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Isolation and potential test of indigenous bacteria from Singolangu, Magetan, Indonesia for bioremediation of organophosphate compounds

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[4]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia
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Abstract. *Pujiati, Prasetyani YW, Ardhi MW, Fatimah, Ramadhan R, Ni'matuzahroh N. 2025. Isolation and potential test of indigenous bacteria from Singolangu, Magetan, Indonesia for bioremediation of organophosphate compounds. Biodiversitas 26: 1029-1038.* The objective of this study was to isolate indigenous bacteria from soil contaminated with pesticide residues and assess their potential for bioremediation of organophosphate compounds, specifically from the profenofos and chlorpyrifos groups. The methodology employed was bacterial isolation, characterization, and ex-situ bioaugmentation testing with a design that included six treatments. The parameters observed included pH, N, P, K, profenofos, and chlorpyrifos pesticide residues. By the fourth week, the bioremediation results demonstrated the exceptional effectiveness of bacteria from the *Pseudomonas* genus, particularly isolate B3, which outperformed other isolates in terms of N, P, and K pH values, which were 90 ppm, 94 ppm, 175 ppm, and 6, respectively. The lowest N, P, K was isolate B1, with consecutive values of 47, 63, 129, and a pH value of 6. The control soil on pesticide residue levels in the chlorpyrifos test was 150 ppm, and profenofos was 29 ppm. In the results of soil testing with Pseudomonas bacteria (isolate B3), chlorpyrifos levels were reduced by 41 ppm to 109 ppm, and profenofos levels were reduced by 21.8 ppm to 7.2 ppm. Isolate B1 was able to reduce profenofos levels by 18 ppm to 132 ppm and decrease profenofos by 9.2 ppm to 18.8 ppm. The test results showed that the type of indigenous bacteria and the length of incubation time have an impact on soil quality and pesticide residue levels.

Keywords: Agricultural soil, bioremediation, chlorpyrifos, indigenous bacteria, profenofos

INTRODUCTION

Agriculture is a significant sector in Indonesia for creating employment opportunities. According to the Indonesian Central Statistics Agency, the agricultural sector in Indonesia employed an average of 38.22 million workers in August 2020 (Ngadi et al. 2023). Despite its importance, many agricultural sectors in Indonesia face challenges in processing agricultural land systems, including soil fertility issues. For instance, in Singolangu Hamlet, Magetan, East Java, Indonesia, where vegetable farming is the primary source of income, farmers commonly use pesticides to protect their crops from pests. Pesticides are chemicals that are applied to agricultural land to control pests, which can reduce crop yields. While pesticides can increase production and are easy to apply, their use can also have negative environmental impacts. Organochlorine insecticides, which were widely used in the past, have left traces in the environmental matrix (Lupi et al. 2019; Gandhi et al. 2021; Ore et al. 2023; Lavandier et al. 2023).

The use of conventional agricultural systems with pesticides can lead to issues such as reduced soil fertility, environmental degradation, and harm to the surrounding ecosystem (Schaeffer and Wijntjes 2022; Mishra et al. 2023). The prolonged application of high concentrations of organophosphate insecticides in soil and water can heighten the negative environmental effects associated with their toxicity. The excessive use of organophosphates can also contribute to the decline of pollinators, including bees, due to unintentional exposure to pollen from treated plants (Chaimanee et al. 2016). The use of these pesticides can have serious consequences because they persist in the environment, can travel long distances, accumulate in the environment in the form of high pesticide residues, and are toxic to non-target organisms and microorganisms (Riyaz et al. 2021; Roy et al. 2022). Pesticides can naturally degrade in soil through processes such as photodegradation, microbial degradation, and bioremediation, although various factors can influence the speed and efficacy of these processes. Microbial degradation is an essentially natural process in which soil microorganisms, including bacteria, fungi, and actinomycetes, metabolize pesticides as a source of carbon, nitrogen, and energy, ultimately resulting in the transformation of these harmful substances into less toxic materials (Getenga et al. 2023; Kaur et al. 2023). Bioremediation, which involves the use of modified microorganisms or organic additives to improve the natural breakdown processes, has demonstrated the potential to decrease pesticide residues in soil. Nevertheless, the presence of numerous agrochemicals, including fungicides, can complicate the process of breaking down these chemicals by influencing the activities of microorganisms and enzymes. This situation highlights the need for improved regulatory risk assessments that more accurately reflect actual agricultural conditions (Schaeffer and Wijntjes 2022; Getenga et al. 2023).

Microorganisms in soil naturally metabolize carbon sources available in their environment. Pesticides are a source of carbon that microbes can use for metabolism. As is well-known, pesticides contain hydrocarbon compounds (H-C), which are macronutrient elements necessary for microbial growth, particularly bacterial growth. Through this process, it can be inferred that microorganisms capable of utilizing contaminant compounds in the environment as substrates for metabolism are potential microbes as remediation agents. Bioremediation is the process of breaking down pollutants in the form of organic/inorganic materials, such as PAHs, hydrocarbons, POPs, pesticides, heavy metals, and so on, using microorganisms, including bacteria, fungi, plants, or enzymes, to control environmental pollution. Microorganisms are bioremediatory agents whose role is to modify toxic pollutants such as pesticides by changing their chemical structure, or what can be referred to as a biotransformation process. This process can be carried out by utilizing potential pesticide biodegradation microorganisms. Sources of pesticide biodegradation microorganisms can be obtained from soil habitats contaminated with pesticide residues. These pesticide biodegradation microorganisms possess great potential to be developed into a formula that can improve soil quality, especially soil that contains pesticide contamination. Based on this background description, researchers aim to develop an environmentally friendly formulation of pesticide-degrading bacteria. This research was carried out by testing the pesticide-degrading bacterial formula isolated from vegetable farming soil in Singolangu Hamlet, Magetan, which has high levels of pesticide residue.

MATERIALS AND METHODS

Study area

This study was undertaken in the partner village of Singolangu Hamlet, Sarangan Village, Plaosan, Magetan District, East Java, Indonesia. The primary agricultural activity in this region is vegetable farming, which is characterized by short crop cycles and frequent use of high levels of pesticides. The region is renowned for its tourist destination, Telega Sarangan, and vegetables are one of the popular souvenirs. Despite this, the use of pesticides and chemical fertilizers cannot be avoided, as the condition of the agricultural land is unhealthy and hardened, leading to decreased productivity. This is the rationale behind the selection of this sampling location. The study area is depicted in Figures 1 and 2.

