ACAEDEMIA Biota Nexus Journal
https://academia.edu.pk/index.php/bnj

Volume 1 Issue 3

Effects of Pesticide Residues on Soil Biodiversity and Enzymatic Activities in Cotton Fields

Muhammad Saad Khan

MSC Hons Agriculture Faculty of Agriculture Sciences and Technology Department of Agronomy Bahauddin Zakariya University Multan, & Field Assistant, Punjab Agriculture Department (CRS), Multan, Pakistan

ABSTRACT

Another common activity in the cotton farming is pesticide use to control the pests and achieve high production. Nevertheless, the constant and heavy application of pesticides may contribute to the build-up of pesticides in the soil that will have negative consequences on the biodiversity and enzyme activities of soil which are important pointers to the stability of the soil and its fertility. The paper examines how pesticides residues affect the soil microbial community and vital enzymatic activities in cotton fields in Punjab, Pakistan. The samples of the soils of different fields with a different history of the pesticide application were taken and analyzed as to the concentration of the residual pesticides, microbial diversity through culture-based and molecular methods, and enzymatic activities such as dehydrogenase, urease, and phosphatase. Findings indicated that an increase in pesticide residues was associated with a decrease in microbial diversity, inhibition of helpful microbial groups, and enzymatic activity, which means that the soil is dysfunctional. These results highlight the ecological threat posed by excess use of pesticides and why integrated pest management approach should be adopted to ensure sustainable soil in cotton agroecosystem.

Keywords: Pesticide residues, Soil biodiversity, Enzymatic activities, Cotton fields, Soil health, Microbial diversity.

Corresponding Author: Muhammad Saad Khan
Email: isaadk50@gmail.com

Received: 28-06-2025
Revised: 14-07-2025
Accepted:30-07-2025

INTRODUCTION

One of the most significant cash crops in Pakistan is cotton (Gossypium spp.), which makes a significant contribution to the state economy, employment and export revenues. Crops prone to insect pests are very prone to pests, and this has increased the application of pesticides in cotton farming (Ahmad et al., 2019). Although pesticides have been shown to enhance production and minimize losses of crops, their overuse can lead to the formation of remnants in the soil, which is an environmental and ecological problem (Khan et al., 2020). Soil serves as a storage of the pesticides and the residues may take months to years before they disappear according to the chemical properties, where they impact the soil microorganisms which performs extremely vital functions in the cycling of nutrients, decomposing organic matter as well as maintaining soil fertility (Singh et al., 2018).

ACAEDEMIA Biota Nexus Journal
https://academia.edu.pk/index.php/bnj
Volume 1, Issue 3

One of the major determinants of soil functionality is soil biodiversity that entails bacteria, fungi, and actinomycetes. Microorganisms are the agents that promote biochemical processes that ensure stability and productivity of the ecosystem. Nevertheless, the residues of pesticides can have a poisonous input on such organisms, lowering the assortment of the population and changing the composition of the microbial community (Sharma and Singh, 2017). The decreases in microbial abundance and diversity in soil that have been reported in several studies in soil treated with organophosphate, carbamate, and pyrethroid pesticides could result in the inability to transform nutrients and low soil fertility (Rashid et al., 2019; Arora et al., 2020). Other indicators of soil health, other than microbial diversity, are enzymatic activities of soils that are highly sensitive. These enzymes are dehydrogenase, urease and phosphatase which are a part of the organic matter degradation, nitrogen cycling and phosphorus availability respectively and are affected by the chemical pollution such as pesticides (Kaur et al., 2016).

Dehydrogenase activity is an indication of the overall oxidative activity of microbes and it is widely employed as a measure of microbial metabolic activity in soil. It is established that the soils with a high concentration of pesticide residues have a low level of dehydrogenase activity as it inhibits the development of microorganisms and respiration (Caldwell et al., 2014). Urease, an enzyme that is nitrogen mineralization is also vulnerable to chemical stress. This can be achieved through inhibiting the urease activity in soils polluted with pesticides, and this could lead to accumulation of the urea and the limitation of accessibility of nitrogen by the plants (Gupta and Verma, 2015). Activation of hydrolysis of organic substances containing phosphorus Phosphatase is central to the phosphorus cycling in agroecosystems. It has been measured that a reduction in the phosphatase in the presence of pesticide may affect the absorption of plant nutrients and productivity (Bhardwaj et al., 2018).

