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Influence of Pesticide Misuse on Soil Microbial Communities in Punjab's Agricultural Zones

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ABSTRACT

The intensive exploitation of the soil for agricultural purposes combined with the intense use of pesticides have been the object of concerns toward their unintended impact on soil health but also on soil microbe diversity. In Punjab, Pakistan, there is a tendency among the agriculture workers to use chemical pesticide in indiscriminate manner and many times for more dosages than recommended and without adhering to advisory rules to safety practices. This study considers the impact of the pesticide abuse of soils in microbial communities of major agriculture zones of Punjab. Soil samples were taken from the fields with a history of high pesticide application and compared to low/zero pesticide applications. Microbial biomass, diversity indices and functional groups were investigated using culture techniques, but also molecular techniques (16S rRNA and ITS). Results showed a significant decrease in microbial diversity and changes in the composition of microbial communities in the pesticide-intensive fields with significant reduction in the populations of beneficial bacteria and fungi. Furthermore, long term exposure to pesticides was associated with the decrease of enzymatic activity and the nutrient cycling patterns of the soil. These findings indicate the ecological risks that are linked with the excessive use of pesticides and is another indication that the sustainability of pest management practices are needed to maintain soil microbial integrity and agricultural productivity.

Introduction

Agricultural intensification in the developing countries including that of Pakistan has resulted in an intensive use of chemical pesticides to control the pests of crops and to increase their yield. Punjab being the most agriculturally fruitful province of the country, thus is responsible for a large portion of wheat, rice and cotton yield of the country. To manage the higher food demands, farmers make intensive use of pesticides, and most of them are not properly trained and do not follow the recommended pesticide application rate (Khan et al., 2018; Malik et al., 2020). While these chemicals are effective in managing pests, the indiscriminate application of pesticides have serious ecological and agronomic implications, especially on soil microbial community essential in nutrient cycle, breakdown of organic matter and soil fertility more broadly (Garcia-Orenes et al., 2010; Imran et al., 2021).

Soil microorganisms in which important ecosystem service functions are carried out, like nitrogen fixation, phosphorus solubilization and decomposition of organic residues (Nannipieri et al., 2017) such as bacteria, fungi and archaea. Changes in microbial abundance, diversity, or the microbial community structure may change these functions that leads to the alteration of soil quality and the productivity of our crops. Studies have ruled out the fact that the excessive application of pesticides can result in the selective repressive effect on the sensitive microbial taxa and increase the frequency of resistant strains leading to the reduction of biodiversity and alteration of the community composition (Singh et al., 2019; Xu et al., 2020). Such disturbances not only cause the soil fertility but could also result in increase in virulence of pesticide resistance among the pathogens and could make the pest managements strategy all the more complicated.

In agricultural areas of punjab, there are fast common pesticides such as organophosphates, carbamates, pyrethroids, neonicotinoids that are used in mixture or we use higher concentration of pesticide than recommended (Qureshi et al., 2017; Tariq et al., 2019). There is evidence that long-term exposure of these chemicals could affect microbial enzymatic activities such as dehydrogenase, phosphatase and urease that have an importance in nutrient cycling (Jiang et al., 2018). Moreover, pesticide residues can persist in the soil and affect microbial recolonisation and alter the functional potential of microbial community with time (Verma et al., 2020).

Despite the known importance of the soil microbes, studies about the effect of pesticide abuse in the Punjab are limited. Most of the past work has been focused either on, crop yield, residue examination or pest death, little work has been done on the ecological implications of the different microbial diversities and soil functioning (Hussain et al, 2021; Shah et al, 2020). Understanding these impacts is critical since soil microbial communities are the representation of long-term agricultural sustainability and quantity against environmental stresses.

This research is going to assess the impact of pesticides misuse on the soil microbial operations in major agricultural areas of Punjab. Specifically, looking at differences in microbial biomass, diversity and functional group composition of fields having intensive pesticide use compared to fields with little or no pesticide application. Furthermore, the importance of pesticide exposure, soil enzymatic activity and nutrient cycling parameters on nutrient cycling and correlation with soil is investigated. This research consequently provides a comprehensive evaluation on the pesticide-induced changes on soil microbiota as a stepping stone to the development of sustainable pest management strategies, a process that striking a balance in between pest control of food crops and environmental environmental protection (Ali et al., 2019; Zhang et al., 2021).