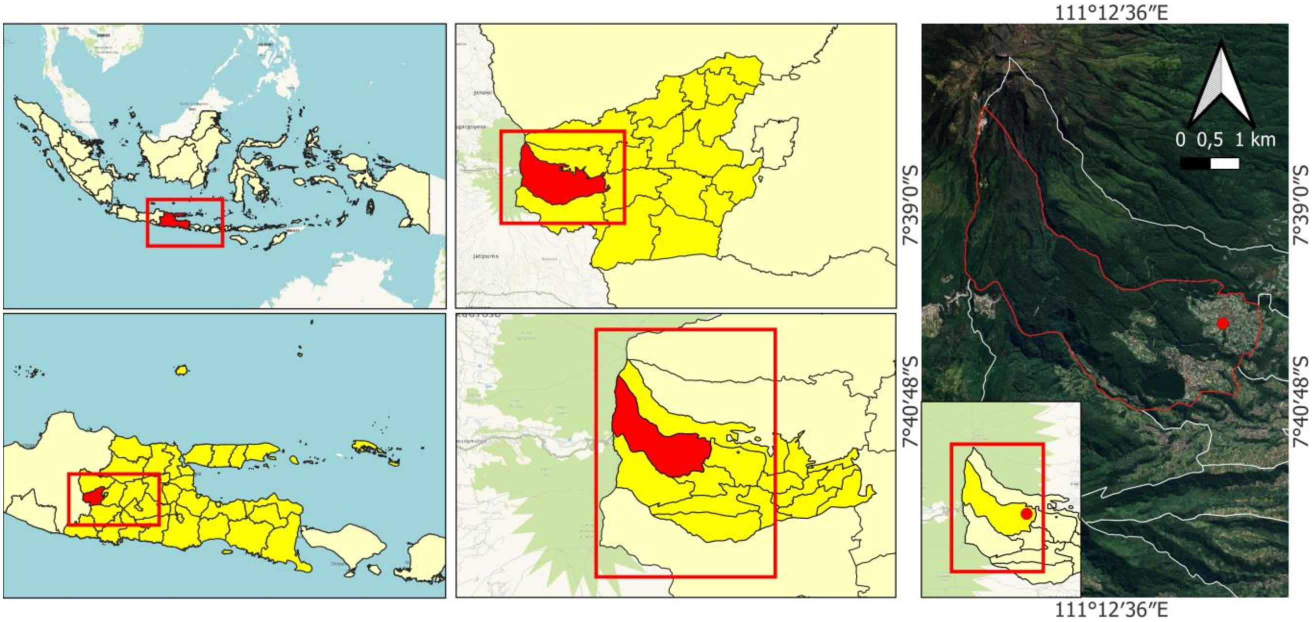

Figure 1. The location of Singolangu Hamlet, Sarangan, Plaosan, Magetan 1,338 masl, East Java, Indonesia

Figure 2. Sampling site and soil condition

Procedures

Soil sampling

Soil sampling was conducted using a simple random sampling method at a vegetable farm in Singolangu Village, Magetan, that had been exposed to pesticides. The soil samples were then transported to the laboratory at Universitas PGRI Madiun. The samples were collected at a depth of 5-30 cm below the surface and stored in a container filled with dry ice. The soil was cleaned of debris and sieved to ensure homogeneity.

Soil sample pretreatment

The soil samples were subjected to pretreatment by exposure to organophosphate pesticides, specifically chlorpyrifos with the brand name Fostin 610 EC and profenofos with the brand name Curacron 500 EC. The soil was left to soak for 30 days, after which it was dried, crushed, and sieved. Soil samples contaminated with pesticide residue were divided into 24 samples, each weighing 50 g, with 4 designated as control samples and 20 designated as treatment samples. Each treatment sample was given a bacterial formula containing isolates B1, B2, B3, B4, and BK.

Sterilization and media preparation

The mixture of NA and distilled water was heated using an electric stove and stirred until homogeneous. The NA solution was transferred to an Erlenmeyer tube, which was covered with sterile cotton and coated with aluminum foil. The media and all laboratory equipment were sterilized using an autoclave at a temperature of 121°C and a pressure of 15 lbs for 15 minutes (Pujiati 2022).

Microbial isolation technique

The agar pour method, involving a series of graded dilutions, was employed to isolate microbes. Soil samples weighing 1 g were suspended in 9 mL of sterile distilled water (10 g/90 mL) for dilution. This process was repeated until the 10^{-9} dilution. Dilutions 10^{-5}, 10^{-6}, and 10^{-7} were selected for bacterial isolation. Next, to commence the bacterial isolation process, 1 mL of sample from dilutions 10^{-5}, 10^{-6}, and 10^{-7} was poured into a petri dish, followed by the addition of 1 mL of griseofulvin solution and an adequate amount of NA media. The mixture was thoroughly combined by gently rotating the petri dish in a clockwise direction eight times on the table. All petri dishes were subsequently covered with clingwrap and stored at room temperature for 2-4 days. Following incubation, the grown microbes were transferred to another NA in test tubes (Pujiati 2022).

Culture stock preparation

The indigenous bacteria obtained from soil samples grown on NA media were revitalized on slanted media for the purpose of creating stock cultures and observation. Each microbial isolate is multiplied fivefold. The process of making culture stock involves pouring out the revitalized bacteria into 600 mL of physiological water and adding 6 g of NaCl (10 g NaCl/L) and a small amount of pesticide. The culture was incubated at room temperature on a shaker set at a speed of 115 rpm for 5 days. If the physiological water becomes turbid, it indicates a higher microbial population (Matos et al. 2021).

Bioremediation process

Six groups of 50-g soil samples contaminated with pesticide residue were treated. The soil samples were cleaned of dirt and sieved to create homogeneous soil. The soil was then weighed and homogenized with a formula specific to each treatment and subsequently placed into sterilized glass bottles. The bottle lids were sealed with cotton and aluminum to prevent contamination (Bahri et al. 2019). The bioremediation formula levels for each treatment are presented in Appendix 2. Observations were conducted every 7 days for 28 days, on days 7, 14, 21, and 28.

Macroscopic and microscopic characterization of indigenous bacteria

Macroscopic examination involved direct observation of microorganisms on agar media in petri dishes. Bacterial characterization, which includes morphological characteristics, such as colony shape, size, color, edge, and elevation of the bacteria, was observed. Microscopic examination of bacterial isolates was performed using the bacterial staining method. The process involved sterilizing a glass object with a Bunsen flame for 3-4 times. Bacterial isolates were aseptically taken using a loop needle and applied to a glass slide. The bacterial isolate was dripped with crystal violet and left for 1 minute, followed by washing with flowing distilled water and drying. Next, the bacterial isolate was dripped with iodine solution and left for 1 minute, followed by washing with running distilled water and drying. The bacterial isolate was then dripped with 95% alcohol for 30 seconds, followed by filling with distilled water and drying. The bacterial isolate was dripped with safranin for 30 seconds and washed with flowing distilled water. Finally, the bacterial isolate was dried with tissue and air dried and observed using a microscope with a magnification of 1000 times.

NPK measurement

NPK levels are determined utilizing a digital ZD soil tester. The process commences with the preparation of a soil sample mixed with water in a 1: 1 ratio to create a testable solution. This solution is subsequently exposed to LED light emitted at specific wavelengths corresponding to nitrogen, phosphorus, and potassium absorption bands. A photodiode is employed to measure the intensity of light reflected from the soil sample, which is then converted into an electrical signal using an optical transducer system. The electrical signal is processed by an Arduino microcontroller, and the NPK levels are displayed digitally, categorized as high, medium, or low**.** Proper calibration is essential to ensure accurate results in various ambient light conditions (Masrie et al. 2018; Fan et al. 2022). The real-time results are then interpreted to assess soil fertility and inform decision-making regarding fertilization strategies.

Data analysis

The characterizing of bacterial isolates involved both macroscopic and microscopic examinations. The macroscopic examination focused on the morphological characteristics observed, including colony shape, size, color, edges, and the elevation of the bacterial colonies. Microscopic observations are conducted to identify the nature of bacterial Grams and the shape of bacterial cells (Salwan et al. 2023). Evaluation of physio-chemical measurements, specifically those pertaining to N, P, and K, entails monitoring alterations in these parameters based on the figures displayed on the tool on a weekly basis. The analysis of pesticide residue levels employs the LC-MS technique executed by PT Angler Biochemlab Surabaya. Observations of pesticide residue levels in the soil focused on organophosphates, specifically chlorpyrifos and profenofos.

