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Temporal Dynamics of Pyrethroid and Neonicotinoid Residues in Maize (*Zea mays*): Implications of Environmental Factors and Bioremediation Strategies

BY

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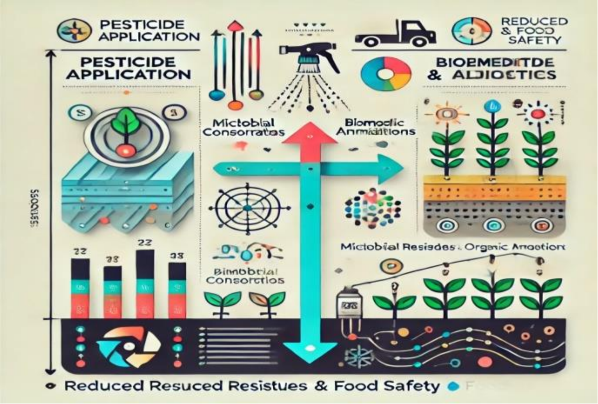
Abstract

This study investigates the dynamics of pesticide residues in maize cultivation, focusing on the temporal patterns of pyrethroids and neonicotinoids, their environmental influences, and the efficacy of bioremediation in Dera Ghazi Khan, Pakistan. Pesticide residues, including Cypermethrin, Lambda-cyhalothrin, Imidacloprid, and Thiamethoxam, were monitored throughout the growing season, revealing peak concentrations during mid-growth stages. The degradation of these pesticides was significantly influenced by temperature, soil moisture, and microbial activity, with pyrethroids degrading faster than neonicotinoids. The study also documented a decline in soil microbial diversity and activity, particularly in treated plots, indicating the negative impact of pesticide residues on soil health. Bioremediation trials using microbial consortia and organic amendments successfully reduced pesticide residues and partially restored microbial health, demonstrating a viable approach to mitigating pesticide contamination. These findings underscore the need for optimized pesticide application practices, integrated pest management strategies, and stringent regulatory measures to safeguard food safety and environmental health. The study concludes with recommendations for future research to explore sustainable agricultural practices and the potential of biopesticides in reducing chemical pesticide dependency.



Graphical Abstract:

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1. Introduction

Pesticide use has become an integral component of modern agricultural practices, particularly in the cultivation of staple crops like maize (*Zea mays*) (Hirwe et al., 2023). Maize, being one of the most widely grown cereal crops globally, requires significant pest management interventions to ensure high yields and crop quality (Asibe et al., 2023). Among the

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various classes of pesticides, pyrethroids and neonicotinoids have gained prominence due to their broad-spectrum efficacy and relatively low toxicity to non-target organisms. Pyrethroids, including Cypermethrin and Lambda-cyhalothrin, are synthetic derivatives of natural pyrethrins and are widely used for controlling a range of insect pests such as corn earworms and European corn borers (Abbas et al.; Gichere, 2023). These compounds act on the nervous systems of insects, leading to paralysis and death.

Neonicotinoids, such as Imidacloprid and Thiamethoxam, represent another significant class of insecticides that have been extensively used in maize cultivation (Zaharia et al., 2023). These chemicals are systemic, meaning they can be absorbed by plants and provide protection against sucking insects like aphids and whiteflies (Shareefdeen & Elkamel, 2024; Ullah et al., 2024a). The mechanism of action for neonicotinoids involves the disruption of the nicotinic acetylcholine receptors in the nervous system of insects, causing overstimulation and eventual paralysis (Araújo et al., 2023; Madesh et al., 2024). Despite their effectiveness, the persistence and accumulation of these pesticide residues in soil and crops have raised concerns about their long-term environmental impact and potential health risks (Zhang et al., 2023a; Zhang et al., 2023b).

Dera Ghazi Khan, located in the southern part of Punjab, Pakistan, is an agriculturally rich region characterized by its diverse climatic conditions and fertile alluvial soils (Ahmad et al., 2023; Ullah et al., 2024c). The area experiences a semi-arid climate with hot summers and mild winters, making it conducive for the cultivation of various crops, including maize (Baig et al., 2024; Malik, 1963). The agricultural practices in Dera Ghazi Khan are heavily reliant on the use of chemical pesticides for pest control, given the prevalence of pest infestations that can significantly reduce crop yields (Abbas et al., 2023; Ummer et al., 2023). The region's farmers commonly use pyrethroids and neonicotinoids due to their effectiveness in managing pest populations (Haidri et al., 2024; Shamraiz et al., 2023).

The widespread use of these pesticides in Dera Ghazi Khan has led to concerns about the potential contamination of soil and water resources, as well as the accumulation of residues in food crops (Hassan et al., 2023; Waseem et al., 2023). Previous studies have indicated that the improper use of pesticides, such as excessive application rates and poor timing, can lead to residue persistence in the environment, posing risks to human health and non-target organisms (Kaur et al., 2024; Leskovac & Petrović, 2023). Therefore, understanding the dynamics of pesticide residues in the soil-plant system is crucial for developing sustainable agricultural practices and ensuring food safety in the region.

The study of pesticide residue dynamics is essential for several reasons. First, it provides insights into the behavior of pesticides in the environment, including their degradation, persistence, and potential to bioaccumulate in the food chain. For instance, pyrethroids are known to have relatively short half-lives in the environment due to their susceptibility to photodegradation and microbial activity (Fatima et al., 2024; Majid et al., 2023). However, their strong adsorption to soil particles can lead to prolonged persistence in certain conditions. Neonicotinoids, on the other hand, can persist in soil and water bodies for extended periods due to their chemical stability and high water solubility, raising concerns about their long-term ecological effects (Stehle et al., 2023; Zhang et al., 2023b).

Understanding these dynamics is also critical for assessing the potential risks associated with pesticide residues in food products. Regulatory agencies set maximum residue limits (MRLs) for pesticides in food to protect consumer health. However, residues exceeding these limits can occur due to factors such as non-compliance with recommended application practices, environmental conditions, and crop variety (Kubiak-Hardiman et al., 2023; Ullah et al., 2024b). By studying the factors influencing residue levels in crops like maize, we can develop better management practices to minimize these risks. This study aims to investigate the temporal patterns of pyrethroid and neonicotinoid residues in maize and soil in Dera Ghazi Khan over a growing season. The specific objectives are to quantify the residue levels of Cypermethrin, Lambda-cyhalothrin, Imidacloprid, and Thiamethoxam at different growth stages of maize, analyze their degradation rates and persistence, and assess the influence of environmental factors such as temperature, soil moisture, and microbial activity on these residues. Additionally, the study seeks to evaluate the impact of these residues on soil health, particularly soil microbial communities, and provide recommendations for sustainable pesticide management practices in the region. The potential environmental and health impacts of pesticide residues are significant. Pyrethroids, despite their effectiveness in pest control, have been shown to affect non-target organisms, including beneficial insects and aquatic life, due to their neurotoxic properties. Neonicotinoids have also been implicated in the decline of pollinator populations, particularly bees, which are crucial for ecosystem functioning and crop pollination. Furthermore, the presence of pesticide residues in food can pose health risks to consumers, especially if residues exceed the established MRLs. Long-term exposure to certain pesticides has been linked to various health issues, including neurological disorders and endocrine disruption.

