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Research Article

Bioremediation of Profenofos-Contaminated Soil using Bio-Slurry, Exogenous, and Indigenous Microorganism Formulation from Puntukdoro Farmland, Indonesia

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Abstract
Pesticide residues in soil present significant environmental and health risks, especially in regions with heavy organophosphate used in vegetable farming. This study examines bioaugmentation, an eco-friendly method for degrading soil pesticide residues, utilizing both indigenous and exogenous microorganisms, as well as bio-slurry from biogas production. Puntukdoro Village, Magetan, Indonesia, generates a substantial quantity of bio-slurry waste, which presents a promising solution to local agricultural challenges, including low crop yields and soil degradation. Puntukdoro Village produces a significant amount of bio-slurry waste, which offers a promising solution to local agricultural issues, including poor crop yield and soil degradation. The study aims to identify and formulate microorganisms from Puntukdoro using bio-slurry and exogenous cellulolytic mold formulations. This involves extracting and characterizing indigenous bacteria, preparing external supplements, and conducting *ex situ* bioaugmentation with seven different treatments. Ten mold isolates, including Penicillium, Monilia, Aspergillus, and Trichoderma, and eight bacterial isolates, including Micrococcus, Pseudomonas, and Bacillus, were identified. Bioremediation assays showed that both indigenous and exogenous microorganisms improved soil quality and reduced pesticide levels. The most effective treatment, P7, with 10% bio-slurry, 10% biofostik, and 10% indigenous microorganisms applied for 28 d (W4), reduced profenofos from 4.718 to 0.000 mg/kg. In contrast, treatment P2W1, with 30% biofostik for 7 d, reduced profenofos by 0.293 mg/kg. These findings indicate that exogenous and indigenous microorganisms can effectively enhance profenofos bioremediation.

Keywords: bioremediation, profenofos, indigenous, molds, bio-slurry

1. INTRODUCTION

Pesticide is a mixture of various chemicals used to eliminate unwanted organisms or pests. Farmers often utilize pesticides to mitigate pest populations and plant diseases, as these substances are perceived as an efficient and rapid solution. Farmers frequently employ pesticide to reduce the number of pests and plant diseases, as it is an effective and speedy solution. In conventional crop rotation systems, the greatest quantity of pesticide residues was discovered in fields cultivating vegetables, followed by cereal-dominated fields, cereal-grass rotations, and grass-dominated fields. Specifically, organophosphate pesticides such as chlorpyrifos and profenofos are commonly used by vegetable plantation farmers to control insect pests. However, the extensive use of pesticides can lead to complications, as they leave residues on plants and pollute the environment, including soil and groundwater [1][2]. Soil quality refers to the capacity of soil to sustain biological productivity, preserve ecological balance, and promote the health and welfare of flora and fauna within an ecosystem [3]-[5].

Soil fertility is a key factor in plant growth and development and is closely related to the nutrient content of the soil. Pesticide residues in the soil can disrupt soil characteristics, nutrients, and microorganisms, leading to a decrease in soil fertility and changes in the physical properties of the soil, such as hardness, odor, and color [6][7]. Organophosphate pesticides are known to harm living organisms [8]. The WHO estimates that pesticides kill 20,000 farmers annually. Alarmingly, pesticide poisoning instances have increased, resulting in 2 million cases and 40,000 fatalities [9] [10]. Pesticide residues may affect the ecology and should be eradicated [11]. Bioaugmentation may break down pesticide residues in soil [12][13]. Bioremediation restores chemically harmed habitats using microorganisms. Fungi and bacteria are essential for soil bioremediation [14][15].