RESULTS AND DISCUSSION

Indigenous bacteria in pesticide-contaminated soil

Data on indigenous bacteria from the soil of Singolangu vegetable farm were collected through bacterial isolation. This process was carried out for three days, followed by observations to gather the necessary data. The results of the bacterial isolation were recorded in the form of macroscopic and microscopic images of the bacteria. The data collected is demonstrated in Figure 3 and Table 1.

Bioremediation test results: N, P, K, and pH levels of remediated soil

Soil quality parameters, including nitrogen (N), phosphorus (P), potassium (K), pH, and moisture levels, were evaluated weekly. The Handled Digital NPK soil tester ZD-1803K Quick-acting Rapid Test was employed to determine N, P, and K values, yielding quantitative results. The specific data obtained through this method are as follows in Table 2 and Figure 4.

Discussion

Indigenous bacteria

The present study successfully isolated indigenous bacteria from soil polluted with pesticides, identifying *Micrococcus* (3 isolates), *Bacillus* (2 isolates), and *Pseudomonas* (1 isolate), which dominated the isolation process. A key methodological innovation of the study was the pretreatment of the soil by adding commercial pesticides. This pretreatment was shown to enhance the adaptability of indigenous bacteria to pesticide pollutants and to increase the potential of the indigenous bacteria themselves as they became accustomed to the presence of pesticides in the soil. Bacteria that can survive in these conditions are potential bacteria, as they can utilize pesticide pollutants as a source of nutrients for growth. Previous studies have indicated that certain bacterial genera, including *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Staphylococcus*, possess the potential to degrade organophosphate pollutants in the environment (Ergüven and Demir 2019; Le et al. 2021)

Table 1. Characterization of indigenous bacteria from Puntukdoro Village, Magetan, Indonesia

Codes	Genus	Colony form	Size	Color	Edge	Elevation	Gram	Cell shape
B1	*Micrococcus*	Irregular	Medium	Yellowish	Entire	Flat	+	Coccus
B2	*Bacillus*	Round	Small	Oren's yellow	Smooth	Raised	+	Basil
B3	*Pseudomonas*	Round	Medium	Yellow	Undulate	Raised	-	Basil
B4	*Micrococcus*	Irregular	Medium	White	Lobate	Flat	-	Coccus
B5	*Micrococcus*	Round	Medium	Cream white	Undulate	Raised	+	Coccus
B6	*Bacillus*	Round	Small	White clear	Smooth	Flat	+	Basil

Table 2. N, P, K, pH levels during bioremediation

Treatments	Incubation time															
	W_1				W_2				W_3				W_4			
	N	P	K	pH	N	P	K	pH	N	P	K	pH	N	P	K	pH
B0	4	5	7	5	4	3	9	5	4	4	11	5	3	2	7	5
B1	20	21	67	5	33	29	77	5	42	46	83	6	47	63	129	6
B2	38	23	117	5	43	39	149	6	56	51	162	6	63	59	180	6
B3	**44**	**55**	68	5	**62**	**60**	86	6	**72**	**83**	120	6	**90**	**94**	175	6
B4	28	33	70	6	44	45	84	5	64	64	134	6	70	70	156	6
BK	33	45	103	6	53	64	132	5	57	71	144	6	72	82	157	6

Notes: B0: Control; B1: Bacterial formula 1; B2: Bacterial formula 2; B3: Bacterial formula 3; B4: Bacterial formula 4; BK: Bacterial consortium formula; W1: Week 1; W2: Week 2; W3: Week 3; W4: Week 4

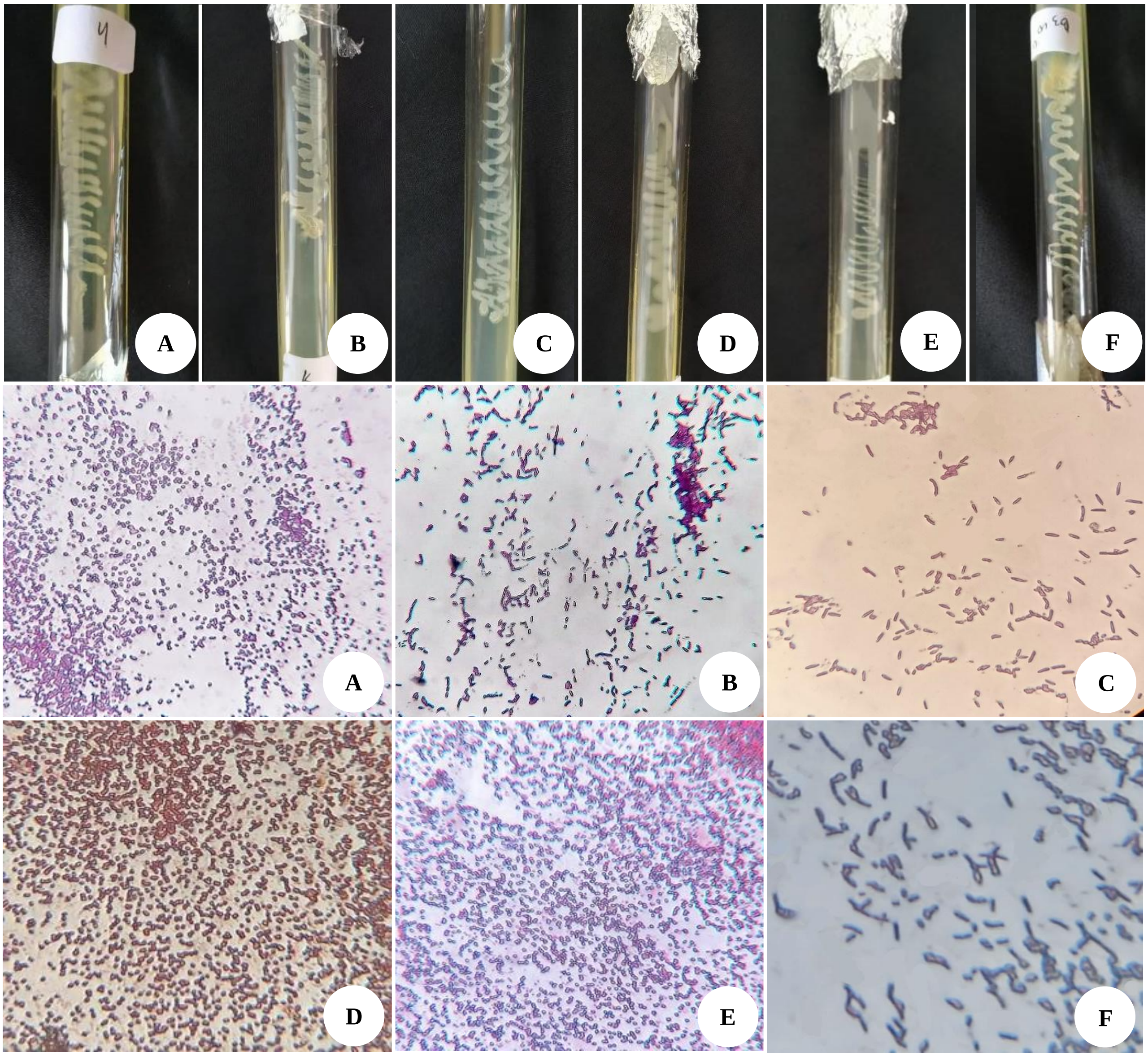

Figure 3. Indigenous bacteria from Singolangu hamlet, Sarangan Village, Magetan, Indonesia. A. B1: Potential bacteria 1; B. B2: Potential bacteria 2; C. B3: Potential bacteria 3; D. B4: Potential bacteria; E. B5: Potential bacteria 5; F. BL6: Potential bacteria 6