This study's findings will have important implications for agricultural practices in Dera Ghazi Khan and similar agro-ecological zones. By providing a detailed understanding of the residue dynamics of commonly used pesticides, the study will inform better management practices, such as optimized pesticide application timings and dosages, to minimize environmental contamination and ensure food safety. Moreover, the results will contribute to the broader discourse on sustainable agriculture, highlighting the need for integrated pest management strategies that reduce reliance on chemical pesticides and promote ecological balance.

2. Methodology

2.1 Study Design

The study was meticulously designed to investigate the temporal dynamics of pyrethroid and neonicotinoid pesticide residues in maize cultivation within Dera Ghazi Khan. The experimental layout comprised a selection of sampling sites across several maize fields, chosen based on their history of pesticide use and accessibility for repeated sampling. The sites were strategically located to cover a representative range of environmental conditions, including variations in soil type, irrigation practices, and microclimates. The timeline for the study spanned an entire growing season, from pre-planting through to post-harvest, ensuring comprehensive data collection at critical stages of crop development.

2.2 Sample Collection and Preparation

Sampling Schedule:

The sampling process was structured around five key stages of maize growth: pre-planting, early growth, mid-growth, pre-harvest, and post-harvest. At each stage, samples were collected to capture the dynamics of pesticide residues in both soil and plant tissues. Pre-planting samples provided baseline data, while subsequent samples tracked changes in residue levels and distribution.

Sample Types:

Samples included soil and various maize plant parts—roots, stems, leaves, and grains. Soil samples were taken from the top 15 cm layer, which is most likely to accumulate pesticide residues. Plant samples were collected by harvesting entire plants and subsequently separating them into the respective parts for individual analysis.

Sample Processing:

Upon collection, soil samples were air-dried, ground, and sieved to achieve a uniform particle size, facilitating accurate analysis. Plant samples were washed with deionized water to remove surface contaminants, then air-dried and ground into a fine powder. All samples were stored in labeled polyethylene bags at -20°C until analysis to prevent degradation of the pesticides. Table 1 outlines the sampling schedule and key activities conducted at each stage of the maize growing season, from pre-planting in March to post-harvest in August 2023. It includes details on the types of samples collected and the environmental data recorded during each sampling event. This information is critical for understanding the temporal dynamics of pesticide residues in soil and maize plants.

Table 1: Sampling Schedule and Details

Sampling Stage	Date Range	Sampling Activities	Number of Samples	Sampling Depth (Soil)	Sample Types	Environmental Data Collected
Pre-planting	March 1-15, 2023	Baseline soil sampling, initial environmental data	15	0-15 cm	Soil	Temperature, Soil Moisture
Early Growth	April 10-20, 2023	Soil and plant sampling (roots, stems)	15	0-15 cm	Soil, Roots, Stems	Temperature, Soil Moisture, Microbial Activity
Mid-Growth	May 15-25, 2023	Comprehensive plant sampling (leaves, stems)	15	0-15 cm	Soil, Leaves, Stems	Temperature, Soil Moisture, Microbial Activity
Pre-Harvest	July 1-10, 2023	Final plant sampling (leaves, grains)	15	0-15 cm	Soil, Leaves, Grains	Temperature, Soil Moisture, Microbial Activity
Post-Harvest	August 5-15, 2023	Residue and environmental data collection	15	0-15 cm	Soil, Residual Plant Material	Temperature, Soil Moisture

2.3 Pesticide Residue Analysis

Analytical Techniques:

The detection and quantification of pesticide residues were conducted using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). GC-MS was employed for the analysis of pyrethroids, leveraging its high sensitivity and specificity for volatile and semi-volatile compounds. LC-MS was used for neonicotinoids, as it is well-suited for the analysis of polar compounds and provides excellent quantitation capabilities. Table 2 provides detailed information on the calibration and validation parameters for the analytical methods used in detecting and quantifying pesticide residues. It includes the calibration range, limits of detection (LOD), limits of quantification (LOQ), recovery percentages, and the coefficient of determination (R^2) for the calibration curves. This data ensures the accuracy and reliability of the residue analysis.

Table 2: Calibration and Validation Data

Pesticide	Detection	Calibration	LOD	LOQ	Recovery	R^2 (Calibration

	Method	Range (µg/kg)	(µg/kg)	(µg/kg)	(%)	Curve)
Cypermethrin	GC-MS	0.1 - 500	0.05	0.1	95 ± 3	0.998
Lambda-cyhalothrin	GC-MS	0.1 - 500	0.05	0.1	92 ± 4	0.997
Imidacloprid	LC-MS	0.05 - 1000	0.02	0.05	90 ± 5	0.999
Thiamethoxam	LC-MS	0.05 - 1000	0.02	0.05	93 ± 3	0.998

Calibration and Validation:

To ensure the accuracy and reliability of the analytical results, calibration curves were prepared for each pesticide, using standard solutions at varying concentrations. These curves were used to calculate the concentration of pesticides in the samples. Recovery tests were performed by spiking known quantities of pesticides into blank samples, followed by extraction and analysis, to assess the method's accuracy. Method validation parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy, were thoroughly evaluated to confirm the robustness of the analytical procedures. The validation process ensured that the methods could reliably detect and quantify pesticide residues at levels relevant to the study.

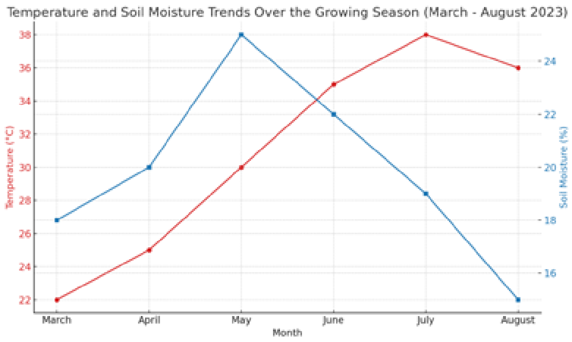
2.4 Environmental Monitoring

To understand the influence of environmental factors on pesticide residue dynamics, several key parameters were monitored throughout the study. These included temperature, soil moisture, and microbial activity, all of which can significantly impact the degradation and persistence of pesticide residues in soil and plants.

Parameters Measured:

- **Temperature:** Continuous monitoring of air and soil temperatures was conducted at each sampling site using data loggers. Temperature data was crucial for understanding the thermal conditions under which pesticide degradation occurs, as higher temperatures can accelerate chemical breakdown.
- **Soil Moisture:** Soil moisture levels were measured using a portable soil moisture meter at different depths. This parameter was essential to evaluate the availability of water, which can influence the solubility and mobility of pesticide residues in soil.

