



Analysis of microbial diversity in pesticide-contaminated soil: A study of culturable microorganisms

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ABSTRACT

Background: Pesticide contamination of soil often leads to significant alterations in the structure and diversity of microbial communities, potentially affecting overall ecosystem function. Understanding these changes is crucial for assessing the ecological impact of pesticide use in agricultural areas. This study analyzes microbial diversity in pesticide-contaminated soil using the Shannon-Wiener diversity index to evaluate the effects of pesticide exposure on microbial populations. **Methods:** A descriptive quantitative approach was used, incorporating the Total Plate Count (TPC) test and Shannon-Wiener Index analysis. The numerical data included the number of microbial individuals (bacteria and fungi) and the relative proportion of each group. Soil samples were purposively collected from three points in a pesticide-contaminated tomato farming area in Dunggala Village, Gorontalo Regency. **Findings:** The microbial community detected in the contaminated soil consisted of bacteria (2.5×10^4 CFU/ml) and fungi (1.35×10^3 CFU/ml). The Shannon-Wiener index value was 0.202, indicating low microbial diversity. This suggests that pesticide contamination negatively impacts microbial richness and evenness in the soil. **Conclusion:** Pesticide contamination significantly reduces microbial diversity, as reflected in the low Shannon-Wiener index value. This decline in microbial richness and evenness highlights the potential ecological consequences of pesticide use in agriculture. To mitigate these negative effects, implementing sustainable pest management practices, such as the use of biopesticides, is recommended. **Novelty/Originality of this article:** This study provides quantitative evidence of the decline in microbial diversity in pesticide-contaminated soil using the Shannon-Wiener index. By focusing on microbial community changes in a specific agricultural setting, the findings contribute to a better understanding of the ecological impacts of pesticide use and emphasize the need for sustainable pest management strategies.

KEYWORDS: microbial diversity; pesticide contamination; shannon-wiener index.

1. Introduction

Rapid population growth demands a rapid and sustainable increase in the agricultural sector. To achieve this, various supporting facilities such as agricultural tools, fertilizers, and chemicals including pesticides are needed. Pesticides are chemical compounds used to control pests, weeds, and plant diseases in agricultural activities (Arsi et al., 2021). However, excessive use of pesticides can cause environmental pollution, especially soil. Pesticides that accumulate in the soil can have a negative impact on soil organisms, including microbes.

Pesticides are defined as substances or compounds, regulators or growth stimulants, including viruses or other microorganisms that are useful for plant protection, as well as a mixture of substances that are useful for preventing, destroying, rejecting, or controlling nuisance pests according to Government Regulation of the Republic of Indonesia/Peraturan Pemerintah Republik Indonesia (PP-RI) No. 6 (1995). In practice, the use of pesticides has

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become an integral part of the crop cultivation process. In Indonesia, the use of pesticides continues to increase every year, both in terms of the number of formulations and volume.

The easy availability of pesticides at affordable prices is one of the main factors for the high use of pesticides. In addition, most farmers still consider pesticides as an effective solution in controlling pests, so there is a dependency to continue using them as a production factor that determines crop yield and quality. However, the uncontrolled use of pesticides with active ingredients such as profenofos and chlorpyrifos on agricultural land, both in dose and frequency, can cause physical, chemical and biological damage to soil (Pratiwi & Asri, 2022).

Although pesticides have significant benefits, their excessive use can lead to pesticide contamination of agricultural land. According to Sinambela (2024), pesticides that exceed the threshold in the environment can act as pollutants that disturb the balance of nature. This is especially true if pesticides are used continuously, with doses that are not in accordance with the rules, and without official supervision from the government or producers (Pratama et al., 2021). The uncontrolled use of pesticides can cause various problems, both in the agricultural, health and environmental sectors. In the agricultural sector, for example, plant pests can become more resistant to pesticides (Joko et al., 2012; Arlinda et al., 2024). Meanwhile, in the environmental scope, increased pesticide residues in the soil can cause pollution (Theresia et al., 2023).

Pesticide contamination of soil ecosystems results in a decrease in soil microbial diversity. This decrease in the number of soil microbes reduces the contribution of microbes to soil fertility. According to Yetgin (2023), the formation of soil nutrients, nutrient cycling, and their availability for plant productivity require the role of soil microbes. Microbes are also involved in nutrient cycling through the transformation of organic and inorganic materials (Bhattacharyya & Furtak, 2022; Wu et al., 2024).