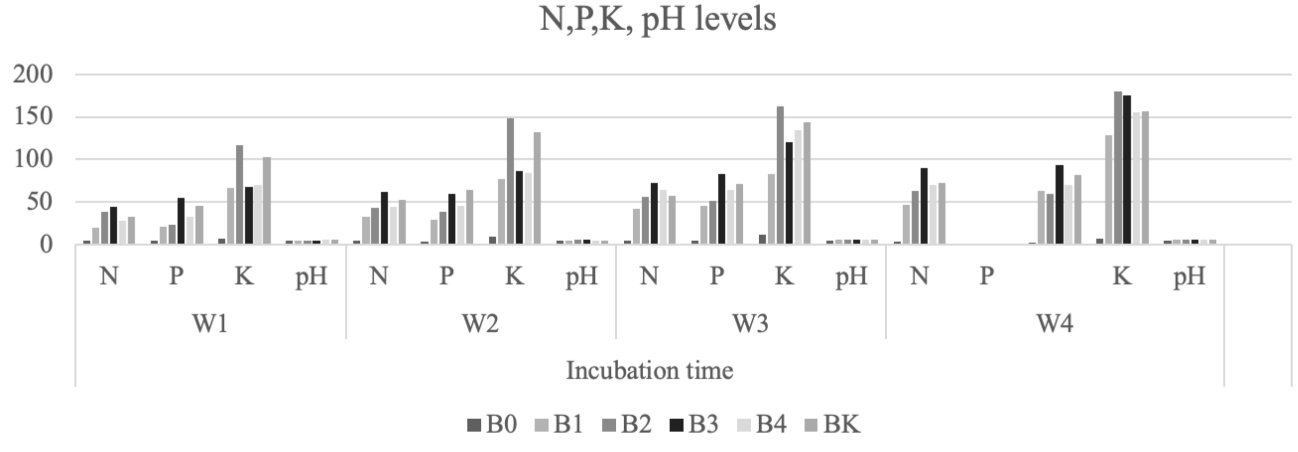

Figure 4. NPK (ppm) and pH levels during bioremediation. B0: Control; B1: Bacterial formula 1; B2: Bacterial formula 2; B3: Bacterial formula 3; B4: Bacterial formula 4; BK: Bacterial consortium formula; W1: Week 1; W2: Week 2; W3: Week 3; W4: Week 4

N, P, K, pH levels

Pesticide exposure in agricultural soils can alter soil quality, depleting nutrients and disrupting microorganisms' activity. These organisms are vital for the breakdown of compounds, the fixation of N, and the dissolution of P and K (Pujiati et al. 2025a,b). Considering this, it was necessary to evaluate the soil's levels of N, P, and K to determine the impact of pesticides on the recovery of these nutrients. Nitrogen (N) levels were evaluated every seven days in this study, namely on days 7, 14, 21, and 28, as illustrated in Table 2 and Figure 4.

The testing of N levels was based on its presence in the soil, which plays a crucial role in plant metabolism, including the formation of nucleic acids, chlorophyll, proteins, and chlorophyll (Cruz et al. 2021). According to the research data, the N levels in the control soil during the first to fourth weeks were 3 ppm to 4 ppm. In contrast, the treated soil showed an increase in N value compared to the control every week, as the control soil did not receive any formula.

The highest N value in the treated soil was observed when the B3 formula was applied, which was evident in the fourth week with a value of 90 ppm. Additionally, the N values increased from the first to the fourth week in the treatments given formulas B1, B2, B4, and BK, with values of 47 ppm, 63 ppm, 70 ppm, and 72 ppm, respectively. This increase in N levels can occur due to the addition of microorganisms in the form of indigenous bacteria (Salama 2015). This demonstrates that the formula used has an impact on the N levels in the treated soil. The elevated levels of nitrogen (N) in soil treated with pesticides are attributed to the activity of microorganisms that possess the capability to fix nitrogen through the utilization of carbon compounds in pesticides (Pujiati et al.2024). Nitrogen is a crucial element for plant growth and development, as it serves as the primary nutrient for plant growth. Free nitrogen is abundant in the air, accounting for approximately 78% of the atmosphere. However, this nitrogen is unavailable to plants in its current form, as it exists as N_2 gas or is inert. Bacteria use this free nitrogen for protein synthesis in their cells, which is then mineralized to make nitrogen available in the soil.

Nitrogen fixation, a crucial process in the ecosystem, can be accomplished by autotrophic bacteria, which utilize light energy to carry out this process. In contrast, bacteria with heterotrophic properties can fix nitrogen anaerobically (Nag et al. 2022). These bacteria, through the nitrification process, transform nitrogen (N_2) into ammonium (NH_3) and nitrate (Rütting et al. 2021). This transformation is made possible by the enzyme nitrogenase, composed of two enzymes: dinitrogenase and dinitrogenase reductase (Sharma et al. 2023b). The weekly rise in the N value in each treatment underscores the significant impact of microorganisms, particularly bacteria, on the N value, highlighting their crucial role in soil nutrient levels of microorganisms, particularly bacteria, has an impact on the N value. Bacteria that can bind nitrogen typically belong to the forms of bacilli and cocci, Gram-negative and Gram-positive bacteria (Matos et al. 2021). *Bacillus*, a genus of bacteria, is a prime example of a nitrogen-fixing bacteria that can significantly increase N levels in the soil. Nitrogen-fixing strains that can also solubilize phosphate can be found in bacteria such as *Pseudomonas monteilii*, *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Pseudomonas mandelii*, *Bacillus altitudinis*, *Klebsiella*, and *Aerobacter* (Cozzolino et al. 2024). These findings indicate that the isolate used as a bioremediation agent possesses the ability to fix nitrogen.

Phosphate testing is essential, as it determines the presence of phosphates in the soil. It plays a crucial role in plant metabolism, such as the formation of ATP, a component of phospholipids, energy storage, and plant height. According to the research data, the P-value levels in the control soil in the first to fourth week were 5 ppm and 2 ppm. However, the treated soil showed an increase in P value levels compared to the control every week, as the control soil was not given a formula (Thi et al. 2022; Tomczyk et al. 2023; Sharma et al. 2023a; Steiner et al. 2024). Therefore, testing N, P, K levels in soil is conducted to assess the recovery of pesticide-contaminated soil through indigenous bacteria.