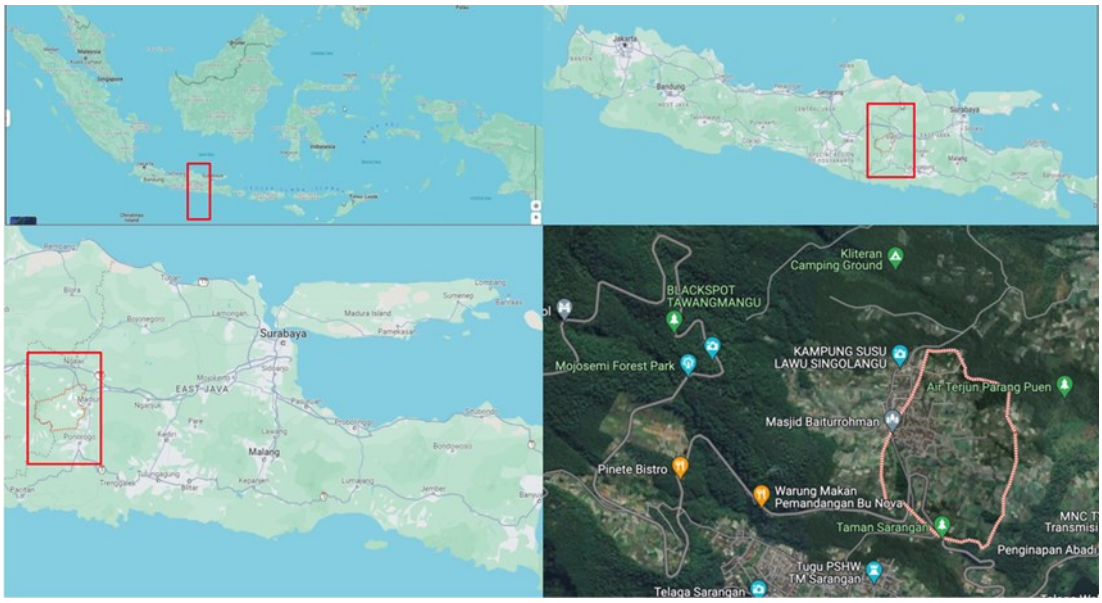


Figure 1. The location where samples were collected in Puntukdoro Village, Plaosan, Magetan. 880 mdpl (24º16'58.96"S, 50º35'00.04"W).

Bioaugmentation begins bioremediation by adding a group of microorganisms to a polluted region. Bioremediation repairs polluted soil with live organisms at low cost and environmental benefit. To improve bioremediation, microorganisms that break down polluted chemicals are needed [16]. These microorganisms might come from organic waste, compost, indigenous and foreign microorganisms, bio-slurry, etc. Based on previous studies, the genera Aspergillus, Penicillium, Trichoderma, Pleurotus, Bacillus, Pseudomonas, and Rhodococcus are among the microorganisms identified as having the capability to degrade organophosphates belonging to the profenofos group [17]-[23]. Both indigenous and exogenous microorganisms have demonstrated the potential to degrade organophosphate compounds through various mechanisms, including hydrolysis, oxidation-reduction reactions, and enzymatic breakdown. Key enzymes such as organophosphate hydrolase and esterases play crucial roles in the degradation process [24]-[26].

As a biogas hamlet, Puntukdoro Village generates a lot of biogas slurry waste. The village's farmers' soil issues can be addressed with this waste. Poor agricultural yield and degraded, infertile soil resulted in financial losses during interviews, as reported by the respondents [27]. Biogas production produces liquid or solid bio-slurry. The presence of microorganisms can be observed in bio-slurry. Notably, this product does not pose any harm to soil or plants, even at substantial concentrations. By incorporating indigenous microorganisms in bioremediation, it is possible to remove pesticide residues from contaminated soil. In this regard, mold and bacteria play a significant role in this process [28][29]. The application of indigenous microorganisms in bioremediation is a promising alternative to reduce pesticide levels in soil [30]. Indigenous microorganisms have exhibited a remarkable capacity to acclimate to a variety of environmental conditions, including diverse pH levels and temperatures, which contributes to their utility in a wide range of agro-ecological contexts [31][32]. Hazardous compounds in the environment can be remediated using exogenous microbes capable of detoxification. These external microbial agents are introduced to enhance or modify biological processes. These microorganisms have the potential to significantly impact various sectors, such as ecological restoration, agriculture, waste management, and oil recovery [33]. Based on this background, this study intends to conduct research on the composition of bio-slurry and the use of indigenous and exogenous microorganisms in the bioremediation of pesticide-contaminated soil. The objective of this research is to evaluate the efficacy of a combination consisting of bio-slurry, indigenous microorganisms, and exogenous

microorganisms in treating soil polluted with pesticides.