Microbes, which include bacteria, fungi, and protozoa, play an important role in soil nutrient cycling, organic matter decomposition, and maintaining the balance of soil ecosystems (Prayudyaningsih et al., 2015). However, the presence of pesticides in soil can affect microbial diversity and abundance, as some microbes are sensitive to pesticides and can experience population declines (Pathak et al., 2022). On the other hand, soils that are frequently exposed to pesticides can form microbial communities that utilize or degrade pesticide residues into their metabolic materials. For example, a study by Kamanavalli & Ninnekar (2000) reported isolates of *Pseudomonas* sp. that were able to hydrolyze propoxur into iso-proxy-phenol and methyl-amine, which were then used as carbon and nitrogen sources by the bacteria. Therefore, the presence of microbial populations in pesticide-contaminated soil can be used to evaluate the impact of contamination.

Contamination of agricultural soils with pesticides causes significant changes in microbial diversity, which can negatively impact soil fertility and ecosystem health. This study aimed to assess the impact of pesticide contamination on soil microbial populations, focusing on the degradation of pesticide residues and its effect on microbial communities. Using Total Plate Count (TPC) methodology, this study investigated the abundance and diversity of soil microbes in pesticide-impacted areas. Preliminary results suggest that pesticide residues affect the composition and metabolic activity of soil microbes, with certain bacterial species being able to degrade pesticide compounds, thus providing insight into the wider ecological consequences of pesticide use.

2. Methods

This research was conducted in March 2024, at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Gorontalo State University. The tools used in this research are Petri dish, Erlenmeyer, stirring rod, hot plate, analytical balance, microscope, preparation glass, cover glass, drop pipette, vortex, test tube, oven, autoclave, bunsen, ose needle, incubator. Materials used in this study were *Nutrient Agar* (NA) media, *Potato Dextrose Agar* (PDA) media, distilled water, crystal violet, lugol, safranin, alcohol, physiological NaCl, and soil taken from Tomato Farm Dunggala

Village, Tibawa District, Gorontalo Regency. The method used in this research is Quantitative descriptive which is based on the TPC test and analyses based on the Shannon-Wiener Index using numerical data, such as the number of microbial individuals (bacteria and fungi) and the relative proportion of each group. The sampling technique uses Purposive sampling technique, namely the determination of sampling at three points of agricultural areas polluted by pesticides.

Bacterial and fungal isolates were taken from soil samples collected from pesticide-contaminated agricultural land in a tomato farming area located in Dunggala Village, Gorontalo Regency. The sampling process used a purposive sampling method, targeting three points in the contaminated area to ensure representative data. Soil samples were taken from a depth of 0–15 cm using a sterile soil drill. Samples were stored in sterile polyethylene containers to prevent contamination. In the laboratory, each sample was prepared by weighing 5 g of soil and dissolving it in 45 mL of sterile distilled water, followed by homogenization using a shaker incubator at 225 rpm for 30 min. A series of serial dilutions were performed up to 10^{-3} , and the diluted samples were incubated at 37°C for 24 hours. Colonies grown on culture media were observed for their macroscopic morphological characteristics, and the data obtained were used to calculate the Shannon-Wiener Index, a metric for measuring species diversity in a community. The Shannon-Wiener diversity index (H') is used to measure the level of diversity in a community by considering the number of species and the distribution of individuals within that community. The value P_i represents the proportion of individuals in the i -th group relative to the total number of individuals in the community, calculated using the Equation 2, where n_i is the number of individuals of the i -th species, and N is the total number of individuals of all species in the community. Additionally, S represents the total number of groups in the community, while \ln is the natural logarithm used in the calculation. The formula used to calculate this index is:

$$H' = - \sum_{i=1}^S P_i \ln(P_i) \quad (\text{Eq. 1})$$

$$P_i = \frac{n_i}{N} \quad (\text{Eq. 2})$$

3. Results and Discussion

The research was conducted in Dunggala village, Tibawa sub-district, Gorontalo district. It was found that the field used pesticides with the active ingredient Diazinon 600 EC to reduce plant pest organisms. The farmland has an area of 250 m² which is planted with tomatoes, as a cultivated crop. Based on interviews with the farm owner, the use of pesticides with the active ingredient Diazinon 600 EC has been used by farmers for approximately three years. This insecticide is used to eradicate insects that attack vegetable cultivation on the farm. It is possible that there will be soil microbes that have adapted and use the active ingredient Diazinon 600 EC as one of their sources of life. Soil samples were then taken at three random points at a depth of 15 cm.

Based on theory, the continuous use of pesticides over a long period of time can lead to the selection of soil microbes that can withstand these toxic compounds (Seghers et al., 2003; Tyas et al., 2015). Some microbes, especially bacteria, can adapt by utilizing pesticides as an energy source or can deconstruct the toxic compounds through the biodegradation process. This is supported by previous studies showing that bacterial groups such as *Pseudomonas* and *Bacillus* have the ability to degrade pesticide compounds, making them more dominant in pesticide-contaminated soil. In contrast, fungi and microbes that are more sensitive to these toxic compounds usually decrease in number, which has an impact on the overall decrease in soil microbial diversity (Rahman et al., 2021; Melki et al., 2017). Therefore, this study aims to understand the extent to which Diazinon pesticide contamination affects soil microbial community structure in agricultural land.