The most effective result in the treated soil was demonstrated by applying formula B3, which comprises *Pseudomonas* bacteria. This is evidenced by the value of 94 ppm in the fourth week. The P levels in the treatment reveal that B3 has the highest value as a result of variations in the enzymes and organic compounds produced by bacteria, which have different capabilities in dissolving P. Additionally, the increase in the P values from the first to the fourth week was observed in the treatments administered with formulas B1, B2, B4, and BK, amounting to 63 ppm, 59 ppm, 70 ppm, and 82 ppm, respectively. According to UPN research conducted in 2011, the classification of phosphorus (P) content is as follows: > 35 ppm (very high), 15-25 ppm (high), 10-15 ppm (medium), and <10 ppm (low). The rise in P levels indicates that the formula employed has an impact on the P levels in the treated soil. The increase in phosphorus (P) occurs due to the activity of microorganisms, particularly bacteria, which are capable of decomposing and dissolving P in the soil.

The degradation of pesticides is a significant process facilitated by the presence of functional enzymes within the bacterial cell structure. The phosphatase enzyme, originating from bacteria, is responsible for hydrolyzing the chemical composition of pesticides, which consist of labile chains, such as chlorpyrifos. However, it is the oxidation process, a crucial part of pesticide degradation, that is carried out by bacteria on pesticide residues, first extracellularly and then intracellularly. This process leads to an increase in the level of dissolved residues and their conversion into organic elements, underscoring the importance of your research in this field.

The phosphatase enzyme operates as a catalyst for the hydrolysis of phosphate-organic compound complexes, facilitating the production of readily available phosphate. The reaction involves the hydrolysis of phosphate sulfate, which is solubilized by phosphate-solubilizing bacteria, with hydrogen sulfide being produced. The binding of Fe ions with phosphate by siderophores from bacteria can lead to the dissolution of phosphate in the soil (Garcia-Sanchez et al. 2023)

Bacteria in the soil depend on available phosphate for their growth and metabolism. The chemical dissolution of phosphate can occur through the production of organic acids by bacteria. These organic acids include succinic, citric, propionate, oxalic, malic, fumaric, acetic, and glutamic acids. The organic acids that are most effective in dissolving phosphate in soil, in descending order, are citrate, oxalate, tartrate, malate, and HCl. The ability of bacteria in the soil to produce different types of organic acids can affect the ease of dissolving phosphate. For instance, if citric acid is produced, the effort to dissolve phosphate is greater. The formation of complex compounds between the various types of organic acids produced by bacteria and Al, Ca, Mg, or Fe ions leads to the release of phosphate (Kuhad et al. 2011).

Based on the outcomes of the P value assessment conducted on a weekly basis, it can be inferred that the employment of this specific bacterial formula is effective in enhancing soil phosphorus levels. Among the various options, the highest phosphorus levels were recorded in response to formula B3, which comprises bacteria from the genus *Pseudomonas*. It is noteworthy that several types of bacteria possess the capability to increase phosphorus levels by facilitating the dissolution of phosphate, including *Pseudomonas striata, P. fluorescens, P.monteilii, P. mandelii, P. putida, Bacillus* sp., *B. altitudinis*, *Achromobactin*, *Bacillus subtilis*, *Micrococcus*, *Flavobacterium*, and *Alcaligenes* (Meng 2019; Ergüven and Demir 2019; Ambreen et al. 2020; Zhang et al. 2021; Wang et al. 2022)

Soil potassium testing is conducted to evaluate its role in plant growth, such as protein synthesis, the translocation of photosynthesis results, and improving the physical and chemical properties of the soil. Research data revealed that the potassium (K) levels in the control soil remained constant at 7 ppm, respectively. However, the K value in the treated soil increased each week, as the control soil did not receive a formula.

The best K value in the treated soil was observed when formula B2 was applied, as demonstrated in the fourth week with a value of 180 ppm. The ability of bacteria to dissolve K varies depending on factors such as the diffusion of organic acids, the attachment time between organic acids and mineral surfaces, the type of organic acid, and the levels of organic acids in the soil (Yadav 2021). Additionally, the K-values increased from the first to the fourth week in the treatments given formulas B1, B3, B4, and BK, respectively, with values of 129 ppm, 175 ppm, 156 ppm, and 157 ppm. Soil potassium levels < 10 ppm are categorized as very low, 10-20 ppm as low, 21-40 ppm as medium, 41-60 ppm as high, and >60 ppm as very high. The weekly increase in potassium levels is influenced by several factors, including the addition of microorganisms to the soil (Dotaniya et al. 2016; Sindhu et al. 2016). This increase in potassium levels indicates that the formula used has an impact on the K levels in the treated soil.

The source of potassium (K) in the soil, primarily from organic matter and the activities of microorganisms, underscores the integral role of these tiny organisms in soil health. Research has shown that the addition of bacteria can enhance the availability of K elements in the soil. Specifically, potassium (K) solubilizing bacteria, which belong to the *Bacillus mucilaginous* type, play a crucial role in aiding the weathering of K elements that are still bound by minerals in the soil. *Bacillus* produces exopolysaccharide, which contributes to the solubilization of potassium elements in soil (Mohite et al. 2017). *Bacillus*, through the production of exopolysaccharide, contributes to the solubilization of potassium elements in soil, highlighting the value of microorganisms in the field of soil science.

The process of potassium dissolution is facilitated by the production of organic acids by bacterial species, such as oxalic, citric, malic, tartaric, acetic, gluconic, and formic acids. These acids break down the bonds between potassium and silicate compounds in the soil. Hydrogen ions in the soil participate in the release of potassium from minerals. When bacteria dissolve potassium, acidolysis and chelation of cations bound to K occur. The pH level decreases, resulting in the release of protons and organic acids by the bacteria. The release of organic acids causes the protonation and acidification of element K, leading to its dissolution. The dissolution or release of potassium elements can be influenced by several factors, such as soil pH, oxygen, sorption processes, ion and water levels, and the bacterial culture provided (Kumar 2022). The application of these microbes is highly beneficial in releasing K elements from the soil. Isolates capable of increasing K levels can be employed as biofertilizers to enhance soil fertility. Potassium-dissolving microbes come from bacterial species, such as *Burkholderia*, *Pseudomonas*, *Azotobacter*, *Bacillus*, *B. mucilaginosus*, *Bacillus licheniformis*, *Rhizobium*, *Serratia marcescens*, and *Paenibacillus* (Jain et al. 2022)

The presence of potassium (K) in the soil is often greater than the elements N and P, as demonstrated in a previous study (Wakeel and Ishfaq 2022). The results indicate that the K value in treated soil surpasses those of N and P. Moreover, various studies have posited that Bacillus exhibits a superior capacity for absorbing potassium when compared to other minerals. This superiority is evident in the B2 formula treatment, which yields a higher K value (Mohite et al. 2017)

According to Table 2 and Figure 4, it can be observed that the control soil maintains a constant pH level of 5, while the treatment soil experiences a change in pH level from 5 to 6. This alteration in pH level can be attributed to the activity of microorganisms and treatment organisms that contribute to restoring the soil's condition. Soil contaminated with pesticide residues becomes more optimal as a result. The increase in pH value can be attributed to the presence of compounds released by bacteria in the soil through the decomposition of organic matter via the hydrogen pump. The breakdown of nitrogen compounds into ammonia resulted in an increase in pH due to the alkaline nature of ammonia (Tian and Niu 2015). Nitrification in nitrogen fixation by bacteria is another factor that influences the increase in pH. In the nitrification process, H+ ions are released from the soil, leading to the replacement of ion content with base cations (Cation Exchange Capacity/CEC). The alkaline cations in the soil are responsible for increasing the pH to alkaline or neutral levels. Additionally, an increase in soil pH values also occurs due to the release of phosphate in the soil (Lv et al. 2015).