The results of this research will hopefully be used to advise farmers, policy makers and extension services about the risks of ecological degradation associated with misuse of pesticides, and the importance of sustaining soil microbial diversity for farming productivity in the long term. A preservation of microbial community is not only good for soil fertility, but a sustainable agricultural practices to mitigate negative effects of chemical feed (Li et al. 2020; Chen et al. 2019). Overall, this study highlights the need for urgent measures to utilise pesticides integrated with other non-toxic methods of pest control to maximise the utilisation of pesticides while protecting the soil ecosystem services of the intensive farming system of Punjab.

Literature Review

Soil populations of microorganisms form complex connections in ecosystem and agricultural productivity in performing critical roles including breakdown of organic matter, cycling of nutrients, and pathogen suppression (Nannipieri et al., 2017; Zhang et al., 2021). Healthy populations of microbes play a part in the structure, fertility and toughness of soils to cope with environmental stresses. However, intensive agricultural practices, primarily abuse of chemical pesticides, have become one of the major problems to the diversity and function of soil microorganisms (Singh et al., 2019; Garcia-Orenes et al., 2010).

Pesticides are being used to a large extent for the elimination of insects, weeds and fungal pathogens; the commonly used pesticides for the control of these crops in the Punjab is organophosphates, carbamates, pyrethroides and neonicotinoids in the case of major crops of wheat, rice and cotton (Qureshi et al., 2017; Tariq et al., 2019). While these chemicals provide a boost to crop yields in the short-term, using these chemicals too frequently and over long periods of time, can have long-term adverse effects for soil microorganisms. However, it has been documented through the studies that there is a high concentration of pesticides to inhibit sensitive bacteria and fungi taxa, reduce the abundances of the microbial life and changes in the community compositions to resistant organisms (Imran et al., 2021; Xu et al., 2020). These shifts could significantly affect the ecological processes such as nitrogen fixations and phosphorus solubilizations which are very essential for maintenance of the soil fertility (Li et al., 2020; Chen et al., 2019).

The ways that pesticides affect microbes communities are multi-fold. Organophosphates and carbamates insecticides for example have the capacity to affect enzymatic processes in soil such as dehydrogenase, phosphatase and urease which are essential in the breakdown of organic matter and nutrient cycling (Jiang et al., 2018; Shah et al., 2020). Similarly, pyrethroids have been described to reduce the respiration process of microbes and inhibit the microorganisms involved in the nitrification processes (i.e. Ali et al., 2019; Malik et al., 2020). Chronic exposure to neonicotinoids can result in a long term persistence of neonicotinoids in soil which have further effects on the inhibition of microbial recolonisation and the alteration of the functional potential of microbial communities (Verma et al., 2020; Khan et al., 2018).

A number of field and laboratory studies also have documented reaching in microbial biomass and diversity related to the use of pesticides. For instance, Garcia-Orenes et al study (2010) showed the reduction of microbial diversity in soils, decrease in populations of beneficial fungi and bacteria resulting from long term pesticide application. Similarly, Imran et al. (2021) reported extensive application of pesticides in rice and wheat field of Punjab had significantly changed community structure of microbes including reduction in nitrogen fixing microbial population and phosphorus solubilizing fungi. These results not only confirm how much chemical stressers are likely to be a threat on microbial ecosystem like the soil, but it also throw some light on how much data on manifestations of micro-organism can be important as warning signs of soil degradation.

The misuse of pesticides is added to a lack of awareness that farmers have as to recommended rates of application, times of application as well as protective measures. In the Punjab, based on surveys, it has been observed that the farmers often use cocktail of pesticides, or use pesticides at higher doses than the recommended dose <= soil contamination and meddling in soil microbial life (Qureshi et al, 2017; Tariq et al, 2019). In addition, overuse not only changes the microorganisms, it can lead to a higher development of pesticide resistant pest populations, which starts a chain of chemical dependency and ecological degradation (Singh et al., 2019; Farooq et al., 2017).

Soil microbial diversity can be determined by culture-based and culture-independent techniques like using high-throughput DNA sequencing 16S rRNA genes for bacteria and ITS regions for fungi (Nannipieri et al., 2017; Xu et al., 2020). These methods are providing insights to the community composition and richness and functional potential. Studies employing these molecular tools have always indicated that fields with a high pandemic of chemical composite (pesticides) possessed lower alpha diversity and alterations in beta diversity, which in turn indicated lower species richness and alterations in the determining microbe taxa (Chen et al., 2019; Li et al., 2020).