3.1 Bacterial and fungal culture results

In this study, microbial culture was conducted to isolate bacteria and fungi from pesticide contaminated soil samples. The culture process began with serial dilutions using concentrations of 10^{-1} , 10^{-2} , and 10^{-3} . This dilution aims to reduce microbial density so as to facilitate the observation of individual colonies. The media used in culture were *Nutrient Agar* (NA) to support bacterial growth, and *Potato Dextrose Agar* (PDA) to support fungal growth. *Nutrient Agar* was chosen due to its general nature and ability to support heterotrophic bacterial growth, while PDA was used due to its high carbon content suitable for fungal growth (Atmanto et al., 2022). After inoculating the soil samples on the media, the Petri dishes were incubated at 28-30°C for 24 hours for bacteria, while incubation for fungi continued until colonies were clearly visible. The results of the exploration of microorganism growth after the incubation period showed the number and type of colonies that developed, as presented in Table 1.

Table 1. Results of bacterial exploration

Exploration	Dilution	Colony Count	TPC Value	Growth
Bacteria	10^{-1}	421	4.21×10^3 CFU/ml	+
	10^{-2}	250	2.5×10^4 CFU/ml	+
	10^{-3}	10	-	-
Fungi	10^{-1}	135	1.35×10^3 CFU/ml	+
	10^{-2}	78	7.8×10^3 CFU/ml	+
	10^{-3}	15	-	-

Notes: (+) growing (-) not growing

Based on table 1, the results of exploration and calculation of *Total Plate Count* (TPC) on soil samples contaminated with pesticides show the presence of microbial populations that vary at different dilution levels. Based on the results of bacterial isolation, the number of colonies obtained was 421 at dilution 10^{-1} , 250 colonies at dilution 10^{-2} , and 10 colonies at dilution 10^{-3} . Meanwhile, the results of fungal isolation found 135 colonies in dilution 10^{-1} , 78 colonies in dilution 10^{-2} , and 15 colonies in dilution 10^{-3} . Referring to the valid TPC calculation standard, which is in the range of 30-300 colonies, an analysis was carried out on dilutions that met these criteria (Yunita et al., 2015).

In the bacterial population, dilution 10^{-2} produced 250 colonies that were within the valid counting range, resulting in a bacterial TPC value of 2.5×10^4 CFU/ml. As for the fungal population, the 10^{-1} dilution produced 135 colonies that met the counting range, with a fungal TPC value of 1.35×10^3 CFU/ml. This difference in TPC values indicates that the bacterial population in pesticide-contaminated soil samples is higher than the fungal population, with a difference of one order of magnitude. This shows the different adaptability between bacteria and fungi to soil conditions polluted with pesticides, where bacteria show a higher growth rate.

3.2 Microbial diversity

The diversity index reflects a combination of species richness and evenness within a community. Based on Table 2, the Shannon-Wiener diversity index calculated for pesticide-contaminated soil was 0.202, indicating a low level of microbial diversity. The microbial community was predominantly composed of bacteria, with a proportion *Pi* of 0.949, while fungi accounted for a much smaller proportion *Pi* of 0.051. This low diversity index suggests that pesticide-contaminated soil creates less favorable environmental conditions for microbial growth, particularly for fungi, which are generally more sensitive to toxic compounds.

The diversity index analysis (Figure 1) illustrates the microbial community structure in pesticide-contaminated soil through the *Pi* values. Bacteria dominate the microbial population with a proportion *Pi* of approximately 0.95, representing 95% of the total community. In contrast, fungi account for a much smaller proportion of *Pi* of about 0.05 or

5% of the community. This imbalance underscores the strong dominance of bacteria over fungi in pesticide-polluted environments, likely due to bacteria's higher adaptability and resistance to toxic compounds compared to fungi.

Table 2. Soil Microbial diversity index

No	Type	Microbial count	Pi (ni/N)	ln pi	Pi.ln Pi
1	Bacteria	25.000	0.948767	-0.05259	-0.04990
2	Fungi	1.350	0.051233	-2.97136	-0.15223
	Total	1.885			

The dominance of bacteria in soil microbial communities polluted by pesticides can be caused by the ability of certain bacteria to adapt to environments containing toxic compounds. Some groups of bacteria are known to have the ability to degrade pesticides through certain enzymes, such as the *Pseudomonas* and *Bacillus* groups, so that they can survive even in environmental conditions that are not favorable for other organisms (Sriningsih & Shovitri, 2015). In contrast, fungi have a lower tolerance to pesticide contamination, leading to lower numbers in polluted soils.