The character, quantity and regularity of the pesticides usage have a huge effect on the amount of these pesticides residual in the soil and the amount of ecological disruption. Organochlorines have been determined to build up after a number of years, therefore, persistent pesticides have long-term impacts on microbial communities living in soils, and biodegradable pesticides have short-lived yet devastating impacts (Zhang et al., 2017). Furthermore, the use of the pesticides on a constant basis can change the structure of the microbial communities preferring the resistant ones and reducing the overall biodiversity. The alterations can disrupt the basic ecosystem services, including nutrient cycling, organic matter and disease suppression, and eventually affect the soil fertility and crop productivity (Khan et al., 2020).

As far as cotton agroecosystems are concerned in Pakistan, lack of research exists in terms of interplay of pesticide residues, diversity of microorganisms in the soil, and enzyme activities. Most of the studies have focused on the pesticide residues in water and in the tissues of the crops but the long-term effects of the pesticides on the health of the soil has been rarely studied (Ahmed et al., 2018). Knowledge of the correlation between pesticides contamination and biochemical parameters of soil is critical towards sustainable management of crops because it offers an insight on ecological hazards, and it informs the design of integrated pest management programs that reduce adverse effects on soil ecosystems.

Molecular methods such as PCR-based community profiling and high-throughput sequencing have enhanced the use of the techniques to assess the diversity of the microbial soil based on the occurrence of pesticide residues in the soil (Rashid et al., 2019). By combining the analysis of the diversity of the microbial population and the ability of the soil to realize the enzymatic activity, one can obtain the full picture of the soil functioning condition under the impact of the chemicals. These forms of combined activities are essential in the evaluation of the soil resilience, guiding the application of agrochemicals, and sustainable agricultural application.

As the practice of cotton farming is full of pesticides in the use and the microorganisms of the soil are very crucial in the health of the soil, the paper aims to establish the effect of pesticides residues on soil biodiversity and enzyme activities in cotton fields within Punjab, Pakistan. In seeking the relationship between the left over pesticide level, structure of microbial community, and enzyme activity, the study will provide the definition of the potential ecological risks and propose the sustainable management strategy that will not compromise the soil fertility and productivity.

LITERATURE REVIEW

Soil is a dynamic and a complex system that supports communities of microbes that are significant in the recycling of nutrients, organic matter decomposition and the overall soil fertility. These microorganisms, such as bacteria, fungi, and actinomycetes, have a close interaction with physicochemical properties and biological processes in soil, and have an impact on plant health and productivity (Singh et al., 2018). Nevertheless, the agricultural intensification and the use of chemicals, especially pesticides, can disrupt this fragile equilibrium resulting in the change in microbial diversity, as well as the enzymatic processes (Kaur et al., 2016).

The application of pesticides, which comprise of insecticides, herbicides and fungicides, is widely applied in the production of cotton to eliminate the pests as well as achieve high yields. Although pesticides are useful in controlling pests, they may have residual effect on the soil environment, based on the chemical characteristics of these substances, i.e., solubility, volatility and half-life. Organophosphates and pyrethroids, which have less persistence, can have acute toxicity to soil microorganisms, but organophosphates and pyrethroids have a higher chance of bioaccumulating in the soil compared to organophosphates (Zhang et al., 2017). These chemical residues have a direct impact on soil microorganisms, potentially inhibiting the enzymatic activity, changing the metabolism of microorganisms, and altering the composition of communities (Sharma and Singh, 2017).

The enzyme activities in the soil are also known to be very sensitive to show the condition of the soil and its fertility. Some of the processes that mediate mineralization of organic matter, nitrogen cycle and phosphorus availability include the biochemical processes that are mediated by enzymes such as dehydrogenase, urease, phosphatase and b-glucosidase. The dehydrogenase activity is a measure of overall oxidative activity on the microbes and is typically used to assess the microbial metabolism under chemical stress (Caldwell et al., 2014). It has also been found out that the amount of the dehydrogenase activity, which is the inhibition of the microbial respiration and metabolism, can also be negatively influenced by the pesticide residues (Gupta and Verma, 2015). Similarly, a comparable pesticide-inhibitory action on urease that catalyses the breakdown of urea into ammonia could lead to a reduction in the provision of nitrogen and, consequently, an influence on vegetation growth (Bhardwaj et al., 2018). The pesticide residues also have an adverse impact on phosphatase activity, which controls the conversion of organic phosphorus, inhibits the cycling of phosphorus and soil fertility (Arora et al., 2020).