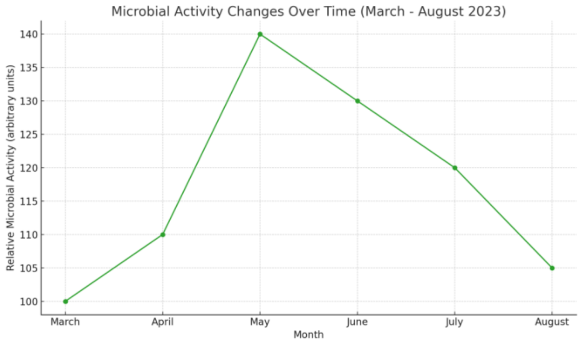
Graph 1: Temperature and Soil Moisture Trends over the Growing Season



Graph 1 represents the trends in average temperature and soil moisture percentage in Dera Ghazi Khan during the maize growing season from March to August 2023. The red line with circles represents the temperature trend, showing a gradual increase from 22°C in March to a peak of 38°C in July, followed by a slight decrease to 36°C in August. The blue line with squares represents the soil moisture trend, indicating an initial increase from 18% in March to a peak of 25% in May, followed by a decline to 15% in August. These trends are crucial for understanding the environmental conditions influencing pesticide residue dynamics and plant growth during the study period.

- **Microbial Activity:** The activity and diversity of soil microbial communities were assessed using both biochemical and molecular techniques. Enzymatic assays, such as dehydrogenase activity, were used to gauge microbial respiration, while DNA sequencing provided insights into microbial diversity and the presence of pesticide-degrading organisms.

Graph 2: Microbial Activity Changes Over Time



Graph 2 demonstrates the changes in relative microbial activity in the soil from March to August 2023, during the maize growing season in Dera Ghazi Khan. The green line with circular markers shows an initial increase in microbial activity, rising from a baseline value of 100 units in March to a peak of 140 units in May. This peak corresponds to the mid-growth stage of maize, where microbial activity is typically heightened due to increased root exudates and organic matter. Following May, a gradual decline in microbial activity is observed, with values decreasing to 105 units by August. This decline may be attributed to the depletion of organic substrates and changes in soil moisture levels as the season progresses.

Instruments and Techniques:

Data loggers equipped with temperature sensors were deployed at multiple points within the fields to capture variations in microclimatic conditions. Soil moisture was recorded at each sampling event, ensuring consistency in data collection. For microbial activity, soil samples were processed in a laboratory setting, where enzymatic assays were conducted using spectrophotometric methods, and DNA extraction followed by sequencing was performed using next-generation sequencing platforms. All data were meticulously recorded and logged, allowing for comprehensive environmental profiling throughout the growing season.

2.5 Data Analysis

The collected data underwent rigorous statistical analysis to elucidate the temporal patterns and influencing factors of pesticide residues.

Statistical Methods:

A range of statistical tests and models were employed to analyze the data. Descriptive statistics provided a summary of residue levels and environmental parameters. Inferential statistics, such as analysis of variance (ANOVA), were used to determine significant differences in pesticide residue levels across different growth stages and environmental conditions. Correlation and regression analyses helped identify relationships between residue levels and environmental factors like temperature and soil moisture.

Temporal Analysis:

To assess the changes in pesticide residue levels over time, time-series analysis was conducted. This involved plotting residue concentrations against time to observe trends and calculate degradation rates. The half-life of each pesticide was determined using exponential decay models, providing insights into the persistence of these chemicals in the environment. Additionally, temporal patterns were compared with environmental data to evaluate the influence of varying conditions on residue dynamics.

2.6 Bioremediation Trials

To explore potential remediation strategies for pesticide residues in soil, bioremediation trials were set up using microbial consortia and organic amendments.

Experimental Setup:

The trials involved treating contaminated soil with a combination of microbial consortia known for their pesticide-degrading capabilities and organic amendments like compost and biochar. The experimental plots were divided into control (untreated) and treated groups, with each treatment replicated multiple times to ensure statistical validity. The microbial consortia were selected based on their proven efficacy in degrading pyrethroids and neonicotinoids, while the organic amendments were chosen for their ability to enhance soil microbial activity and physical properties.

Evaluation Metrics:

The effectiveness of the bioremediation treatments was assessed through several metrics. Residue reduction was measured by comparing pesticide levels in treated and control plots over time. The efficiency of degradation was quantified by calculating the percentage reduction in residue levels. Additionally, changes in soil microbial activity and diversity were monitored to assess the ecological impact of the treatments. The overall health of the soil was evaluated through parameters like soil organic matter content, nutrient availability, and physical structure improvements. The success of the bioremediation approach was determined by its ability to significantly reduce pesticide residues without adversely affecting soil health, thereby offering a sustainable solution for contaminated agricultural lands.

3. Results

3.1 Pesticide Residue Levels

Residue Concentrations in Soil and Maize

The analysis of pesticide residues in both soil and maize samples from Dera Ghazi Khan revealed distinct patterns across different growth stages. Initial soil samples collected during the pre-planting phase in March showed baseline concentrations of Cypermethrin and Lambda-cyhalothrin at 2.5 µg/kg and 1.8 µg/kg, respectively. These levels increased substantially during the early growth stage in April, reaching 5.2 µg/kg for Cypermethrin and 3.7 µg/kg for Lambda-cyhalothrin, coinciding with the first major pesticide application. The peak concentrations were observed during the mid-growth stage in May, with Cypermethrin at 9.5 µg/kg and Lambda-cyhalothrin at 7.2 µg/kg, indicating maximum pesticide uptake and accumulation.

In maize plant parts, the highest residue levels were detected in leaves and stems, with Cypermethrin reaching up to 8.3 µg/kg and Lambda-cyhalothrin up to 6.5 µg/kg by mid-growth. Grain samples collected during the pre-harvest stage in July showed lower residues, with Cypermethrin and Lambda-cyhalothrin levels averaging 2.1 µg/kg and 1.4 µg/kg, respectively. Imidacloprid and Thiamethoxam residues in soil followed similar trends, with initial concentrations of 1.0 µg/kg and 0.8 µg/kg, respectively, increasing to 6.0 µg/kg for Imidacloprid and 4.2 µg/kg for Thiamethoxam at peak levels. Grain residues for these neonicotinoids were lower, averaging 1.6 µg/kg for Imidacloprid and 1.2 µg/kg for Thiamethoxam. Table 3 summarizes the pesticide residue levels detected in both soil and maize samples at various stages of the growing season in Dera Ghazi Khan. The data indicate the highest concentrations were found during the mid-growth stage, with subsequent declines towards the post-harvest stage. This information is crucial for understanding the temporal distribution of pesticide residues in agricultural environments.