2. MATERIALS AND METHODS

2.1. Study Area

This study utilized soil specimens that had been polluted with pesticide residues and these samples were obtained from vegetable plantations in Puntukdoro Village, Plaosan District, and Magetan Regency. The research was carried out in the laboratory of the Biology Education Department, Universitas PGRI Madiun. The soil samples were collected from the top 5–30 cm of the soil surface. For each treatment, 140 g of soil was used, which was cleaned and sieved to create homogeneous soil. The study area is presented in Figure 1, and its condition is depicted in Figure 2.

2.2. Procedures

2.2.1. Soil Sampling Procedure

Soil samples were taken at a single point within the vegetable plantation that had been exposed to pesticides through simple random sampling. The soil samples were transported to the laboratory at PGRI Madiun University for analysis. The soil samples were collected at a depth of 5–10 cm below the surface and were collected in stool container and stored in a dry ice box (4 °C). The collected soil was then cleaned of any dirt and sieved to obtain a homogeneous sample [34].

2.2.2. Soil Sample Pretreatment

The soil samples were pretreated using organophosphate pesticides, specifically chlorpyrifos with the trademark Fostin 610 EC and profenofos with the trademark Curacron 500 EC. A 2 mL of each pesticide were dissolved in one liter of water [35][36]. The soil samples were then soaked in the pesticide solution for 7 d, after which they were dried, crushed, and sieved. The soil samples contaminated with pesticide residue were divided into eight equal portions, weighing 140 g each. One of the portions was designated as the control, while the remaining seven were treated with different levels of bio-slurry, biofostikcs, and indigenous microorganisms. The levels of bio-slurry, biofostikcs, and indigenous microorganisms used were 30%, 15%, and 10% , respectively [37]-[39]. All of these procedures are performed aseptically using LAF to minimize contamination.

2.2.3. Growth Medium

Preparing agar media involves making two types of media: Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media. To prepare NA media, weigh 2.8 g of NA and mix it with 100 mL of distilled water, Heat and stir the mixture until it becomes homogeneous. Transfer the NA solution to an Erlenmeyer flask, cover it with sterile cotton, and wrap it with aluminum foil. Sterilize the NA media using an autoclave at 121 ºC for 15 min. To make PDA media, weigh 3.9 g of PDA and mix it with 100 mL of distilled water. Heat the mixture on an electric stove until it boils, creating a clear solution. Transfer the PDA solution to an Erlenmeyer flask, cover it with sterile cotton, and wrap it with aluminum foil. Sterilize the PDA using an autoclave at 121 ºC for 15 min, with a pressure of 1–2 atm [40].

2.2.4. Microbial Isolation

The agar pour method, which involves making a

Figure 2. The condition of agricultural soil and bio-slurry produced from Puntukdoro Village, Plaosan, Magetan.a) soil condition; b) Collecting bioslurry.

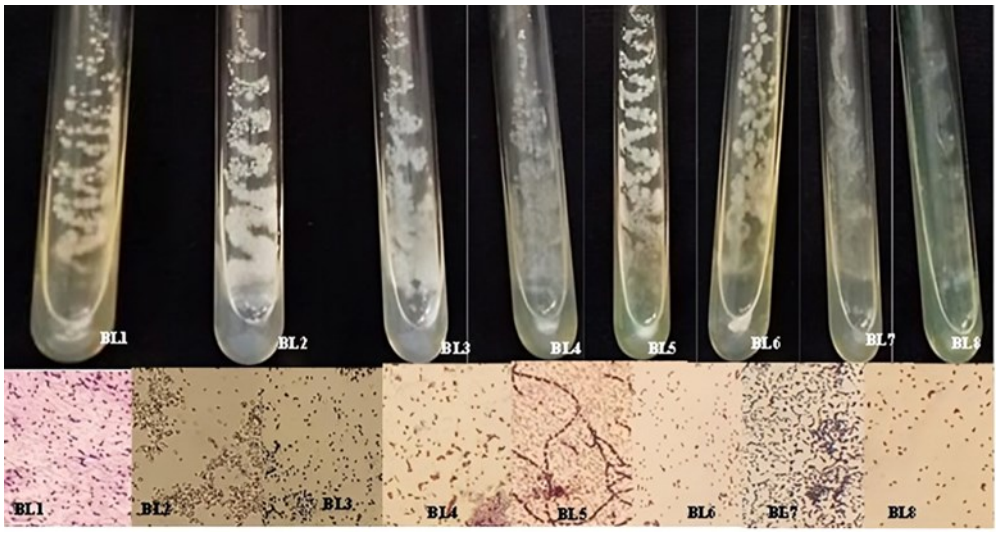


Figure 3. Indigenous bacteria from Puntukdoro Village, whereas BLx is potential bacteria x.