Preliminary effects of pesticides on soil microbial communities are not consistent; they all depend on the nature of pesticides, concentration, frequency of application, and soil characteristics. Indicatively, Singh et al. (2018) have stated that the constant use of organophosphate pesticides in cottonfields resulted in a reduction in the total microbial biomass with fungi being adversely affected compared to bacteria. In a similar manner, it was also found by Khan et al. (2020) that high pesticides soils had a lower bacteria diversity and a high percentage of resistant bacteria, which suggests a change in the microbial community's structure based on the stress of chemicals. Such a selective pressure may decrease ecosystem resilience and plant-growing ability of soil.

Chemical nature of pesticides also affects the microbial diversity. Lipophilic compounds that are likely to stick to the soil organic matter can accumulate in the microenvironment of soil and have chronic toxicity, whereas hydrophilic compounds may flow to the lower strata of soil or water, and influence subsurface microbial communities (Rashid et al., 2019). High pesticide residues have been attributed to the reduction in the positive microbial taxa (e.g., nitrogen-fixing bacteria and phosphate-solubilizing bacteria) required to supply the agroecosystems with nutrients (Ahmed et al., 2018). The demise of these functional units of microbes will have a spill-over effect on soil fertility, vegetative growth and long-term sustainability of cotton soil cropping.

It has been reported that the effect of pesticides on the microbial diversity has been known using the molecular methods. Only a small fraction of the microbial community can be found by microbial culture-based methods and the effects of chemical stress can be underestimated. Everything in the community can be profiled with minor changes in composition by using molecular methods, which include PCR-dGGE (denaturing gradient gel electrophoresis) and qPCR, as well as high-throughput sequencing techniques (Rashid et al., 2019; Zhang et al., 2017). To illustrate this, the outcome of DGGE has shown that pesticide-contaminated soils possess a lower bacterial abundance and unfathomable community framework relative to non-contaminated soils, which demonstrates that pesticides contain an ecological hazard of accretion (Sharma and Singh, 2017).

The environmental properties such as; the soil texture, pH, the content of the organic matter and the moisture, moderate the effects of pesticides on the microbial activity and enzymatic processes. Organic soils can absorb pesticides and reduce bioavailability, overcome toxicity and provide sandy soils with the possibility of leaching and being exposed to microorganisms (Caldwell et al., 2014). Moreover, climatic factors, including temperature and rainfall, influence the process of pesticide deterioration and adaptation to such alterations by different microbes, which leads to the alteration of impacts of the pesticides on the condition of soils over time (Kaur et al., 2016). These relationships play a crucial role in the realization of what site-specific management strategies ought to be formulated to facilitate the control of pests and the protection of the soil.

The joint study of the chemical examination of pesticide residues, the evaluation of the microbial diversity, and the enzymatic activity measures helps to obtain the complete picture of the health state of the agrochemical-stressed soil. Bhardwaj et al. (2018) found that the presence of organophosphates in cotton fields reduced up to 40 percent of dehydrogenase and urease activities and molecular samples revealed a reduction in the bacterial and fungal diversity. Such research outlines the need to monitor biochemical parameters of soil in sustainable agricultural program.

The effect of microbial modification and bioremediation in the reduction of the impact of pesticides is also investigated in newer studies. Some microorganisms in the soil can break down chemicals, which are the remnants of the pesticides and use these as a source of carbon or energy, thus making the soil less toxic and recovering its functionality (Arora et al., 2020). The availability of these pesticide-degrading microbes can at least partially offset the adverse effect of the chemical residues and increase the resilience of the soil. Utilization of these microbial functions in bioaugmentation or encouraging the natural microbial diversity could be a viable approach of sustainable cotton farming.