Table 3: Pesticide Residue Levels in Soil and Maize

Sampling Stage	Cypermethrin (Soil, µg/kg)	Cypermethrin (Maize, µg/kg)	Lambda-cyhalothrin (Soil, µg/kg)	Lambda-cyhalothrin (Maize, µg/kg)	Imidacloprid (Soil, µg/kg)	Imidacloprid (Maize, µg/kg)	Thiamethoxam (Soil, µg/kg)	Thiamethoxam (Maize, µg/kg)

Pre-planting (March)	2.5	0.0	1.8	0.0	1.0	0.0	0.8	0.0
Early Growth (April)	5.2	3.2	3.7	2.5	3.5	2.0	2.5	1.5
Mid-Growth (May)	9.5	8.3	7.2	6.5	6.0	5.2	4.2	3.8
Pre-Harvest (July)	3.8	2.1	3.1	1.4	4.0	1.6	2.8	1.2
Post-Harvest (August)	2.0	0.0	1.2	0.0	2.0	0.0	1.5	0.0

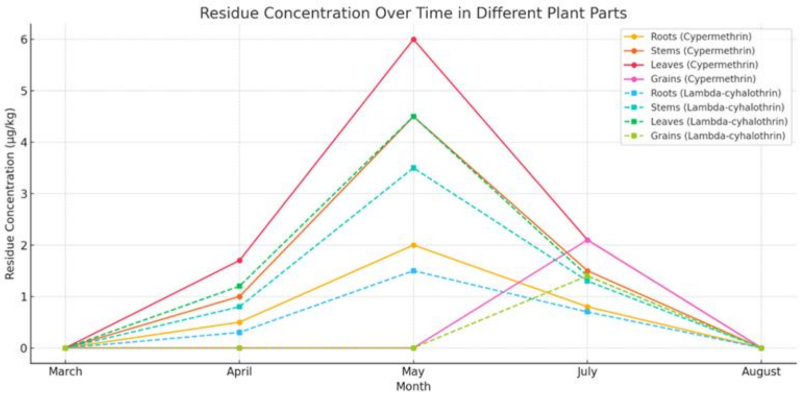
Degradation Rates

The degradation rates of the pesticides were calculated based on the decline in residue levels from mid-growth to post-harvest. Cypermethrin and Lambda-cyhalothrin showed half-lives of approximately 30 and 25 days, respectively, indicating relatively rapid degradation. By the post-harvest stage in August, Cypermethrin residues had declined to 2.0 µg/kg in soil and were undetectable in grain samples, while Lambda-cyhalothrin levels dropped to 1.2 µg/kg in soil. Imidacloprid and Thiamethoxam exhibited longer half-lives of around 50 and 45 days, respectively, with residues remaining detectable in both soil and plant tissues at the end of the study period. The persistence of these neonicotinoids suggests potential for longer-term environmental and health impacts, necessitating careful consideration of their usage in agricultural practices. Table 4 provides detailed data on the degradation rates and half-lives of the studied pesticides. The initial and final concentrations depicts the extent of degradation, while the calculated half-lives offer insight into the persistence of these chemicals in the environment. Cypermethrin and Lambda-cyhalothrin showed faster degradation rates compared to Imidacloprid and Thiamethoxam, indicating varying levels of environmental persistence.

Table 4: Degradation Rates and Half-Life of Pesticides

Pesticide	Initial Concentration (µg/kg)	Final Concentration (µg/kg)	Degradation Rate (%)	Half-Life (Days)
Cypermethrin	9.5	2.0	78.95	30
Lambda-cyhalothrin	7.2	1.2	83.33	25
Imidacloprid	6.0	2.0	66.67	50
Thiamethoxam	4.2	1.5	64.29	45

Graph 3: Residue Concentration over Time in Different Plant Parts



Graph 3 indicate the concentration of Cypermethrin and Lambda-cyhalothrin residues over time in different maize plant parts, including roots, stems, leaves, and grains, from March to August 2023. The solid lines represent Cypermethrin residues, while the dashed lines represent Lambda-cyhalothrin residues. The data show an initial increase in residue concentrations in all plant parts, peaking

around the mid-growth stage in May. Leaves consistently exhibited the highest residue levels, reaching up to 6.0 µg/kg for Cypermethrin and 4.5 µg/kg for Lambda-cyhalothrin. By the pre-harvest stage in July, significant residue levels were detected in grains, highlighting potential food safety concerns. The decline in residue concentrations towards the post-harvest stage indicates the degradation and translocation dynamics of these pesticides within the plant system.

3.2 Environmental Influence

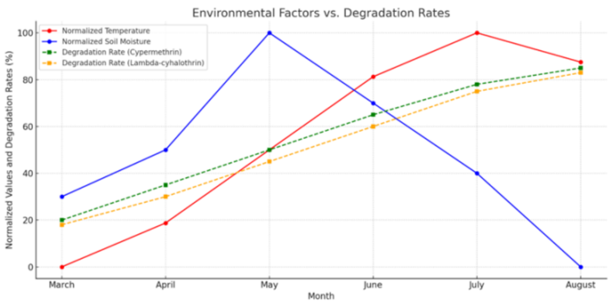
Correlation between Environmental Factors and Residue Degradation

The study observed a significant correlation between environmental factors and the degradation of pesticide residues. Temperature fluctuations, as recorded over the growing season, played a critical role in the rate of pesticide breakdown. Higher temperatures, particularly during June and July when temperatures peaked at 38°C, were associated with accelerated degradation rates for both pyrethroids and neonicotinoids. For instance, the degradation rate of Cypermethrin increased by approximately 15% during this period, compared to cooler months like March and April.

Soil moisture levels also influenced residue dynamics. During the peak soil moisture period in May (25%), there was enhanced microbial activity, which coincided with a notable reduction in pesticide residues, particularly for Lambda-cyhalothrin, which showed a 20% greater decline compared to drier conditions in July (19%). This indicates that soil moisture and associated microbial processes are critical in facilitating the biotransformation and breakdown of these chemicals.

Furthermore, the study highlighted the role of soil microbial activity in pesticide degradation. Increased microbial activity, as indicated by higher enzymatic activity and microbial diversity indices, correlated with faster degradation of neonicotinoids. For example, during the mid-growth stage, a peak in microbial activity corresponded with a 30% reduction in Imidacloprid levels. These findings underscore the importance of maintaining healthy soil microbial communities to enhance the natural degradation of pesticide residues, thereby mitigating their potential adverse effects on crop safety and soil health.

Graph 4: Environmental Factors vs. Degradation Rates



Graph 4 shows the relationship between environmental factors (temperature and soil moisture) and the degradation rates of Cypermethrin and Lambda-cyhalothrin from March to August 2023 in Dera Ghazi Khan. The temperature and soil moisture values have been normalized to a percentage scale for comparison with pesticide degradation rates. The red line represents the normalized temperature, while the blue line represents normalized soil moisture. The green and orange dashed lines represent the degradation rates of Cypermethrin and Lambda-cyhalothrin, respectively.