Functional effects of pesticide misuses are far beyond the composition of the microbes. Enzymes biochemical activity of (Dehydrogenase, Phosphatase and urease) in soil closely associated with the health of microorganisms and nutrient cycle (Jiang et al. 2018; Shah et al. 2020). Declines in these enzyme activities have been reported within soils experiencing pesticide intensive farming with reduction in the metabolic activities of the microbe and potential limitation of nutrients for crops. Additionally, the pesticide residues can interact with soil organic matter and clay mineral which can affect the bioavailability and persistence and subsequently influence the recolonisation of microbes and recovery of microbial community (Verma et al., 2020; Khan et al., 2018).

Integrated pest management (IPM) strategies have been proposed as a way of mitigating the ill effects of abuse of pesticides on soil microorganisms. The principle of IPM involves the use of biological control agents, crop rotation, resistant cultivars, judicious use of chemicals, minimizing the use of harmful pesticides and simultaneously maintaining the crop production (Ali et al., 2019; Farooq et al., 2017). Studies have been conducted in the Punjab that has shown the possibility of reestablishing the microbial diversity and enzyme activity by implementing the IPM practices during the high-intensity and farming systems (Malik et al., 2020; Imran et al., 2021).

The interactions among pesticides and soil micro-organismies have greater implications for the agricultural exemplified existence. Reduced microbial diversity and imperfect enzymatic activities may not only influence soil fertility, nutrient recycling and crop yields, it will also result in the issues of food security (Garcia-Orenes et al., 2010; Zhang et al., 2021). Moreover soil microbial communities play a role in ecosystem resiliency such as resistance to pathogen invasion and resistance to abiotic stresses such as drought and salinity. Thus, the maintenance of the population of microbes with its diversity and functionality is of utmost importance for long-term agricultural sustainability of the Punjab and other like agro-ecological zones (Li et al., 2020; Chen et al., 2019).

In Conclusion, available literature shows the misuse of pesticide has a great influence on soil microbial diversity, composition and function in agricultural zone of Punjab. While chemical pesticides are still significant to the management of the pest problem, their abuse create ecological risks to soil health and productivity. Understanding these impacts influence a scientific basis for promotion of sustainable pest management practices, such as IPM, reduction of pesticide application and soil monitoring programs. Future studies should have the focus of conducting longitudinal studies on recovery of microbes after the change in pesticide practices and considering the use of microbial indicators in the decision-making process about sustainable agriculture (Ali et al., 2019; Verma et al., 2020; Shah et al., 2020).

Methodology

This research has been performed to establish the effects of pesticide abuse on microbial communities in soil flourishing in major agricultural areas of Punjab, Pakistan. A total of six districts with diverse cropping systems (wheat, rice, cotton, and sugarcane) were selected for sampling in such a manner that there would be a variation in the pesticide application intensity and history. Soil samples were collected during the post-harvest period where the cumulative effect of the exposure to pesticides during the growing season was collected. Each sampling location was selected based on farmer's pesticide usage records and high, medium and low pesticide application intensity fields were selected.

At each site composite soil samples were collected from the upper 15cm (0-15 cm) soil layer using a sterile auger. Each composite sample consisted of five subsamples because of spatial variation and to account for the field taken randomly across the field. Samples were placed in sterile polyethylene bags and were transported to the laboratory stored in 4degC inside cool carriers where they were stored till analysis. Soil physicochemical properties include pH, organic matter, texture, and moisture which were determined using standard techniques (Jackson 1973) in order to try to control for some potential confounding factors for microbial communities.

Microbial analysis both included the culture both dependent and independent approach. Culture-based techniques were plate counts for total bacteria, fungi and actinomycetes by nutrient agar, potato dextrose agar and actinomycete isolation agar, respectively. Colony-forming units (CFU) were calculated for quantifying the microbial abundance. Functional groups, i.e., nitrogen fixing bacteria and phosphate solubilizing microbes were enumerated using the selective media with the help of the established protocols (Alef & Nannipieri, 1995).

For molecular analysis, extraction of the DNA from soil was performed with the MoBio Power Soil DNA Isolation Kit. Bacterial community was studied using 16S rRNA gene sequencing and fungal community using the gene sequencing of the ITS region. HTS was performed from Illumina MiSeq using genomic DNA. Bioinformatic analysis was performed which included quality filtering, the clustering operation taxonomic unit (OTU) similarity with 97% similarity and calculation of alpha diversity and beta diversity between the pesticide application categories to differentiate microbial richness and microbial community compositions.

Soil enzymatic activities were determined in order to assess the microbial functional potential. Dehydrogenase activity was done by triphenyl tetrazolium chloride (TTC) method and phosphatase by using p-nitrophenyl phosphate as substrate and Urease activity using the colorimetric method of ammonium release (Tabatabai, 1994). These analyses gave some insight as to how the process of nutrient cycling is affected by pesticide misuse.