Although there has been heavy application of pesticides in cotton agroecosystems in Pakistan, little has been done to relate the combined effects of the pesticide residues on biodiversity of soil and enzymatic activity. The majority of studies are contributed to personal chemical studies or crop residue evaluation with little combination of microbial and enzymatic values (Ahmed et al., 2018). The gap in the knowledge highlights the necessity of systematic research that can assess the ecological effects of using pesticides, detect susceptible microbial taxa, and determine amounts of functional deficiencies in soil ecosystems. Policy development, integrated pest management (IPM) and sustainable soil and crop productivity are areas where such information is necessary.

Overall, the current sources show that pesticide residues have adverse impacts on the soil biodiversity and enzymatic processes and have implications on nutrient cycling, soil fertility, and crop performance. The extent of such effects varies according to the type of pesticide, concentration, persistence and soil properties. Combining molecular microbial analysis with enzyme activity analysis can be viewed as an effective method to assess soil health in the environment with chemical stress. This research is meant to fill knowledge gaps in cotton growing areas of Punjab, Pakistan, by examining the correlation between the residual concentration of pesticides, microbial diversity, and enzymatic activities, which will eventually be used to inform sustainable soil management and ecological protection.

METHODOLOGY

Study Area and Site Selection

The experiment was done in cotton-producing areas of Southern Punjab, Pakistan, that is, in Multan, Bahawalpur, and Muzaffargarh districts. The climatic conditions of these regions are arid-to-semi arid, the soils are sandy-loam. The cotton cultivation is intensive. Sampling fields were chosen depending on the history of pesticide application, crop management practices and accessibility. They sampled 15 cotton fields that embody low, moderate, and high patterns of pesticide use in order to determine the effects of residual pesticides on the soil biodiversity and soil enzymatic activities.

Preparation and Sampling of Soils

A state of 0–20 cm deep soil samples were taken during the high cotton growing season through a sterilized auger. To consider the spatial heterogeneity, five composite samples of soil were selected at random in each of the fields to create a representative sample of soil per field. Samples were put in sterile polyethylene bags, frozen in ice and then separated in two parts, one put in 4 o C to analyse microbes and the other dried by air and sieved (2 mm mesh) to analyse pesticide residues and enzyme activity.

Pesticide Residue Analysis

The analysis of the soil pesticides residues was conducted by means of the Gas Chromatography-Mass Spectrometry (GC-MS) according to the standard protocols (Kaur et al., 2016). A bout 10 g of air-dried soil was dried using acetone and the solid-phase extraction (SPE) column. Leftover pesticides that are widely used in cotton such as organophosphates (chlorpyrifos), carbamates (carbofuran), and pyrethroids (cypermethrin) were measured. Analytical grade standards were used to prepare calibration curves and limits of detection (LOD) and quantification (LOQ) limits had been determined of each compound. The results of residue were in mg/kg of soil.

Diversity Assessment of Microbial Diversity

The presence of microbial communities in the soil was compared using both culture-dependent and molecular methods.

1. **Culture-based analysis:**
- Bacteria: Soil was serially diluted and placed on nutrient agar and allowed to incubate at 28degC over a period of 48 hours. To determine the abundance of bacteria, colony-forming units (CFU) were counted.
- Fungi: Soil dilutions were plated on potato dextrose agar (PDA) to select the growth of bacteria that were inhibited by antibiotics. Plates were incubated at 25degC with 5-7 days and fungal growths were counted and morphologically observed.

2. **Molecular analysis:**
- A 0.5 g of soil was subjected to DNA extraction with the help of a commercial soil DNA extraction kit (Qiagen, Germany).

ACAEDEMIA Biota Nexus Journal
https://academia.edu.pk/index.php/bnj
Volume 1, Issue 3

- To profile microbial diversity, bacteria and fungi were subsequently amplified using PCR with 16S rRNA genes and ITS regions respectively.
- Community composition was determined by denaturing gradient gel electrophoresis (DGGE), and the band richness and intensity were determined as a measure of microbial diversity.

Soil Enzymatic Activities

There were three enzymes that were used to determine the functional health of soil:

Dehydrogenase activity: The activity was determined through triphenyl tetrazolium chloride (TTC) reduction that is applied to estimate the microbial oxidative activity (Caldwell et al., 2014). The results were written in mg triphenyl formazan (TPF)/g soil/h.

Urease activity: It was measured through method of hydrolysis of urea wherein the quantity of ammonium elements liberated in the soil extracts was ascertained. The activity was reported in terms of the value mg NH4+-N/g soil/h (Gupta and Verma, 2015).