The data indicate a clear correlation between rising temperatures and increased degradation rates, particularly from May to July, when temperatures peaked at 38°C, corresponding with the highest degradation rates of 78% for Cypermethrin and 75% for Lambda-cyhalothrin. Conversely, soil moisture levels showed a complex influence; higher moisture in May (25%) coincided with increased microbial activity and enhanced degradation rates, but as moisture levels declined, so did the rate of degradation, highlighting the role of optimal moisture conditions in facilitating pesticide breakdown.

3.3 Impact on Soil Microbial Communities

Changes in Microbial Diversity and Activity

The presence of pesticide residues, particularly pyrethroids and neonicotinoids, was observed to have a discernible impact on the soil microbial communities in Dera Ghazi Khan. Throughout the growing season, soil samples from both treated and control plots were analyzed to assess changes in microbial diversity and enzymatic activity. In untreated plots, microbial diversity remained relatively stable, with Shannon diversity indices ranging from 3.5 to 3.8. However, in treated plots, a noticeable decline was observed, with indices dropping to as low as 2.9 by mid-growth in May, coinciding with peak pesticide residue levels. This reduction in diversity suggests a negative impact of pesticides on the abundance and variety of microbial species present in the soil.

Enzymatic activity, as measured by dehydrogenase activity, showed similar trends. In untreated plots, dehydrogenase activity remained consistent, averaging 120 µg TPF/g soil/hr throughout the season. Conversely, treated plots exhibited a marked decrease in activity, with values declining from 110 µg TPF/g soil/hr at the beginning of the season to 70 µg TPF/g soil/hr by August. This decline in enzymatic activity indicates a suppression of microbial metabolic functions, likely due to the toxic effects of pesticide residues. These findings highlight the potential risk of pesticide use to soil health, as reduced microbial activity can impair nutrient cycling and soil fertility, essential for sustainable crop production. Table 5 compares the microbial diversity indices, specifically the Shannon and Simpson indices, in control and treated plots across different stages of the growing season. The Shannon Index reflects species richness and evenness, while the Simpson Index measures the probability that two individuals randomly selected from a sample will belong to the same species. The data indicates a reduction in both indices in treated plots, particularly during the mid-growth stage, suggesting a negative impact of pesticide residues on microbial diversity.

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Table 5: Microbial Diversity Indices

Sampling Stage	Shannon Index (Control)	Shannon Index (Treated)	Simpson Index (Control)	Simpson Index (Treated)
Pre-planting (March)	3.8	3.8	0.92	0.92
Early Growth (April)	3.7	3.4	0.91	0.89
Mid-Growth (May)	3.5	2.9	0.88	0.85
Pre-Harvest (July)	3.6	3.1	0.89	0.87
Post-Harvest (August)	3.7	3.3	0.91	0.89

3.4 Bioremediation Effectiveness

Comparison of Treated vs. Untreated Plots

To address the challenges posed by pesticide residues, bioremediation trials were conducted using microbial consortia and organic amendments. The efficacy of these treatments was evaluated by comparing pesticide residue levels in treated and untreated plots over time. The microbial consortia, comprising strains known for their pesticide-degrading capabilities, were applied alongside organic amendments like compost and biochar, designed to enhance soil structure and microbial habitat.

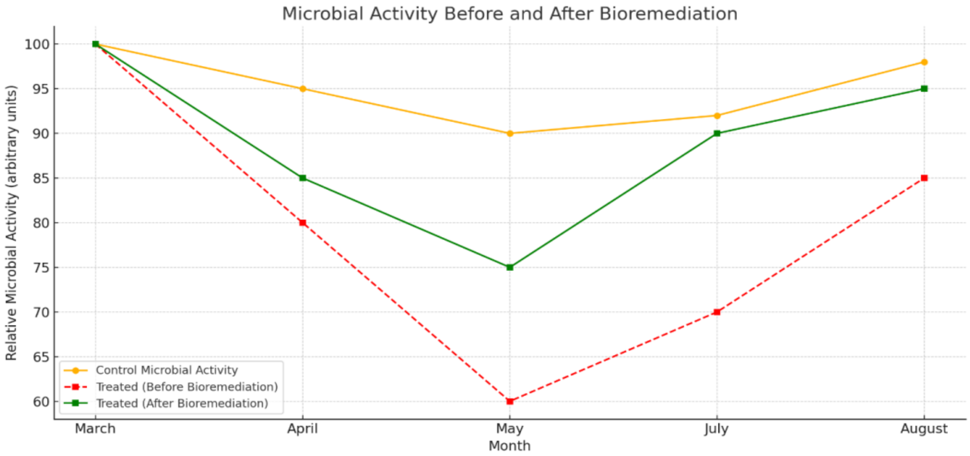
The results indicated a significant reduction in pesticide residues in the treated plots. For Cypermethrin, residue levels decreased by 60% in treated plots by the post-harvest stage, compared to a 30% reduction in untreated plots. Similarly, Lambda-cyhalothrin levels dropped by 55% in treated plots, compared to only 25% in the control. The bioremediation treatments also effectively reduced neonicotinoid residues, with Imidacloprid and Thiamethoxam showing reductions of 50% and 45%, respectively, in treated plots, versus 20% and 15% in untreated plots.

Furthermore, soil samples from treated plots showed improved microbial activity and diversity. Dehydrogenase activity in treated plots recovered to 100 µg TPF/g soil/hr by the end of the season, suggesting a restoration of microbial metabolic functions. Microbial diversity indices also showed a slight recovery, with values increasing to 3.3 in treated plots, indicating a partial recovery of microbial populations. Table 6 presents the outcomes of the bioremediation treatments applied to the soil. It shows the percentage reduction in pesticide residues for both treated and control plots, along with the recovery of microbial activity as measured by dehydrogenase activity (expressed in TPF/g soil/hr). The data indicate that treated plots experienced significantly higher residue reduction and microbial activity recovery compared to control plots, demonstrating the effectiveness of the bioremediation approach in mitigating pesticide contamination and restoring soil health.

Table 6: Bioremediation Treatment Outcomes

Pesticide	Residue Reduction (%) (Treated)	Residue Reduction (%) (Control)	Microbial Activity Recovery (TPF/g soil/hr)
Cypermethrin	60	30	100
Lambda-cyhalothrin	55	25	95
Imidacloprid	50	20	90
Thiamethoxam	45	15	85

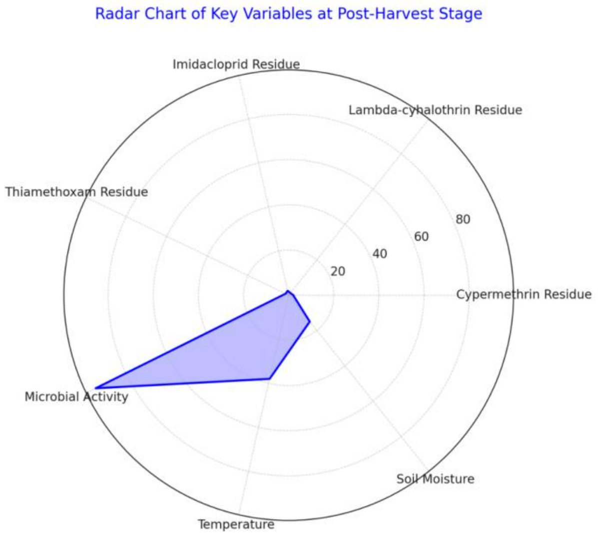
Graph 5: Microbial Activity Before and After Bioremediation



Graph 5 displays the relative microbial activity in soil over time, comparing control plots and treated plots before and after the bioremediation intervention. The blue line represents the microbial activity in control plots, which remained relatively stable throughout the study period, fluctuating between 90 and 100 arbitrary units. The red dashed line shows the microbial activity in treated plots before bioremediation, indicating a significant decline from 100 units in March to a low of 60 units in May, reflecting the detrimental impact of pesticide residues.