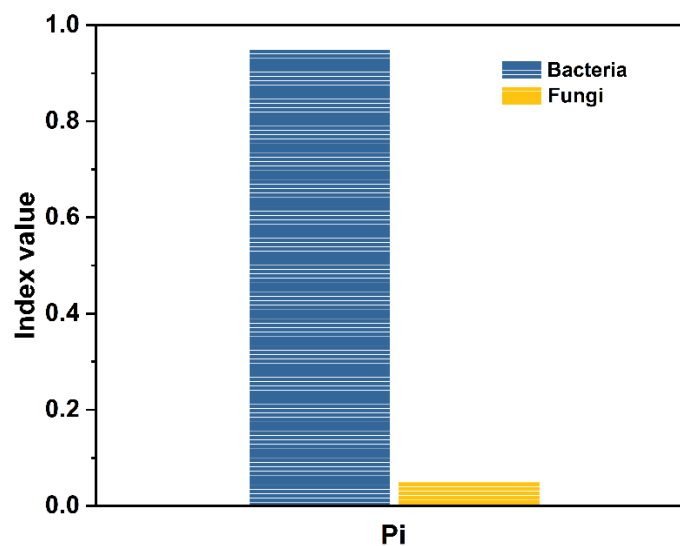


Fig. 1. Pesticide contaminated soil microbial community values

This low Shannon-Wiener index value also indicates that the distribution of species in the soil microbial community is uneven. This indicates that the presence of pesticides not only reduces the total number of microbes but also inhibits the development of certain species that are sensitive to these toxic compounds. This condition can have a negative impact on soil ecosystem function, considering that microbes have an important role in nutrient cycling, organic matter decomposition, and soil ecosystem balance.

According to Diniariwisan & Rahmadani (2023), the Shannon-Wiener Diversity Index is an important tool for measuring the complexity of microorganism communities because this index provides information that combines species richness (total number of types of microorganisms) and the uniformity of individual distribution between species in a community. The value of this index provides an in-depth picture of the structure of the microbial community, including how certain species dominate or how individuals are distributed among the species present. Physical Factors, Temperature, oxygen content, osmotic pressure, and pH are some of the physical factors that affect the growth of microorganisms. For example, increased temperature can accelerate the rate of enzymatic reactions in microorganism cells to a certain extent before causing damage. Chemical Factors, Toxic compounds or other chemical compounds that serve as food ingredients also affect the growth of microorganisms. The availability of nutrients such as metals, iron, sulphur, phosphorus, hydrogen, nitrogen, and oxygen are essential for microbial growth.

The Shannon-Wiener diversity index (H') has a range of values between 0 and $\ln(S)$, where S is the total number of species in the analyzed community. A minimal value ($H'=0$) indicates no diversity, i.e. the entire community consists of only one species with no other diversity. Conversely, a maximal value ($H'=\ln(S)$) indicates maximum diversity, where each species in the community has the same number of individuals, or the distribution is very even. The higher the H' value, the greater the diversity of species and the more evenly distributed individuals are among species. Conversely, the lower the H' value, the lower the species diversity and the more dominant one species is compared to the others. For example, in a community of 10 species ($S=10$), the maximum value of ' H' ' is about 2.30. Lower values of H' , such as 0.5 or 1.5, indicate dominance by a few species, with an uneven distribution of individuals among species. Therefore, higher ' H' ' values reflect better diversity and uniformity in a community (Roswell et al., 2021; Patel & Halder, 2022).

4. Conclusions

The results showed that pesticide pollution of soil in tomato farmland in Dunggala Village, Gorontalo Regency, significantly reduced soil microbial diversity. The microbial community detected consisted of bacteria at 2.5×10^4 CFU/ml and fungi at 1.35×10^3 CFU/ml. The low Shannon-Wiener diversity index value (0.202) reflects the low richness and uniformity of the microbial community due to pesticide exposure. This decrease in microbial diversity can have a negative impact on ecosystem functions, including organic decomposition processes and soil fertility. Therefore, it is necessary to implement more sustainable pest management, such as the use of biopesticides, to reduce the negative impact of pesticide pollution on soil and maintain ecosystem balance.

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Author Contribution

Conceptualization, A.P.L.; Methodology, P.L.A.; Validation, A.P.L. and P.L.A.; Investigation, A.P.L.; Resources, A.P.L. and P.L.A.; Data Curation, A.P.L.; Writing – Original Draft, P.L.A.; Writing – Review and Editing, A.P.L. and P.L.A.; Visualization, P.L.A.; Supervision, A.P.L.; Project Administration, A.P.L.

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The authors declare no conflict of interest.

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