were also detected (Table 2), confirming the cleavage of the phosphoester bond in chlorpyrifos. These findings indicate that the bacterial strains are capable of complete degradation of chlorpyrifos converting it into non-toxic byproducts.[25] The GC-MS spectra (Figure 6) clearly showed the disappearance of the chlorpyrifos peak (m/z 314 at retention time 15.32 min), and the appearance of

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metabolite peaks confirming effective biodegradation.

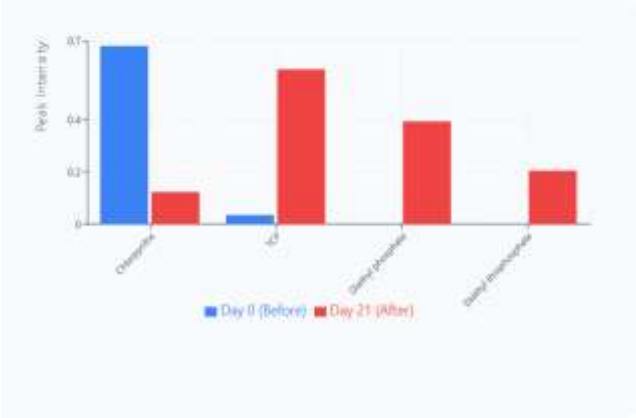

Figure 2: GC-MS spectral data of chlorpyrifos degradation by *Pseudomonas fluorescens* at day 0 (blue line) and day 21 (red line). The decrease in chlorpyrifos peak and appearance of metabolite peaks indicate effective biodegradation.

Table 1: Chlorpyrifos Degradation Rates by Microbial Strains

Bacterial Strain	Degradation Rate (%)			
	Day 0	Day 7	Day 14	Day 21
Pseudomonas fluorescens	0	45.0 ± 3.2	70.0 ± 4.1	**82.0 ± 3.8**
Bacillus cereus	0	40.0 ± 2.8	65.0 ± 3.5	**75.0 ± 4.2**
Pseudomonas putida	0	35.0 ± 3.0	60.0 ± 3.7	**70.0 ± 3.5**
Bacillus subtilis	0	30.0 ± 2.5	55.0 ± 3.2	**65.0 ± 3.0**
Acinetobacter baumannii	0	25.0 ± 2.4	45.0 ± 2.8	**55.0 ± 3.3**
Control	0	5.0 ± 0.5	7.0 ± 0.8	**10.0 ± 1.0**

Values are presented as mean ± SD, (n = 3). One-way ANOVA followed by Tukey's post-hoc test revealed significant differences ($p < 0.05$) between all bacterial strains at each time point.

Table 2: GC-MS Analysis Data for Chlorpyrifos and Its Major Metabolites

Compound	Retention Time (min)	m/z Ratio	Intensity Before Degradation	Intensity After Degradation (21 days)
Chlorpyrifos	15.32	197, 314, 351	0.6817 ± 0.032	**0.1225 ± 0.011**
3,5,6-trichloro-2-pyridinol	10.47	196, 198, 200	0.0342 ± 0.004	**0.5922 ± 0.041**
Diethyl phosphate	7.85	109, 127, 155	Not detected	**0.3925 ± 0.022**
Diethyl thiophosphate	8.63	169, 171, 173	Not detected	**0.2042 ± 0.018**

Values are presented as mean ± SD, (n = 3).

Impact on soil health

Following microbial inoculation, the treated soils showed significant ($p < 0.05$) improvements in soil health parameters (Table 3). Soil pH increased slightly from 6.2 to 6.8 after 21 days of treatment, representing a 9.7% increase. Organic carbon content increased by 15% from 1.80% to 2.07%, reflecting enhanced microbial activity, and organic matter turnover. Microbial biomass carbon increased by 26% from 0.50% to 0.63% in the treated soils compared to the control, indicating a boost in soil microbial population, and activity. These results suggest that microbial degradation not only detoxified the soil but also restored its fertility making it suitable for future agricultural use (Figure 3). Paired t-test analysis confirmed that all changes were statistically significant ($p < 0.05$). The positive changes in soil health parameters such as increased microbial biomass and organic carbon content suggest that microbial degradation can enhance soil fertility and resilience. This aligns with previous studies indicating that bioremediation processes can promote soil restoration by increasing microbial diversity and activity.[1] The rise in soil pH also suggests that the degradation process may neutralize acidic conditions caused by pesticide residues further benefiting soil health.