Phosphatase activity: Phosphatase activity was determined by using p-nitrophenyl phosphate as this is used to measure the amount of p-nitrophenol that is released with a given time metric. The results were also given through mg pNP/g soil/h (Arora et al., 2020).

The assays were performed on a triplicate basis with their blank controls being taken to control the abiotic responses.

Statistical Analysis

The SPSS version 25 was used to perform the data analysis. The post hoc test was to compare the level of pesticide residues, abundance of microbes and enzyme activities in the fields with different records of pesticides application at a significant level of $p < 0.05$ which was used to proceed with one-way ANOVA. The Pearson correlation coefficients were estimated to find out relationships between pesticide residues and microbial diversity and enzymatic activities. GraphPad Prism 9 was used to prepare the graphical representations, and the BioNumerics software to examine the DGGE banding patterns to analyse the changes in the microbial communities.

DATA ANALYSIS & FINDINGS

Pursuit of Pesticide Residual in Soil

Pesticide residues of 15 cotton fields were detected in soil samples such as chlorpyrifos (organophosphate) and carbofuran (carbamate) and cypermethrin (pyrethroid). Findings showed that the level of pesticide residue differed greatly based on the past application history of the pesticides (Table 1). Fields that contained the highest level of pesticide usage contained the highest level of residues, and fields, which contained the lowest level of pesticide usage contained the lowest level of pesticide residues.

Table 1: Mean Pesticide Residue Concentrations in Cotton Field Soils (mg/kg)

Field Category	Chlorpyrifos	Carbofuran	Cypermethrin	Total Residues
Low Pesticide Use	0.12 ± 0.03	0.08 ± 0.02	0.05 ± 0.01	0.25 ± 0.04
Moderate Use	0.35 ± 0.06	0.22 ± 0.04	0.18 ± 0.03	0.75 ± 0.08
High Use	0.78 ± 0.09	0.55 ± 0.07	0.42 ± 0.05	1.75 ± 0.12

Interpretation

The outcome of ANOVA indicated significant differences ($p < 0.01$) of pesticide residues in fields that

ACAEDEMIA Biota Nexus Journal
https://academia.edu.pk/index.php/bnj
Volume 1 Issue 3

had different application histories. Viewed in absolute terms, high use fields contained almost seven times the amount of residues as low use fields, demonstrating that soils that had been exposed to intensive application of pesticides had accumulated the products in the soils.

Soil Microbial Diversity

The counts of microbes using culture based methods showed a high reduction in bacterial and fungal numbers with an increase in pesticide residues (Table 2).

Table 2: Microbial Abundance in Cotton Field Soils (CFU × 10^6/g soil)

Field Category	Bacteria	Fungi	Bacteria:Fungi Ratio
Low Use	9.2 ± 0.6	3.1 ± 0.4	2.97
Moderate Use	6.8 ± 0.5	2.2 ± 0.3	3.09
High Use	3.9 ± 0.4	1.1 ± 0.2	3.55

Bacteria and fungi counts reduced 57 and 65 percent in fields with large use of pesticides and low-use fields, respectively. The small factual increase in the ratio of the bacteria to fungi indicates that fungi are more vulnerable to pesticide residues as compared to bacteria. The correlation test using Pearson analysis revealed a strong negative relationship between total pesticides residue and microbial abundance ($r = -0.82$ with bacteria, -0.88 with fungi, $p < 0.01$).

The same findings were supported by molecular analysis (DGGE) whose results showed a lower richness and intensity of bands in the high use fields, which pointed to a reduced microbial diversity and loss of sensitive microbial taxa. DGGE profile cluster analyses demonstrated that different community changes occurred, where high-use fields could be distinguished with low- and moderate-use fields according to the pesticide selecting resistant microbial strains.

Soil Enzymatic Activities

The enzyme dehydrogenase, urease and phosphatase activities reduced with rise in pesticide residues (Table 3).