Chlorpyrifos and profenofos degradation

Based on the physio-chemical data obtained during observations within four weeks, it was determined that formula B3 had a higher value. Consequently, it was deemed that soil with the B3 treatment had the best treatment, so a pesticide residue level test was conducted. Soil treated with the bacterial consortium was not selected for testing of pesticide residue levels due to having N, P, and K values that were lower than the B3 formula. According to the authors, this inhibition is due to the presence of certain compounds, such as streptomycin, amphotericin, nystatin, and natamycin, which are released by bacteria to inhibit the growth of other bacteria. Then, to determine if there is a decrease in pesticide residue levels in the soil, tests were also conducted on the control soil. The LC-MS test results on B3 treatment soil, which contained bacteria from the *Pseudomonas* genus, revealed chlorpyrifos levels at 109 mg/kg or 109 ppm and profenofos levels at 7.2 ppm. In contrast, the control soil showed chlorpyrifos residue at 150 mg/kg or 150 ppm and profenofos at 29 mg/kg or 29 ppm (Figures 5 and 6).

The reduction in pesticide residues is attributed to the activity of bacteria that secrete organic acids and enzymes, which facilitate pesticide degradation through compound mineralization. These bacteria, demonstrating remarkable resilience, have adapted to the presence of pesticides and use the residue as a metabolic substrate. Bacteria from the *Pseudomonas* genus possess the capability to degrade organ chemical compounds, including organophosphates (Aswathi et al. 2019; Subsanguan et al. 2020) are a prime example of this adaptation.

Bacterial species from the genera *Pseudomonas*, *Alcaligenes*, *Rhodococcus*, *Bacillus*, *Micrococcus*, and *Flavobacterium* release functional enzymes, including phosphatase and esterase, to hydrolyze and break labile chemical bonds in pesticides. Increasing the microbial population is closely related to changes in element levels in the constituent compounds of pesticides. The compounds found in pesticides serve as a source of nutrients, minerals, carbon, and electron recipients in respiration reactions during metabolism for microorganisms.

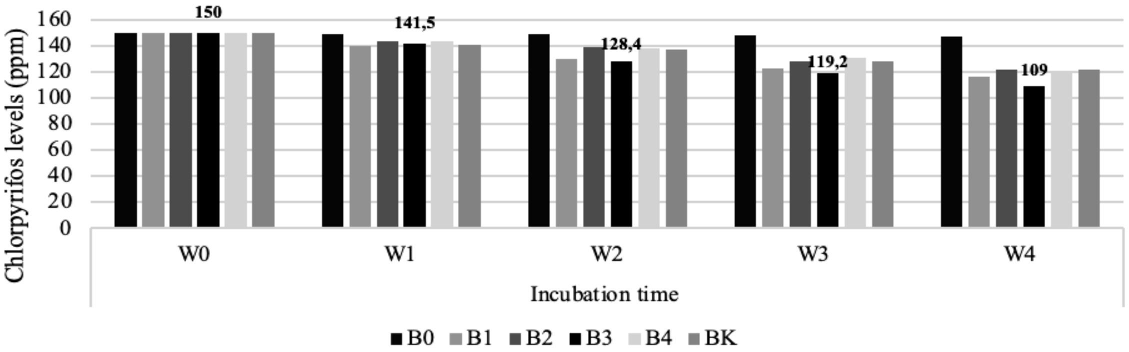

Figure 5. Chlorpyrifos levels during bioremediation. B0: Control; B1: Bacterial formula 1; B2: Bacterial formula 2; B3: Bacterial formula 3; B4: Bacterial formula 4; BK: Bacterial consortium formula; W1: Week 1; W2: Week 2; W3: Week 3; W4: Week 4

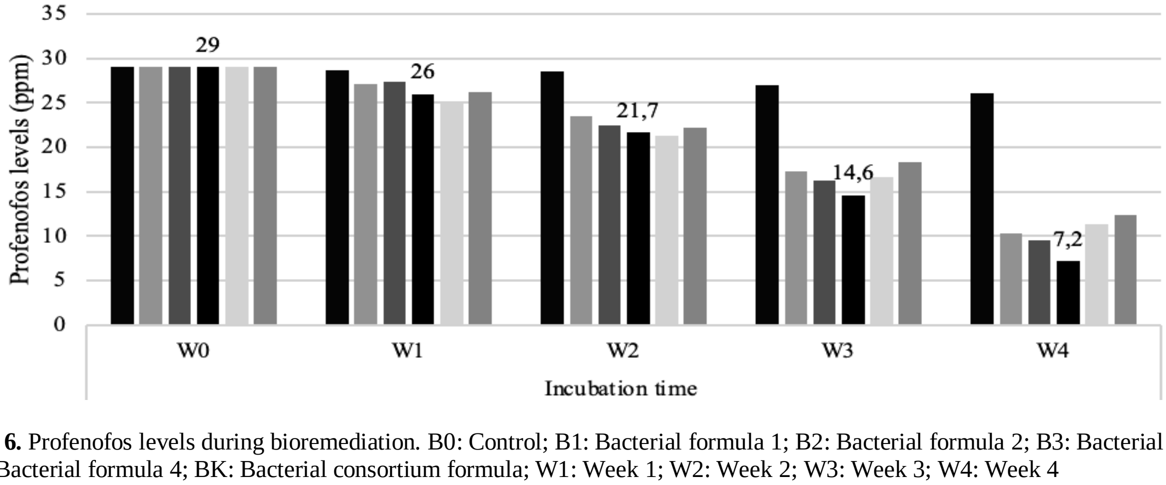

Figure 6. Profenofos levels during bioremediation. B0: Control; B1: Bacterial formula 1; B2: Bacterial formula 2; B3: Bacterial formula 3; B4: Bacterial formula 4; BK: Bacterial consortium formula; W1: Week 1; W2: Week 2; W3: Week 3; W4: Week 4

The concentration of chlorpyrifos in the soil decreases due to degradation and adsorption by bacteria. Various bacterial species, such as those in the genera *Bacillus*, *Micrococcus*, and *Pseudomonas*, can carry out bioremediation in soil contaminated with chlorpyrifos by utilizing and breaking down pesticide compounds as a source of nutrition and energy for growth and reproduction. The factors that influence the degradation of pesticides, such as oxidation, hydrolysis, reduction, and isomerase reactions, include several variables like organic and inorganic compounds, pH, temperature, ionic strength, and degradation time. The duration of bioremediation significantly affects the levels of pesticide degradation. The longer the bioremediation time, the greater the reduction in pesticide residue levels.

In conclusion, three genera of indigenous bacterial isolates were identified in pesticide-contaminated soil, namely *Micrococus*, *Bacillus*, and *Pseudomonas*. There is a relationship between the provision of indigenous bacteria and bioremediation time and the physio-chemical levels of the soil, specifically in terms of N, P, and K values. The results indicated that soil quality improved from the first week to the fourth week. The use of formula B3, which contains bacteria from the genus *Pseudomonas*, resulted in the best N and P values. In comparison, formula B2, which contains bacteria from the genus *Bacillus*, showed the best K values. There is also a relationship between the provision of indigenous microorganisms and bioremediation time and pesticide residue levels. By the fourth week, the administration formula B3 led to a significant decrease in pesticide residue levels, specifically a decrease in profenofos levels by 41 mg/kg or 41 ppm and a decrease in chlorpyrifos levels by 21.8 mg/kg or 21.8 ppm compared to the control soil.