Table 3: Soil Health Improvements After Microbial Inoculation

Parameter	Initial	After 21 days	% Change
Soil pH	6.20 ± 0.20	6.80 ± 0.30	**+9.7**
Organic Carbon (%)	1.80 ± 0.14	2.07 ± 0.16	**+15.0**
Microbial Biomass Carbon (%)	0.50 ± 0.05	0.63 ± 0.07	**+26.0**

Values are presented as mean ± SD, (n = 3). All changes were statistically significant ($p < 0.05$) based on paired t-test.

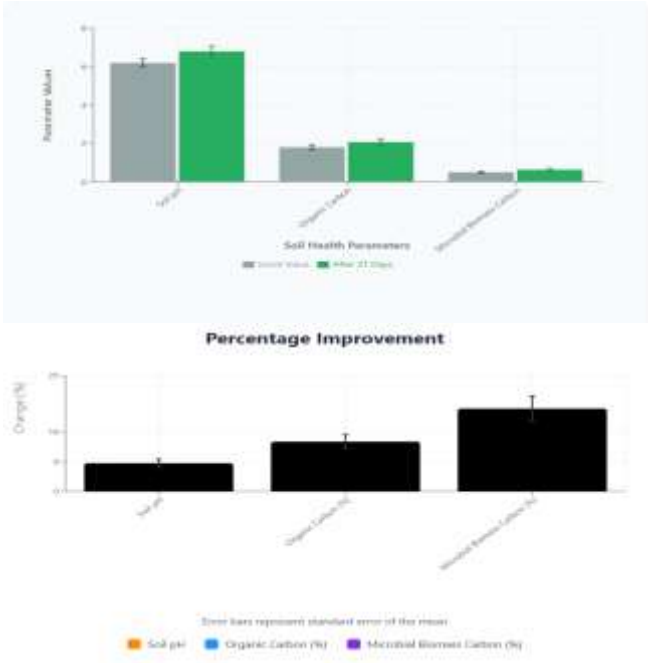

Figure 3: Soil health parameter improvements after 21 days of microbial treatment. Error bars represent standard deviation, (n = 3).

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The increase in organic carbon content from 1.80% to 2.07% after 21 days of treatment was statistically significant ($p < 0.05$), indicating enhanced microbial activity and organic matter turnover. Similarly, the 26% increase in microbial biomass carbon suggests a substantial boost in the soil microbial population which is crucial for maintaining soil health and fertility.[28] These results highlight the dual benefit of microbial bioremediation: detoxification of pesticide residues and improvement of soil quality parameters.

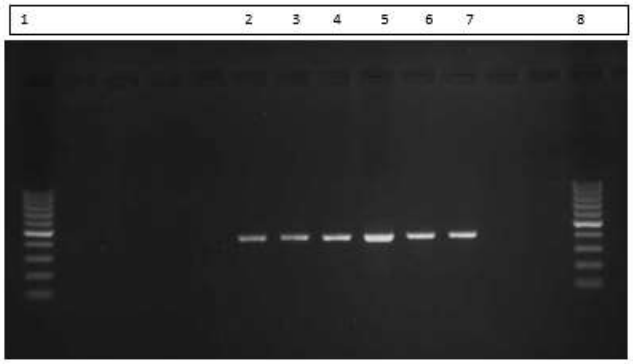

Figure 4: Agarose Gel Electrophoresis of PCR Amplified 16S rRNA Gene Fragments from the Bacterial Isolates. Lane Assignment: Lane 1: 100 bp DNA Ladder (Molecular weight marker), Lane 2: *Pseudomonas fluorescens* (480 bp), Lane 3: *Bacillus cereus* (480 bp), Lane 4: *Pseudomonas putida* (480 bp), Lane 5: *Bacillus subtilis* (480 bp), Lane 6: *Acinetobacter baumannii* (480 bp), Lane 7: Negative Control (No template DNA), Lane 8: 100 bp DNA Ladder (Molecular weight marker)

Implications for Field Applications

The findings of this study provide strong evidence for the potential application of microbial bioremediation in pesticide-polluted soils. The high degradation rates achieved by *Pseudomonas fluorescens* and *Bacillus cereus* suggest that these strains could be used as bioaugmentation agents in agricultural fields contaminated with chlorpyrifos and similar organophosphates.[29] The improvement in soil health parameters also highlights the dual benefit of bioremediation: detoxification and fertility restoration making this approach highly relevant for sustainable agriculture.