Table 3: Soil Enzymatic Activities in Cotton Field Soils

Field Category	Dehydrogenase (µg TPF/g/h)	Urease (µg NH_4^+-N/g/h)	Phosphatase (µg pNP/g/h)
Low Use	45.8 ± 2.5	32.4 ± 1.8	27.6 ± 1.5
Moderate Use	32.1 ± 2.1	21.7 ± 1.5	18.9 ± 1.2
High Use	18.4 ± 1.6	12.3 ± 1.1	9.5 ± 0.8

There was a reduction of 60 percent of the dehydrogenase activity of the high use fields over the low use fields and this indicated that the oxidative metabolism of the microbes was largely suppressed. The urease and phosphatase were decreased by 62 and 66 percent indicating a lack of nitrogen and phosphorus cycling. ANOVA proved that no enzymatic activities were the same across the categories of pesticide-use ($p < 0.01$). Pearson correlation analysis revealed that total pesticide residues and the enzyme activities had a strong negative correlation ($r = -0.85$ to -0.91, $p < 0.01$).

Connection among the residues of Pesticides, the diversity of microorganisms, and the activity of the enzyme.

Correlation indicated significant interaction of the pesticide residues, microbial abundance and soil

ACAEDEMIA Biota Nexus Journal
https://academia.edu.pk/index.php/bnj
Volume 1, Issue 3

enzyme activities (Table 4).

Table 4: Pearson Correlation Coefficients Between Pesticide Residues, Microbial Diversity, and Enzymatic Activities

Parameter Pair	Correlation (r)	Significance (p)
Total Residues – Bacteria	–0.82	<0.01
Total Residues – Fungi	–0.88	<0.01
Total Residues – Dehydrogenase	–0.91	<0.01
Total Residues – Urease	–0.85	<0.01
Total Residues – Phosphatase	–0.89	<0.01
Bacteria – Dehydrogenase	0.87	<0.01
Fungi – Phosphatase	0.84	<0.01

The negative interference between pesticide residues, microbial and enzymatic parameters are indicative of the fact that the introduction of chemical load into soil inhibits the microbial multiplicity and the functionality of enzymes. There are positive relationships between microbial abundance and enzyme activity implying that healthy microbial communities are needed to sustain soil biochemical processes.

Overall Findings

Cotton fields that are being intensively controlled have the highest levels of pesticides accumulation with the residues of up to 1.75 mg/kg.

The higher levels of pesticides the less microbial diversity, and the fungi are more sensitive than bacteria.

The enzymatic activities in the soil are reduced tremendously in the presence of high pesticide residues, which means that the process of nutrient-cycling is impaired.

The close relationships between the pesticide residues, abundance of microbes, and enzyme activities indicate that intensive pesticide application presents an ecological risk and hence the necessity of adopting sustainable management practices.

These findings present extensive proof that the over use of pesticides impacts negatively the health of the soil by interfering with the biologic diversity as well as biochemical processes within the cotton agro ecology.

CONCLUSION AND RECOMMENDATIONS

The current research proves that the pesticide residues in the soils of cotton fields in Southern Punjab, Pakistan, have a remarkable effect on the soil biodiversity and enzymatic activity, which influence the soil health and ecological sustainability. Sectors with greater proportions of pesticide application had more chlorpyrifos, carbofuran, and cypermethrin which were closely linked with decreases in the abundance and diversity of microbes. Culture and molecular studies were found to have shown that fungus populations are more vulnerable to pesticide residues than bacteria, which lead to changes in the community structure of the microbes and decreased ecosystem stability.

Enzyme activities in the soil such as dehydrogenase, urease and phosphatase were significantly reduced in the soils that had high pesticide residues. This decrease implies poor microbial metabolism and derailments in the cycling of nitrogen and phosphorus that are essential in soil fertility as well as cotton output. Good negative relationships between the overall residue of pesticides and the microbial counts as well as the enzymes activity substantiate the fact that too much of a chemical in use is harmful to the soil

ACAEDEMIA Biota Nexus Journal
https://academia.edu.pk/index.php/bnj
Volume 1 Issue 3

functionality. The results indicate that though pesticides play a vital role in controlling pests, their excessive use may cause the long-term ecological impacts of soil, and the loss of useful soil microorganisms and their decrease in the turnover of nutrients.

On behalf of these findings, it is possible to offer a number of recommendations:

Integrated Pest Management (IPM): Farmers are encouraged to embrace IPM where biological, cultural control methods are used in conjunction with selective application of chemicals to ensure that there are minimal pesticide left-overs in the soils without compromising on the ability to control pests.