series of graded dilutions, is used to isolate microbes. Soil samples were obtained and weighed at 1 g before being suspended in 9 mL of sterile distilled water for graded dilutions [41]. The process was repeated until the 10^{-9} dilution. Dilutions 10^{-3}, 10^{-4}, and 10^{-5} were chosen for the isolation of bacteria and molds. To isolate bacteria, 1 mL of sample from dilutions 10^{-3}, 10^{-4}, and 10^{-5} was poured into a petri dish, followed by the addition of 1 mL of griseofulvin solution and sufficient NA media [42]. For the isolation of molds, 1 mL of sample from dilutions 10-3, 10-4, and 10^{-5} was poured into a petri dish, and then 1 mL of chloramphenicol solution and sufficient PDA media were added then homogenized. The mixture was made homogeneous by slowly rotating the petri dish in a figure-eight motion eight times on the table. All petri dishes were then wrapped in cling wrap and stored at room temperature for 3–7 d [43]. After the isolation medium had allowed microbes to grow, they were transferred to other NA and PDA media contained in test tubes.

2.2.5. Preparation of Microbial Culture Stock

Preparing a microbial culture stock involves rejuvenating indigenous microorganisms from soil samples that have grown on NA (bacteria) and PDA (molds) media and observing them in slanted media for stock culture. Each microbial isolate is then replicated twice. To prepare the culture stock, pour the rejuvenated microbes into 600 mL of physiological water, add 6 g of NaCl, and a small amount of pesticide. Incubate the culture at room temperature on a shaker at a speed of 115 rpm for 4 d. The degree of turbidity in the physiological water indicates the microbial population.

2.2.6. Bioremediation Procedure

The treatment was conducted on seven groups of soil samples contaminated with pesticide residues, each weighing 140 g. The soil was cleaned of debris and sifted to achieve homogeneity. The homogenized soil was then placed into 250 mL heat -resistant plastic bottles, and the mouth of the bottle was sealed to prevent contamination [44]. The bioremediation formula was administered to each treatment group, and observations were conducted at seven-day intervals over a 28-day period, specifically on day-7, -14, -21, and -28.

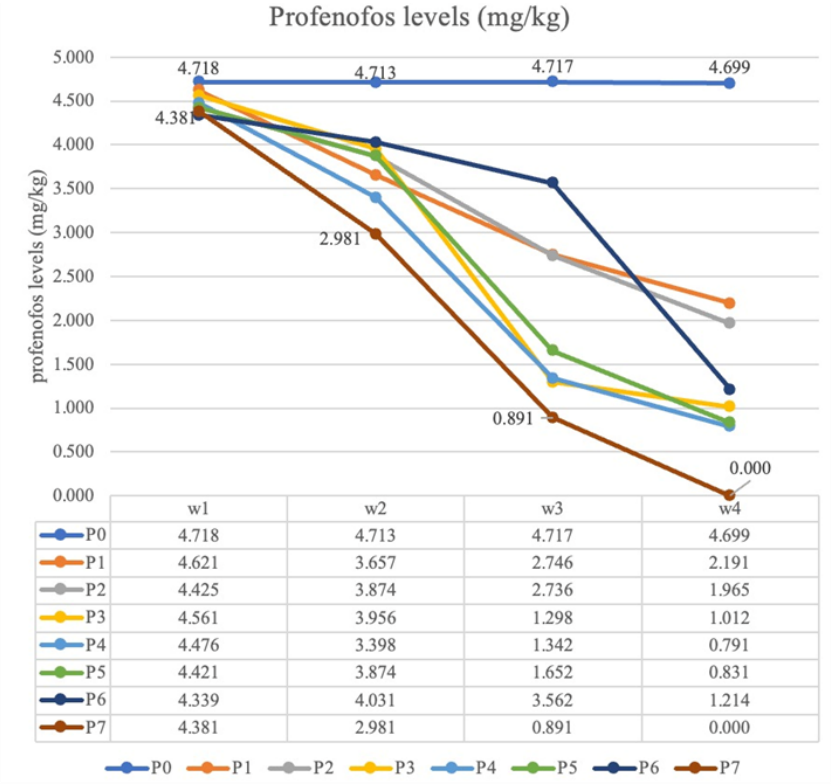
2.2.7. Macroscopic and Microscopic Characterization of Microorganisms