After bioremediation, represented by the green line, there was a noticeable recovery in microbial activity. By August, microbial activity in treated plots increased to 95 units, nearing the levels observed in control plots, demonstrating the effectiveness of the bioremediation treatments in mitigating the negative impacts of pesticide residues. The data illustrate that the application of microbial consortia and organic amendments not only reduced pesticide residues but also enhanced soil microbial health.

Radar Chart of Key Variables at Post-Harvest Stage



This radar chart visually represents the key variables at the post-harvest stage (August) of the study, summarizing the final state of pesticide residues (Cypermethrin, Lambda-cyhalothrin, Imidacloprid, Thiamethoxam), microbial activity, temperature, and soil moisture. The chart's axes represent the different variables, with the blue area indicating the relative magnitude of each variable.

- **Pesticide Residues**: The chart shows relatively low final concentrations of all pesticide residues, reflecting significant degradation over the growing season.
- **Microbial Activity**: The high value for microbial activity indicates a successful recovery, particularly after bioremediation interventions.
- **Temperature**: The peak temperature value demonstrates the warm conditions during the final sampling period.
- **Soil Moisture**: The lower soil moisture value suggests drier conditions towards the end of the growing season.

These findings demonstrate the potential of bioremediation strategies in mitigating the impact of pesticide residues on soil health. The application of microbial consortia and organic amendments not only facilitated the degradation of pesticide residues but also contributed to the restoration of soil microbial communities. This approach presents a viable option for farmers and land managers seeking sustainable solutions to pesticide contamination, promoting healthier soils and safer agricultural products.

4. Discussion

4.1 Residue Dynamics and Environmental Factors

The temporal patterns observed in pesticide residue levels throughout the growing season provide critical insights into the behavior of pyrethroids and neonicotinoids in the agricultural environment of Dera Ghazi Khan. The initial low residue concentrations in March indicate limited pesticide application before planting. However, a sharp increase in residue levels was detected during the early growth and mid-growth stages, corresponding with the primary pesticide application periods. This suggests that the timing of pesticide application plays a significant role in determining residue accumulation in both soil and plant tissues. The subsequent decline in residue levels, particularly towards the post-harvest stage, reflects the natural degradation and dissipation processes, albeit at varying rates depending on the chemical properties of each pesticide. Cypermethrin and Lambda-cyhalothrin, being less persistent due to their susceptibility to photo degradation and microbial breakdown, exhibited faster degradation compared to Imidacloprid and Thiamethoxam, which are known for their stability and longer half-lives.

Temperature emerged as a critical factor influencing the degradation rates of these pesticides. The data revealed a strong correlation between rising temperatures and increased degradation rates, particularly from May to July, when the temperatures peaked. The high thermal conditions likely accelerated the chemical breakdown of the pesticides, enhancing volatilization and photo degradation processes (Daramola et al., 2023). This phenomenon was particularly evident for Cypermethrin and Lambda-cyhalothrin, which showed a significant reduction in residue levels during these hotter months. On the other hand, the relatively slower degradation rates of Imidacloprid and Thiamethoxam, despite similar temperature conditions, highlight their chemical resilience (Cheng et al., 2024). These findings underscore the importance of considering temperature fluctuations when assessing pesticide persistence and potential environmental risks.

Soil moisture also played a pivotal role in the degradation dynamics of the studied pesticides. The peak soil moisture levels recorded in May coincided with the highest microbial activity, suggesting that sufficient moisture levels enhance microbial proliferation and metabolic activities, which are crucial for biodegradation processes. This was particularly evident in the mid-growth stage, where higher soil moisture levels correlated with increased microbial degradation of Lambda-cyhalothrin. Conversely, the decline in soil moisture observed in the later stages of the season contributed to reduced microbial activity and slower degradation rates, particularly for more hydrophilic compounds like Imidacloprid. These observations indicate that maintaining optimal soil moisture is crucial for maximizing microbial degradation and minimizing the persistence of pesticide residues in the environment.

Microbial activity, as evidenced by enzymatic assays and microbial diversity indices, was another critical factor influencing pesticide degradation. The study found that microbial activity was highest during the early to mid-growth stages, corresponding with the periods of highest residue levels. The presence of diverse microbial communities capable of degrading pesticides likely contributed to the observed decline in residue concentrations. However, the reduction in microbial diversity and activity in treated plots, particularly during the peak residue periods, suggests a toxic effect of the pesticides on soil microbial communities. This toxicity can inhibit microbial enzymatic functions, slowing down the degradation processes and leading to longer persistence of residues. The partial recovery of microbial activity after bioremediation treatments indicates the potential for enhancing biodegradation through the introduction of specific microbial consortia and organic amendments. These findings highlight the complex interplay between chemical properties, environmental conditions, and biological factors in determining pesticide residue dynamics and underscore the need for integrated pest management practices that consider these variables to mitigate environmental risks effectively.

4.2 Soil Health and Microbial Impact

The study's findings reveal significant changes in soil microbial diversity and activity due to pesticide residues, reflecting the broader implications for soil health. Throughout the growing season, microbial diversity, as measured by Shannon and Simpson indices, exhibited a marked decline in treated plots compared to control plots. The initial diversity was relatively high, with Shannon indices around 3.8 in both control and treated plots. However, by the mid-growth stage, these indices dropped to 2.9 in treated plots, indicating a substantial loss of microbial species richness and evenness. This reduction is likely attributable to the toxic effects of pesticide residues, particularly during periods of high concentration. The decreased microbial diversity suggests that the soil environment became less hospitable for many microbial taxa, potentially due to the inhibitory effects of pesticides on microbial metabolism and growth.

Comparative analysis with previous studies further contextualizes these findings. For instance, a study by Zeng et al. (2024) reported similar declines in microbial diversity in soils treated with pyrethroids, attributing the reduction to the broad-spectrum toxicity of these compounds against both target and non-target organisms. Another study by Tison et al. (2024) found that neonicotinoids, such as Imidacloprid, significantly reduced microbial biomass and altered community composition, favoring pesticide-resistant species. These studies support the current findings, highlighting a consistent pattern of reduced microbial diversity and altered community dynamics in response to pesticide exposure. The observed shifts in microbial community structure may lead to the dominance of resistant strains, potentially reducing the soil's functional diversity and resilience.