Pesticide Residues: There is a need to monitor the level of pesticides in cotton field regularly to avoid an accumulation of pesticides and to protect soil health. Residual loads can be assessed with the help of simple field-based assays and periodic analysis with the help of GC-MS.

Enhancement of Soil Microbial Health: To strengthen the soil resilience and microbial enzymatic actions, the practices which enhance microbial diversity including organic amendments (compost, biofertilizers), crop rotation, and decreased chemical applications should be promoted.

Farmer Education and Awareness: Awareness should be carried out through education of the farmers on the environmental effects of using large amounts of pesticide and the advantages of using sustainable farming methods.

Policy and Regulation: The authorities in agriculture ought to promote regulation to the use of pesticides, encourage the use of environmentally friendly products and encourage the types of farming that will preserve the soil biodiversity and functionality.

To sum up, it is possible to emphasize that pesticide residues, the diversity of microorganisms in the soil, and enzyme activity in cotton agroecosystems have a critical relationship in this study. To reduce adverse impacts of pesticide accumulations, to maintain the health of soil, and the long-term cotton productivity, sustainable management strategies are necessary. The findings can be used to make sound ecological policies and guidelines that farmers can implement in ensuring that crop protection does not affect environmental protection.

REFERENCES

Ahmed, S., Ali, M., & Khan, R. (2018). Assessment of pesticide residues in agricultural soils and their effects on microbial diversity. *Environmental Monitoring and Assessment, 190*(2), 85. https://doi.org/10.1007/s10661-017-6391-2

Ahmad, M., Hussain, A., & Khan, S. (2019). Cotton production challenges and pesticide usage in Pakistan: A review. *Journal of Agricultural Science, 11*(3), 120–132. https://doi.org/10.5539/jas.v11n3p120

Arora, N., Singh, J., & Sharma, P. (2020). Impact of pesticide residues on soil enzyme activities and microbial biomass. *Soil and Tillage Research, 199*, 104605. https://doi.org/10.1016/j.still.2020.104605

Bhardwaj, D., Mehta, P., & Kumar, R. (2018). Effects of organophosphate pesticides on soil enzymatic activities in cotton fields. *Environmental Science and Pollution Research, 25*(15), 14567–14578. https://doi.org/10.1007/s11356-018-1612-9

Caldwell, D., Rice, J., & Simons, T. (2014). Soil dehydrogenase activity as an indicator of microbial metabolism under chemical stress. *Soil Biology and Biochemistry, 70*, 100–107. https://doi.org/10.1016/j.soilbio.2013.12.004

Gupta, V., & Verma, S. (2015). Influence of pesticide residues on soil urease activity and nitrogen mineralization. *Journal of Environmental Management, 150*, 229–236. https://doi.org/10.1016/j.jenvman.2014.12.026

Kaur, R., Singh, P., & Sharma, S. (2016). Monitoring pesticide residues in agricultural soils using GC-MS techniques. *Journal of Environmental Chemistry and Ecotoxicology, 8*(4), 29–38. https://doi.org/10.5897/JECE2016.0303

Khan, H., Ali, Q., & Farooq, M. (2020). Effects of pesticide residues on soil microbial communities in cotton agroecosystems. *Applied Soil Ecology, 147*, 103364. https://doi.org/10.1016/j.apsoil.2019.103364

Rashid, M., Shah, S., & Ahmed, N. (2019). Molecular characterization of soil microbial communities under pesticide stress. *Journal of Soil Science and Plant Nutrition, 19*(3), 639–652. https://doi.org/10.1007/s42729-019-00120-7

Sharma, A., & Singh, K. (2017). Pesticide impact on soil microbial diversity: A review. *Environmental Science and Pollution Research, 24*(5), 4027–4040. https://doi.org/10.1007/s11356-016-8262-5

Singh, R., Mehta, P., & Kumar, S. (2018). Soil microbial diversity and enzymatic activities under pesticide application in cotton fields. *Ecotoxicology and Environmental Safety, 163*, 567–576. https://doi.org/10.1016/j.ecoenv.2018.07.012

Zhang, Y., Li, X., & Wang, Q. (2017). Persistence and ecological effects of pesticides in agricultural soils: A global review. *Science of the Total Environment, 586*, 566–576. https://doi.org/10.1016/j.scitotenv.2017.02.165