Macroscopic observations were conducted by directly examining microorganisms on agar media in petri dishes. The morphological characteristics of mold were assessed by observing its top and bottom surfaces, color, edges, and elevation. In contrast, the characteristics of bacteria, including their colony shape, size, color, edge, and elevation, were also examined. Microscopic observations were conducted on mold to examine its reproductive organs and spores. This was done by thinly slicing the mold and agar medium with a razor blade, placing it on a glass slide, adding one drop of distilled water, and covering it with a slide. The slide was then observed using a binocular

microscope. The bacterial staining method was used to observe the microscopic characteristics of bacterial isolates. This process involved sterilizing a glass slide by passing it 3–4 times over a Bunsen flame. Aseptically, bacterial isolates were taken using a loop needle, applied to a glass slide, and dripped with crystal violet. The bacterial isolate was then washed with flowing distilled water and dried. Subsequently, the bacterial isolate was dripped with iodine solution, washed with flowing distilled water, and dried. Next, the bacterial isolate was dripped with 95% alcohol for 30 s, rinsed with distilled water, and dried. Finally, the bacterial isolate was dripped with safranin for 30 s, washed with flowing distilled water, and observed using a binocular microscope with a magnification of 1000 times.

2.2.8. Biochemical Test of Indigenous Bacteria

The indole test was performed using 10 g of *p*-dimethylaminobenzaldehyde, 150 mL of isoamyl alcohol, and 50 mL of concentrated HCl in Tryptone broth. For the methyl red test, 0.1 g of methyl red dye and 300 mL of ethyl alcohol were mixed with 200 mL of water, using MR-VP broth. The Voges-Proskauer test utilized 40% KOH and alpha-naphthol in glucose phosphate broth. The citrate utilization test involved bromothymol blue at 0.4 g/mL and 0.05 M NaOH in a citrate agar slant. Gram staining was performed according to standard protocols. For the indole test, 4 mL of tryptophan broth were inoculated with a 24-h culture and incubated at 37 °C for 24 h, followed by the addition of 0.5 mL of Kovac's reagent. The methyl red test involved inoculating MR-VP broth with 2 loopfuls of bacterial cultures, incubating at 37 °C for 48 h, and adding a small amount of methyl red indicator. For the Voges-Proskauer test, cultures were inoculated into glucose phosphate broth and incubated for 48 h. Subsequently, 0.6 mL of alpha-naphthol and 0.2 mL of a 40% KOH solution were added and mixed thoroughly. For the citrate utilization test, 5-mL tubes of medium were prepared as slants. Cultures were streaked on citrate



	w1	w2	w3	w4
P0	4.718	4.713	4.717	4.699
P1	4.621	3.657	2.746	2.191
P2	4.425	3.874	2.736	1.965
P3	4.561	3.956	1.298	1.012
P4	4.476	3.398	1.342	0.791
P5	4.421	3.874	1.652	0.831
P6	4.339	4.031	3.562	1.214
P7	4.381	2.981	0.891	0.000

Figure 4. Profenofos levels during bioremediation.

Notes: P0 = control treatment (soil+pesticide only); P1 = treatment with 30% bio-slurry, P2 = treatment with 30% biofostik, P3 = treatment with 30% indigenous microorganisms, P4 = treatment with 15% bio-slurry and 15% biofostik, P5 = treatment with 15% bio-slurry and 15% indigenous microorganisms, P6 = treatment with 15% biofostik and 15% indigenous microorganisms, P7 = treatment with 10% bio-slurry, 10% biofostik, and 10% indigenous microorganisms, wx = week x.

Table 1. Characterization of indigenous bacteria from Puntukdoro Village.