REFERENCES

Ambreen S, Yasmin A, Aziz S. 2020. Isolation and characterization of organophosphorus phosphatases from *Bacillus thuringiensis* MB497 capable of degrading chlorpyrifos, triazophos and dimethoate. Heliyon 6 (7): e04221. DOI: 10.1016/j.heliyon.2020.e04221.

Aswathi A, Pandey A, Sukumaran RK. 2019. Rapid degradation of the organophosphate pesticide-Chlorpyrifos by a novel strain of *Pseudomonas nitroreducens* AR-3. Bioresour Technol 292: 122025. DOI: 10.1016/j.biortech.2019.122025.

Bahri S, Rokhim S, Prasiska YS. 2019. Kontaminasi bakteri *Escherichia coli* pada sampel daging. J Health Sci Preven 3: 62-67. DOI: 10.29080/jhsp.v3i1.195. [Indonesian]

Chaimanee V, Evans JD, Chen Y, Jackson C, Pettis JS. 2016. Sperm viability and gene expression in honey bee queens (*Apis mellifera*) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate acaricide coumaphos. J Insect Physiol 89: 1-8. DOI: 10.1016/j.jinsphys.2016.03.004.

Cozzolino ME, Córdoba ME, Ramos PD, Ferrari SG, Silva PG. 2024. Phosphate solubilization by *Bacillus* isolates and its influence in a cyanobacterial co-culture. Malays J Microbiol 20 (3): 289-295. DOI: 10.21161/mjm.220150.

Cruz C, Vishwakarma K, Choudhary DK. 2021. Soil Nitrogen Ecology. Springer Nature Switzerland, Switzerland. DOI: 10.1007/978-3-030-71206-8.

Dotaniya ML, Meena VD, Basak BB, Meena RS. 2016. Potassium Uptake by Crops as Well as Microorganisms. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds). Potassium Solubilizing Microorganisms for Sustainable Agriculture. Springer India, New Delhi. DOI: 10.1007/978-81-322-2776-2_19.

Ergüven GÖ, Demir G. 2019. Evaluation of the detoxification potential of *Micrococcus* strains and plants for bioremediate organochlorine herbicides. Intl J Innov Approach Agric Res 3 (3): 353-364. DOI: 10.29329/ijiaar.2019.206.1.

Fan W, Kam KA, Zhao H, Culligan PJ, Kymissis I. 2022. An optical soil sensor for NPK nutrient detection in smart cities. 18th Intl Conf Intell Environ. DOI: 10.1109/IE54923.2022.9826759.

Gandhi K, Vasudeva C, Singh V, Umekar M. 2021. Immobilised TiO_2 application for pesticides degradation using a solar still. Clean Eng Technol 4: 100163. DOI: 10.1016/j.clet.2021.100163.

Garcia-Sanchez M, Bertrand I, Barakat A, Zeroual Y, Oukarroum A, Plassard C. 2023. Improved rock phosphate dissolution from organic acids is driven by nitrate assimilation of bacteria isolated from nitrate and $CaCO_3$-rich soil. PLoS One 18 (3): e0283437. DOI: 10.1371/journal.pone.0283437.

Getenga ZM, Mogusu EO, Ngige AN, Kimosop SJ, Mutua GK, Kengara F, Reiner S, Ulrike D. 2023. A review of the enhanced degradation of pesticides in tropical agricultural soils. J Environ Eng Sci 19: 9-17. DOI: 10.1680/jenes.22.00096.

Jain D, Saheewala H, Sanadhaya S, Joshi A, Bhojiya AA, Verma AK, Mohanty SR. 2022. Potassium solubilizing microorganisms as soil health engineers: An insight into molecular mechanism. In: Dubey RC, Kumar P (eds). Rhizosphere Engineering. Academic Press, London. DOI: 10.1016/B978-0-323-89973-4.00007-7.

Kaur R, Singh D, Kumari A, Sharma G, Rajput S, Arora S, Kaur R. 2023. Pesticide residues degradation strategies in soil and water: A review. Intl J Environ Sci Technol 20: 3537-3560. DOI: 10.1007/s13762-021-03696-2.

Kuhad RC, Singh S, Lata, Singh A. 2011. Phosphate-Solubilizing Microorganisms. In: Singh A, Parmar N, Kuhad RC (eds). Bioaugmentation, Biostimulation and Biocontrol. Springer Berlin Heidelberg, Berlin, Heidelberg. DOI: 10.1007/978-3-642-19769-7_4.

Kumar D. 2022. Bioremediation of Imazetharpyrin in Soil/Water System. [Thesis]. Rani Lakshmi Bai Central Agricultural University, Jhansi.

Lavandier RC, Arêas J, Lemos LS, de Moura JF, Taniguchi S, Montone R, Quinete NS, Hauser-Davis RA, Siciliano S, Moreira I. 2023. Trophic chain organochlorine pesticide contamination in a highly productive upwelling area in Southeastern Brazil. Intl J Environ Res Public Health 20 (14): 6343. DOI: 10.3390/ijerph20146343.

Le TH, Hoang QC, Vu DD, Vo THT. 2021. Biodegradation of organophosphorus insecticide methyl parathion by soil microorganisms. E3S Web Conf 265: 03002. DOI: 10.1051/e3sconf/202126503002.

Lupi L, Bedmar F, Wunderlin DA, Miglioranza KSB. 2019. Levels of organochlorine pesticides in soils, mesofauna and streamwater from an agricultural watershed in Argentina. Environ Earth Sci 78: 3-9. DOI: 10.1007/s12665-019-8579-3.

Lv YC, Xu G, Sun JN, Brestič M, Živčák M, Shao HB. 2015. Phosphorus release from the soils in the yellow river delta: Dynamic factors and implications for eco-restoration. Plant Soil Environ 61: 339-343. DOI: 10.17221/666/2014-PSE.

Masrie M, Rosman MSA, Sam R, Janin Z. 2017. Detection of Nitrogen, Phosphorus, and Potassium (NPK) nutrients of soil using optical transducer. Proceeding of the 4th IEEE International Conference on Smart Instrumentation, Measurement and Applications (ICSIMA) 28-30 November 2017, Putrajaya, Malaysia. DOI: 10.1109/ICSIMA.2017.8312001

Matos IF, do Carmo Silva B, de Souza SB, Bertolazi AA, de Souza Pedroni NL, Intorne A, Ribeiro DP, Ramos AC. 2021. Ecophysiology of Nitrogen in Symbiotic Relationships of Plants and Microorganisms. In: Cruz C, Vishwakarma K, Choudhary DK, Varma A (eds). Soil Nitrogen Ecology. Springer International Publishing, Cham. DOI: 10.1007/978-3-030-71206-8_2.

Meng D. 2019. An alkaline phosphatase from *Bacillus amyloliquefaciens* YP6 of new application in biodegradation of five broad-spectrum organophosphorus pesticides. J Environ Sci Health B 54: 336-343. DOI: 10.1080/03601234.2019.1571363.