Statistical analyses were performed using the software package of the statistical package and computer programme, the Statistical Analysis and Data Mining (SPSS) v26. One-way analysis of variance (ANOVA) was applied for the identification of differences for microbial abundance, diversity and enzyme activity of biological communities between fields with different intensities of pesticides. Pearson correlation coefficients was determined to find out relations between pesticide application rates, microbial diversity indexes and enzymatic activities. Principal coordinate analysis (PCoA) based on the sequencing data in order to visualize the differences of the community composition. Significance at $p < .05$ accepted.

Through this combined method of sampling in the field, microbial enumeration from the environment, molecular studies and use of enzymatic assay provided a comprehensive assessment of the effect of pesticide misuse on the soil microbial population and their functional ability in the agricultural zones of the Punjab area.

Data Analysis & Findings

The analysis of the soil samples resampled from the six major agricultural districts of Punjab showed significant difference in microbial communities of the sampled fields with high, moderate and low pesticide application intensities. Culturebased methods revealed very significant reduction of bacteria and fungi total abundance with pesticide intensive fields. For example, high pesticide fields had a mean bacterial CFU of 3.4x10^7 per gram of dry soil compared to the low pesticide fields which had a CFU of 7.8x10^7 (Table 1). Similarly, fungi populations have been diminished by almost 45% in high intensity fields indicating the negative effect of the continued chemical exposure on sensitive microbial taxa. Actinomycetes one of the most important mechanisms involved in decomposition of organic matter also were significantly reduced in the pesticide intensive soils leading to falsus key nutrient management processes.

Table 1. Microbial abundance (CFU g^{-1} dry soil) across fields with different pesticide application intensities

Pesticide Intensity	Bacteria ($\times 10^7$)	Fungi ($\times 10^5$)	Actinomycetes ($\times 10^5$)
High	3.4 ± 0.2	1.8 ± 0.1	2.1 ± 0.2
Moderate	5.6 ± 0.3	3.2 ± 0.2	3.9 ± 0.3
Low	7.8 ± 0.4	3.3 ± 0.2	4.5 ± 0.3

Molecular analyses with the use of 16S rRNA and ITS sequencing revealed more details about microbial community composition. High pesticide intensity fields showed decrease of alpha diversity indexes, while opposite trend was observed for Shannon diversity from 5.8 for low intensity fields to 3.9 for high intensity field for bacterial community and therefore, in high pesticide intensity field, the microbial richness and evenness were lost. Beta diversity analysis revealed distinct segregation of microbial communities according to the pesticide intensity suggesting the significant influence of chemical exposure on the reconfiguration of the soil microbiota (Figure 1). Fungal community composition also differed where beneficial taxa such as Trichoderma and Glomus became suppressed in the high pesticide fields, and the pesticide resistant genera such as Fusarium were more abundant.

Soil enzymatic activity coincided with these trends in microbe activity. Dehydrogenase activity, which is an indicator of general microbial metabolic activity, revealed a reduction of 42% of enzyme activity in high pesticide fields when compared with low pesticide fields (Table 2). Phosphatase and urease activities which are indispensable for the cycling of phosphorus and nitrogen were also significantly lower in high-intensity fields, indicating that mis-use of pesticides does not only impact on microbial abundance, but also on their functional potential. Pearson correlation analysis confirmed that there were highly positively correlated values between microbial diversity parameters and enzymatic activity ($r = 0.78$--0.85, $p < 0.01$) which point out the interdependence of the community structure and soil's functionality.

Table 2. Soil enzymatic activities across fields with varying pesticide intensities

Pesticide Intensity	Dehydrogenase (µg TPF g^{-1} h^{-1})	Phosphatase (µg PNP g^{-1} h^{-1})	Urease (µg NH_4^+ g^{-1} h^{-1})
High	12.5 ± 1.1	8.3 ± 0.7	9.4 ± 0.8
Moderate	19.6 ± 1.3	12.7 ± 0.9	15.2 ± 1.0
Low	21.8 ± 1.5	14.3 ± 1.0	17.5 ± 1.2

Functional group analysis revealed nitrogen-fixing bacteria (e.g. Azotobacter and Rhizobium) to be especially sensitive to high exposure to pesticides by reducing 50% of their abundance. Similarly, phosphate solubilizing bacteria declined significantly due to which P availability may be reduced for crops. According to fungal guild analysis, there were decrease of arbuscular mycorrhizal fungi which have an important functions for nutrient uptake and soil structure stabilization. These results are in line with previous studies indicating that inappropriate use of pesticides may selectively inhibit the beneficial microbial community and favour opportunistic or resistant community species (Imran et al., 2021; Singh et al., 2019).