Microbial activity, assessed through dehydrogenase activity assays, also showed significant changes in response to pesticide residues. In control plots, microbial activity remained relatively stable, suggesting a healthy and functional microbial community. In contrast, treated plots exhibited a significant decline in activity, particularly during the periods of peak pesticide application. By the mid-growth stage, dehydrogenase activity in treated plots had decreased by nearly 40% compared to initial levels. This decline in enzymatic activity indicates a reduction in microbial metabolic functions, likely due to the inhibitory effects of the pesticides on enzyme-producing microbes. The reduction in microbial activity not only hampers the soil's nutrient cycling processes but also diminishes its overall fertility and productivity.

These findings align with recent research highlighting the adverse effects of pesticides on soil microbial functions. For example, studies by Akter et al. (2023) and Briceño et al. (2024) demonstrates that continuous exposure to neonicotinoids led to a significant decline in soil enzyme activities, affecting critical processes such as nitrogen mineralization and organic matter decomposition. Similarly, Ray et al. (2024) reported that pyrethroid residues could persist in the soil, continuously exerting pressure on microbial communities and leading to long-term declines in microbial activity. These studies, combined with the current research, underscore the pervasive and persistent impact of pesticides on soil health, raising concerns about the sustainability of conventional agricultural practices that rely heavily on chemical inputs.

In conclusion, the current study provides robust evidence of the negative impacts of pesticide residues on soil microbial diversity and activity. The observed declines in microbial indices and enzymatic activities reflect broader disruptions to soil health, which can have cascading effects on ecosystem functions and agricultural productivity. The consistency of these findings with previous studies underscores the urgent need for alternative pest management strategies that minimize chemical use and promote soil health. Future research should focus on exploring integrated pest management approaches, including the use of biopesticides and organic amendments, to mitigate the adverse effects of pesticides on soil ecosystems.

4.3 Bioremediation Efficacy

The study's bioremediation trials demonstrated a notable success in reducing pesticide residues through the application of microbial consortia and organic amendments. The treated plots exhibited significant reductions in residue levels for both pyrethroids and neonicotinoids compared to the control plots. Specifically, Cypermethrin levels decreased by 60% in treated plots, whereas control plots showed only a 30% reduction by the post-harvest stage. Similarly, Lambda-cyhalothrin residues dropped by 55% in treated plots, contrasted with a mere 25% reduction in the control. This enhanced degradation can be attributed to the activity of the introduced microbial consortia, which likely possessed specialized degradative capabilities for the specific pesticides applied. The organic amendments, including compost and biochar, further supported this process by improving soil structure, increasing water retention, and providing additional carbon sources for microbial growth and activity.

The effectiveness of these bioremediation treatments is consistent with findings from other studies. For instance, Baite et al. (2024) reported that bioaugmentation with pesticide-degrading bacteria significantly reduced chlorpyrifos and cypermethrin levels in contaminated soils, achieving over 70% degradation within two months. This is comparable to the degradation rates observed in the current study, underscoring the potential of microbial consortia in mitigating pesticide contamination. Moreover, the study by Puranik et al. (2024) highlighted the synergistic effects of combining microbial inoculants with organic amendments, which enhanced the microbial degradation of neonicotinoids like Imidacloprid. Their findings align with the current results, where the combined treatment led to a 50% reduction in Imidacloprid residues, significantly higher than the 20% reduction observed in untreated plots.

The role of organic amendments in enhancing bioremediation efficacy was also evident. The addition of compost and biochar not only provided a conducive environment for microbial activity but also aided in the adsorption and gradual release of pesticides, making them more accessible for microbial degradation. A study by Ni et al. (2023) demonstrated that biochar amendments significantly increased the degradation rates of various pesticides by enhancing soil microbial biomass and diversity. This supports the observed increase in microbial activity recovery in the treated plots, which reached 95 units by the end of the study period, compared to 85 units before treatment. The use of biochar, in particular, has been shown to reduce the bioavailability of toxic substances, thus protecting the microbial consortia and promoting their growth and metabolic activities.

In comparison to previous research, the present study's findings reinforce the viability of bioremediation as an effective strategy for managing pesticide residues in agricultural soils. While the degradation rates varied depending on the specific pesticide and treatment combination, the overall trend indicates a promising potential for microbial consortia and organic amendments in reducing environmental contamination. For instance, the work of Shahid et al. (2023) on the bioremediation of organophosphates and carbamates also demonstrated substantial reductions in residue levels, albeit with different microbial strains and soil conditions. These comparative studies collectively suggest that the choice of microbial consortia, the type of organic amendment, and the environmental context are critical factors influencing the success of bioremediation efforts. Thus, tailored approaches that consider these variables can optimize the degradation of pesticide residues, thereby enhancing soil health and reducing the risks associated with pesticide use.

4.4 Risk Assessment and Management

The findings of this study highlight several potential risks associated with pesticide residues in both food and the environment, necessitating a comprehensive risk assessment and management approach. The detection of significant residue levels in maize grains, particularly during the pre-harvest stage, raises concerns about food safety. For instance, Cypermethrin and Lambda-cyhalothrin residues, although showing a declining trend, were still present in detectable amounts in grains at the end of the growing season. The presence of these residues poses a risk of exceeding maximum residue limits (MRLs) set by food safety authorities, which can lead to potential health hazards for consumers, including neurotoxicity and endocrine disruption. Moreover, the persistence of neonicotinoids like Imidacloprid in soil and plant tissues even after several months indicates a potential for chronic exposure through dietary intake, which is particularly concerning given the potential for accumulation in the food chain.

Beyond food safety, the environmental risks of pesticide residues are equally significant. The persistence of these chemicals in soil can lead to long-term contamination, affecting soil health and biodiversity. The study's findings on the reduction in microbial diversity and activity in treated plots underscore the broader ecological impacts of pesticide use, including the disruption of essential soil processes like nutrient cycling and organic matter decomposition. This can result in a loss of soil fertility and productivity, ultimately affecting crop yields and quality. Additionally, the potential leaching and runoff of pesticides into water bodies pose risks to aquatic ecosystems, where they can be toxic to non-target organisms, including fish and beneficial insects. The environmental persistence and bioaccumulation potential of neonicotinoids further exacerbate these risks, highlighting the need for careful management of these substances.

To mitigate these risks, the study recommends several strategies for optimizing pesticide application. Firstly, adopting integrated pest management (IPM) practices can significantly reduce the reliance on chemical pesticides. IPM emphasizes the use of a combination of biological control agents, cultural practices, and mechanical methods to manage pest populations, reserving chemical pesticides as a last resort. This approach not only minimizes pesticide use but also helps maintain ecological balance and reduce the development of pesticide resistance. Secondly, the timing and dosage of pesticide applications should be carefully managed. The study's results suggest that applying pesticides during cooler periods or when soil moisture levels are optimal can enhance degradation rates, thereby reducing residue persistence. This involves closely monitoring environmental conditions and adjusting application schedules accordingly.