Mishra SK, Tripathi KM, Pandey S. 2023. Effect of chemical pesticides on soil health and its physio-chemical properties. Novel Perspect Geogr Environ Earth Sci 13 (8): 156-167. DOI: 10.9734/bpi/npgees/v8/6115E.

Mohite BV, Koli SH, Narkhede CP, Patil SN, Patil S V. 2017. Prospective of microbial exopolysaccharide for heavy metal exclusion. Appl Biochem Biotechnol 183: 582-600. DOI: 10.1007/s12010-017-2591-4.

Nag M, Lahiri D, Ghosh S, Ghosh S, Ray RR. 2022. Autotrophic nitrification in bacteria. In: Shah MP, Rodriguez-Couto S (eds). Development in Wastewater Treatment Research and Processes. Elsevier, Cambidge. DOI: 10.1016/B978-0-323-91901-2.00003-6.

Ngadi N, Zaelany AA, Latifa A, Harfina D, Asiati D, Setiawan B, Ibnu F, Triyono T, Rajagukguk Z. 2023. Challenge of agriculture development in Indonesia: Rural youth mobility and aging workers in agriculture sector. Sustainability 15 (2): 922. DOI: 10.3390/su15020922.

Ore OT, Adeola AO, Bayode AA, Adedipe DT, Nomngongo PN. 2023. Organophosphate pesticide residues in environmental and biological matrices: Occurrence, distribution and potential remedial approaches. Environ Chem Ecotoxicol 5: 9-23.

Pujiati P. 2022. Teknik Pengamatan Mikroba, 1st edn. UNIPMA Press, Madiun. [Indonesian]

Pujiati P, Kiswardianta RB, Dewi NK, Fadillah N. 2024. Evaluate the quality of compost fertilizer with additional bio-slurry on mustard plants (*Brassica rapa* L.). AIP Conf Proc 3095: 020006. DOI: 10.1063/5.0205088

Pujiati P, Fatimah, Ramadhan R, Ni'matuzahroh N. 2025a. Mycoremediation of pesticide-contaminated soil: A review. BIO Web Conf 148: 1-16. DOI: 10.1051/bioconf/202414802020.

Pujiati P, Hertanti, Kiswardianta RB, Fatimah, Ramadhan R, Ni'matuzahroh N. 2025b. Isolation and potential evaluation of organophosphate-indigenous degrading fungi from Singolangu Farmland, Magetan, Indonesia. Biodiversitas 26: 166-177. DOI: 10.13057/biodiv/d260118.

Riyaz M, Shah RA, Sivasankaran K. 2021. Pesticide residues: Impacts on fauna and the environment. Pesticide Residues: Impacts on Fauna and the Environment. Intech Open, London. DOI: 10.5772/intechopen.98379.

Roy A, Roy M, Alghamdi S, Dablool AS, Almakki AA, Ali IH, Yadav KK, Islam MR, Cabral-Pinto MMS. 2022. Role of microbes and nanomaterials in the removal of pesticides from wastewater. Intl J Photoenerg 2022 (1): 2131583. DOI: 10.1155/2022/2131583.

Rütting T, Schleusner P, Hink L, Prosser JI. 2021. The contribution of ammonia-oxidizing archaea and bacteria to gross nitrification under different substrate availability. Soil Biol Biochem 160: 108353. DOI: 10.1016/j.soilbio.2021.108353.

Salama AA. 2015. Response of rice plants to inoculation with indigenous strains of cyanobacterial along with different levels of inorganic n-fertilizers. Adv Biochem Biotechnol 1 (1): 1-14.

Salwan R, Rana A, Sharma V. 2023. Chapter 4-Microscopy and imaging analysis of microorganisms. In: Salwan R, Sharma V (eds.) Laboratory Methods in Microbiology and Molecular Biology. Academic Press, London.

Schaeffer A, Wijntjes C. 2022. Changed degradation behavior of pesticides when present in mixtures. Eco-Environ Health 1: 23-30. DOI: 10.1016/j.eehl.2022.02.002.

Sharma C, Sharma P, Kumar A, Walia Y, Kumar R, Umar A, Ibrahim AA, Akhtar MohdS, Alkhanjaf AAM, Baskoutas S. 2023a. A review on ecology implications and pesticide degradation using nitrogen fixing bacteria under biotic and abiotic stress conditions. Chem Ecol 39: 753-774. DOI: 10.1080/02757540.2023.2253220.

Sharma P, Kanta Pandey K, Lepcha A, Sharma S, Maurya N, Kumar Sharma S, Pradhan R, Kumar R. 2023b. Elucidating the potential of nitrifying bacteria in mitigating nitrogen pollution and its industrial application. Microsphere 2: 246-259. DOI: 10.59118/xfkd8065.

Sindhu SS, Parmar P, Phour M, Sehrawat A. 2016. Potassium-Solubilizing Microorganisms (KSMs) and Its Effect on Plant Growth Improvement. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds). Potassium Solubilizing Microorganisms for Sustainable Agriculture. Springer India, New Delhi.

Steiner M, Falquet L, Fragnière AL, Brown A, Bacher S. 2024. Effects of pesticides on soil bacterial, fungal and protist communities, soil functions and grape quality in vineyards. Ecol Solutions Evidence 5: 1-12. DOI: 10.1002/2688-8319.12327.

Subsanguan T, Vangnai AS, Siripattanakul-Ratpukdi S. 2020. Aerobic and anoxic degradation and detoxification of profenofos insecticide by *Pseudomonas plecoglossicida* strain PF1. Ecotoxicol Environ Saf 190: 110129. DOI: 10.1016/j.ecoenv.2019.110129.

Thi Hieu Thu N, Cao Son T, Thu Trang D, Thi My Le N, Duy Toi N, Thi Van N, Thuy Hang D. 2022. Indigenous diazotrophs and their effective properties for organic agriculture. Viet J Biotechnol 20: 751-760. DOI: 10.15625/1811-4989/17070.

Tian D, Niu S. 2015. A global analysis of soil acidification caused by nitrogen addition. Environ Res Lett 10 (2): 024019. DOI: 10.1088/1748-9326/10/2/024019.

Tomczyk NJ, Rosemond AD, Kaz A, Benstead JP. 2023. Contrasting activation energies of litter-associated respiration and P uptake drive lower cumulative P uptake at higher temperatures. Biogeosciences 20: 191-204. DOI: 10.5194/bg-20-191-2023.

Wakeel A, Ishfaq M. 2022. Potassium dynamics in soils. In: Wakeel A, Ishfaq M (eds). Potash Use and Dynamics in Agriculture. Springer Singapore, Singapore.

Wang Z, Zhang H, Liu L, Li S, Xie J, Xue X, Jiang Y. 2022. Screening of phosphate-solubilizing bacteria and their abilities of phosphorus solubilization and wheat growth promotion. BMC Microbiol 22 (1): 296. DOI: 10.1186/s12866-022-02715-7.

Yadav AN. 2021. Soil Microbiomes for Sustainable Agriculture. Springer, Cham.

Zhang X, Zhan Y, Zhang H, Wang R, Tao X, Zhang L, Zuo Y, Zhang L, Wei Y, Li J. 2021. Inoculation of phosphate-solubilizing bacteria (*Bacillus*) regulates microbial interaction to improve phosphorus fractions mobilization during kitchen waste composting. Bioresour Technol 340: 125714. DOI: 10.1016/j.biortech.2021.125714.