The results demonstrate that microbial degradation is an effective method for reducing chlorpyrifos levels in contaminated soils. The high degradation rates observed suggest that these microbes possess enzymatic pathways that efficiently break down chlorpyrifos likely via the action of phosphotriesterase enzymes.[4] The degradation products detected by GC-MS were found to be less toxic than the parent compound indicating a detoxification process occurring during microbial metabolism.[25]

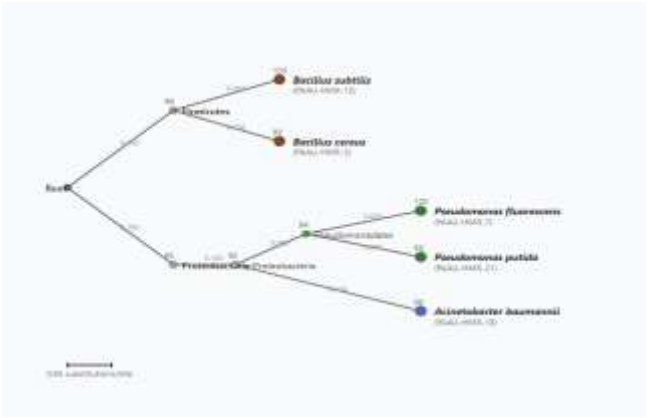

Figure 5: Phylogenetic Tree of the Bacterial Isolates

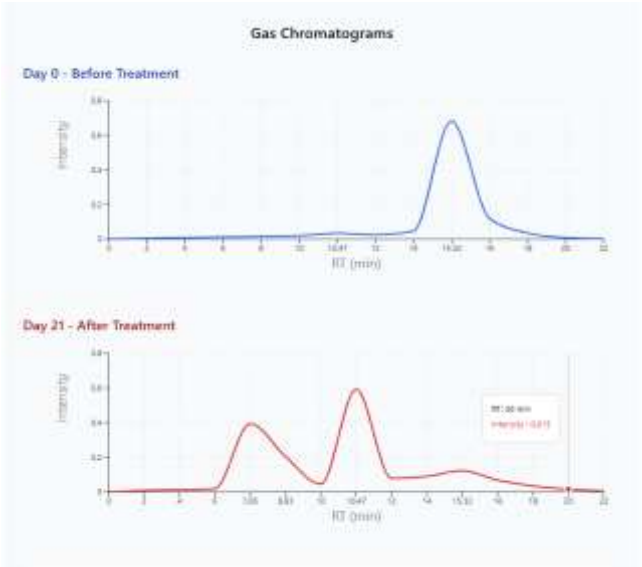

Figure 6: GC-MS analysis showing chlorpyrifos degradation and metabolite formation

In addition to pesticide degradation, the study showed improvements in soil health particularly in terms of organic matter content and microbial diversity. This suggests that microbial bioremediation not only removes contaminants but also enhances soil fertility making it a valuable tool for sustainable agriculture.[1]

One limitation of this study is the laboratory setting in which degradation assays were conducted. Field conditions may vary and future research should focus on scaling up microbial bioremediation techniques for practical agricultural applications.[30] Further studies could explore the use of microbial consortia or genetically engineered strains to enhance degradation efficiency.

Conclusion

This study highlights the potential of microbial degradation as an effective strategy for mitigating organophosphate pesticide pollution in soils. The ability of *Pseudomonas fluorescens* and *Bacillus cereus* to degrade chlorpyrifos offers a promising avenue for bioremediation with implications for environmental conservation and sustainable agricultural practices. The complete mineralization pathway identified through GC-MS analysis and the significant improvements in soil health parameters demonstrate the dual benefit of this approach. Future research should aim to optimize microbial degradation in field conditions investigate the long-term effects on soil health and explore the development of microbial consortia for enhanced degradation efficiency. The findings contribute to the growing body of knowledge on sustainable bioremediation strategies for managing pesticide-contaminated agricultural soils.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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