1. Overall, the results suggest pesticide misuse in agricultural zones of the Punjab results in:
2. Decreased levels of microbial biomass/genetic diversity.
3. Changed make-up of the bacterial and fungal community.
4. Reduced enzymatic activities which play an important part in nutrient cycling
5. Suppression of the important functional groups like nitrogen fixing and phosphates solubilising microbes

These changes all together represent a threat to soil health, fertility and sustainability. The data suggests fields with minimum pesticide application have greater and functionally more diverse microbial community and ecosystem resilience and agricultural productivity. This shows the need of urgent application of sustainable pest management practices to reduce the negative ecological impact of pesticide misuse.

Discussion

The results of this investigation have clearly showed that misuse of pesticides has a major role in soil microbial community of agricultural zones in Punjab. Fields with an elevated pesticide use pattern had significant changes in bacterial, fungal and actinomycete populations in accordance with the suspicion that if chemicals are excessively used, this can negatively affect the abundance of the microbial population. As well as suggestions of decreases in the microbial diversity, where sensitive organisms were suppressed by the pesticides there were benefits for pesticide-resistant organisms where they became dominant, molecular analyses also suggested. This alteration in community structure is in accordance with previous research showing that chemical stressors represent for resistant species in the microbial community, and also restrict the general ecosystem diversity (Singh et al., 2019; Imran et al., 2021).

Soil enzymatic activities including dehydrogenase, phosphatase and urease were significantly decreased in pesticide intensive fields because of the altered metabolic activities of the microorganisms. Reduced enzyme activity suggests the nutrient cycling processes (e.g. nitrogen fixation, phosphorous mobilisation) are affected with high stress from pesticides. The high levels of linear correlations determined between microbial diversity and the enzymatic activity ($r = 0.78 - 0.85$, $p < 0.01$) show the functional dependence of soil processes on the microbial health. These findings are consistent with previous studies which demonstrate that pesticide overuse during the use phase not only decreases microbial abundance but also functional potential, which can result in a long-term outcome of pesticide application on soil fertility and crop productivity (Jiang et al., 2018; Verma et al., 2020).

Functional group analysis was used to demonstrate significant decreases of abundance of nitrogen fixing and phosphate solubilizing microbes or arbuscular mycorrhizal fungi important for plant acquisition of nutrients and soil structure. The elimination of these beneficial organisms suggests that abuse of pesticide(s) may result in long term degradation of soil fertility and ecological resiliency. On the contrary, the low pesticide use fields had higher level of microbial richness and function, which indicate that lower level of chemical inputs can preserve the ecosystem services for soil and/or sustainable agriculture (Ali et al., 2019; Chen et al., 2019).

Conclusion

This study has concluded that misuse of pesticide in agricultural zones of Punjab has significant negative impacts on microbial communities of soil. High intensity use of pesticides reduces the abundance, community diversity and enzymatic activity of microorganisms and alters the community of microorganisms and inhibits important functional groups. These changes have inventory on the fertility of soils, nutrient cycling and long term agricultural sustainability. Maintaining the low or judicious application of pesticides to preserve the microbial diversity and ensuring continued functionality is the need for the productivity of the crop and ecological balance.

Recommendations

Based on the findings of the study a number of recommendations are stated to could mitigate the adverse impacts of the misuse of pesticides:

1. Adoption of Integrated Pest Management (IPM): Farmers should adopt Integrated Pest Management (IPM) strategies based on use of biological controls and resistant varieties of crops along with minimum use of chemical, which will sustain the health of microbes and keep the pest in check.
2. Training and Awareness Programs: Agricultural extension services should conduct programs to give training on recommended pesticide doses, times of application and safety measures to minimize indiscriminate use.
3. Soil Monitoring: Monitoring of microbial diversity and enzymatic activity of soil on a regular basis can also be used as an early indicator of soil health deterioration and appropriate management interventions.
4. Promotion of Organic Amendments - Addition of Organic Matter - Incorporation of Organic amendments in soil e.g. compost or Green manure can therefore buffer the negative effects of pesticides, increase microbial resilience, and nutrient cycling.
5. Policy and Regulation: Strengthening policy and regulation with regards to distribution, labelling and applications of pesticides to prevent over applications and protect soil ecosystems.

Implementing these strategies could be fruitful to guarantee for sustainable maintenance of soil microbial diversity, nutrient cycling and long term agricultural productivity in the Punjab intensive farming systems.

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