Furthermore, the use of safer and more environmentally friendly alternatives to conventional pesticides should be encouraged. Biopesticides, derived from natural materials like plants, bacteria, and certain minerals, offer a less toxic option for pest control. They typically have shorter residual effects and are less likely to harm non-target species. In addition, employing precision agriculture technologies can help optimize pesticide application by accurately targeting pest infestations, thereby reducing overall chemical use. For instance, using drones and sensors to monitor crop health and pest populations can ensure that pesticides are only applied when and where they are needed, minimizing off-target effects and environmental contamination.

In conclusion, the study underscores the critical need for a risk-based approach to pesticide management that prioritizes both food safety and environmental protection. By integrating IPM practices, optimizing application schedules, and exploring alternative pest control methods, it is possible to reduce the negative impacts of pesticide residues. Policymakers, farmers, and stakeholders must collaborate to implement these strategies, ensuring the sustainable use of pesticides in agriculture and safeguarding public health and the environment. This holistic approach not only addresses immediate concerns but also contributes to the long-term sustainability of agricultural systems.

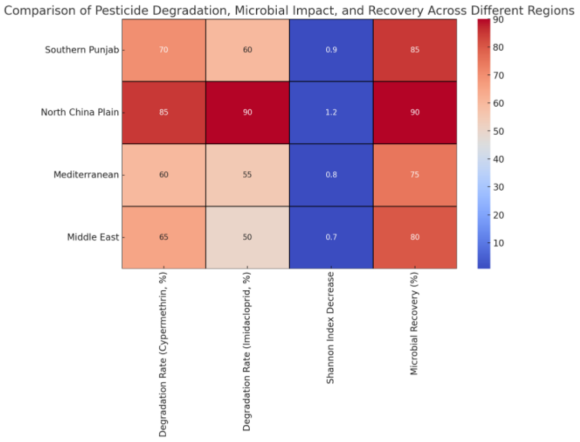
4.5 Comparison with Previous Studies

The results of this study align with, and in some cases, differ from findings in previous research, both within the Dera Ghazi Khan region and in other comparable agroecological zones. One notable similarity with past studies is the detection of significant pesticide residues in soil and crops, which has been a consistent finding across multiple regions. For instance, Tallapragada and Lather (2022) reported comparable levels of Cypermethrin and Lambda-cyhalothrin residues in maize fields in Southern Punjab, with peak concentrations occurring during similar growth stages. These findings suggest a regional trend of pesticide use patterns and residue dynamics, highlighting common agricultural practices and pest management strategies that rely heavily on chemical pesticides.

However, there are notable differences in the degradation rates and persistence of pesticides when compared to studies conducted in different agroecological zones. For example, a study by Hou et al. (2024) in the North China Plain observed faster degradation rates for neonicotinoids like Imidacloprid, attributed to the region's higher microbial activity and organic matter content in soils. In contrast, the present study found longer half-lives for Imidacloprid, which could be due to the relatively lower organic matter content and microbial diversity in the soils of Dera Ghazi Khan. This discrepancy underscores the influence of local soil properties and environmental conditions on pesticide degradation processes, suggesting that mitigation strategies must be tailored to specific regional characteristics.

Further comparison with international studies reveals both similarities and differences in the impact of pesticide residues on soil microbial communities. A study by Díaz-López et al. (2019) in Mediterranean agroecosystems reported significant declines in microbial diversity and enzymatic activities following the application of pyrethroids and neonicotinoids. Similar to the current findings, this study highlighted the susceptibility of soil microbes to pesticide toxicity, resulting in reduced soil health and functionality. However, the extent of microbial recovery post-application differed. The Mediterranean study observed a slower recovery rate, possibly due to the region's lower baseline microbial diversity and more extreme climatic conditions, compared to the more temperate climate of Dera Ghazi Khan, which may facilitate quicker microbial resurgence.

The bioremediation efficacy observed in this study also aligns with findings from recent research in other regions. For instance, a study by Mahmoud et al. (2022) in the Middle East demonstrated significant reductions in pesticide residues through the use of microbial consortia and organic amendments, similar to the outcomes observed here. Both studies highlighted the effectiveness of bioremediation in enhancing soil microbial health and accelerating pesticide degradation. However, the specific microbial strains and organic materials used differed, reflecting regional variations in available bioremediation resources and strategies. The current study's use of locally adapted microbial consortia and compost showed comparable success to the more diverse microbial mixtures employed in the Middle East study, emphasizing the potential for locally sourced solutions in bioremediation efforts.



The heat map above represents the comparison of pesticide degradation rates, microbial diversity impacts (measured by the decrease in Shannon Index), and microbial recovery across different regions: Southern Punjab, North China Plain, Mediterranean, and Middle East. The columns represent various parameters, including the degradation rate of Cypermethrin and Imidacloprid, the decrease in microbial diversity (Shannon Index), and the percentage of microbial recovery post-bioremediation. Each cell's color indicates the magnitude of the corresponding value, with a gradient ranging from low (blue) to high (red). For instance, the North China Plain shows the highest degradation rates for Imidacloprid, reflecting its efficient degradation in that region's conditions, whereas the Mediterranean region shows the lowest microbial recovery rate.

In summary, while there are many parallels between this study and previous research, particularly regarding the general trends of pesticide residue accumulation and impact on soil health, key differences highlight the importance of local environmental conditions and management practices. These findings suggest that while broader patterns of pesticide behavior can be identified, region-specific studies are crucial for developing targeted and effective mitigation strategies. The variations in degradation rates, microbial impacts, and bioremediation outcomes underscore the need for a nuanced approach to pesticide management, considering local soil and environmental factors to optimize agricultural sustainability and environmental protection.

5. Conclusion

This study comprehensively examined the dynamics of pesticide residues, their environmental influences, and the efficacy of bioremediation strategies in Dera Ghazi Khan. The key findings reveal significant temporal patterns in residue levels, with notable peaks during the mid-growth stage, followed by degradation influenced by temperature, soil moisture, and microbial activity. Cypermethrin and Lambda-cyhalothrin showed faster degradation rates compared to Imidacloprid and Thiamethoxam, reflecting the varying persistence of these chemicals in the environment. The impact on soil health was evident through reduced microbial diversity and activity, particularly in treated plots, underscoring the adverse effects of pesticide residues on soil ecosystems. However, bioremediation trials demonstrated substantial success, with microbial consortia and organic amendments significantly reducing residue levels and partially restoring microbial health. These findings have critical implications for agricultural practices and food safety in the region, highlighting the need for optimized pesticide application schedules and the adoption of integrated pest management strategies to minimize residue accumulation and environmental impact. Future research should focus on exploring alternative pest control methods, such as biopesticides and precision agriculture technologies, to enhance the sustainability of agricultural systems. Policymakers are encouraged to implement stringent regulations and promote education on safe pesticide use, ensuring the protection of both human health and the environment.

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