Codes	Colony Form	Size	Pigment	Edge	Elevation	Gram	Cell Shape	Genus
BL1	Round	Medium	Yellow	Entire	Raised	+	Cocus	Micrococcus
BL2	Irregular	Medium	White	Undulate	Flat	+	Cocus	Micrococcus
BL3	Irregular	Medium	Yellow	Undulate	Flat	+	Cocus	Micrococcus
BL4	Irregular	Medium	Yelow-green	Undulate	Flat	-	Rod	Pseudomonas
BL5	Irregular	Medium	White	Lobate	Raised	+	Coccus	Steptococcus
BL6	Irregular	Medium	Yellow	Entire	Raised	-	Cocus	Pseudomonas
BL7	Irregular	Medium	Beige	Lobate	Flat	+	Basil	Bacillus
BL8	Irregular	Medium	Beige	Undulate	Flat	-	Cocus	Pseudomonas

agar slants and incubated at 37 °C for 24 h [45].

2.2.9. Pesticide Reduction Analysis

Profenofos reduction was evaluated using liquid chromatography-mass spectrometry (LC-MS) analyzed by Pesticide and Fertilizer Laboratory, Department of Agriculture and Food Security, East Java, an effective method for separating, identifying, and analyzing compounds. This technique can be utilized for quality control purposes and can also be combined with other analytical methods to further elucidate the components of mixtures. In the present study, a matrix-matched method was employed to analyze samples with the aid of a Micromass Quattro Micro API Triple Quadrupole Mass Spectrometer (UK). The Waters Sunfire C18 Column 100 Å, 150 mm × 2.1 mm × 1.5 µm was used as the column specification, and 20 µL samples were injected into the device [46].

2.2.10. Data Analysis

In this study, the bioremediation of pesticide-contaminated soil was accomplished through bioaugmentation. This involved the addition of microorganisms, including both indigenous and exogenous populations, as well as other sources such as bioslurry and biofostik. The indigenous microorganisms were isolated and determined by macroscopic and microscopic characterization. The evaluation of the bioremediation procedure is contingent upon the following parameters: N, P, K, and pH, and the degradation level of profenofos ascertained through the utilization of LC-MS. The pH, N, P, and K levels were determined by observing changes in the color of the KIT solution based on a color chart that was observed every week.

3. RESULTS AND DISCUSSIONS

This research utilized indigenous microorganisms isolated from Puntukdoro Village in Magetan regency, comprising bacteria and fungi (Figures 3 and 4). The exogenous microbes employed were a consortium of cellulolytic fungi from previous research conducted by authors, designated as biofostik [47]. Biofostik has been produced on a large scale in the partner village and

applied by farmers to their lands. Another microbial source utilized was bioslurry, an abundant waste product in Puntukdoro village used by many residents for agriculture. The use of these three microbial sources aimed to determine their effectiveness in reducing pollutants, particularly pesticides, and assess how these formulations can improve soil health. These microbial sources are also abundant in Puntukdoro Village.

3.1. Indigenous Microorganism Data from Treatment Soil

The data collected on indigenous microorganisms includes information on the bacterial and mold populations from the treatment soil. Microbial isolation was performed every seven days, namely on day-7, -14, -21, and -28. The isolation process was conducted for 5 d, after which observations were made to collect data in the form of macroscopic and microscopic images, and microbial characteristics. The data collected are presented in Figure 3 and Table 1.

In the untreated soil, a total of 8 bacterial isolates and 10 fungi isolates were identified, representing the indigenousnative microorganisms present. Images of the bacterial isolates can be observed in Figure 3, while Tables 3 lists the mold isolates. A comparison was conducted between the characteristics of the identified indigenous bacteria and molds and the descriptions provided in Table 1, Table 2 and Table 3. Bioremediation test results, i.e., N, P, K, and pH levels of remediated soil in the treatment soil were evaluated on day-7, -14, -21, and -28. The testing was done using an NPK kit, which produced qualitative data results. The data obtained can be found in Table 4.

3.2. Discussion

3.2.1. Indigenous Microorganism from Puntukdoro Farmland

The studies have identified certain indigenous microorganisms, including fungi and bacteria, that possess the ability to degrade profenofos. These conclusions are drawn from observations of their growth in NA and PDA media, derived from soil samples previously exposed to profenofos. This procedure aims to enrich the soil with the contaminant (profenofos) to facilitate the adaptation of indigenous microorganisms to these conditions. It is well recognized that the specific characteristics and metabolic processes of indigenous bacteria are shaped by their environmental context, including the presence of contaminating compounds. The indigenous fungi identified are classified within the genera Aspergillus, Trichoderma, Monilla, Penicillium, and Fusarium, as detailed in Table 3. The genera of indigenous bacteria identified include Bacillus, Pseudomonas, Streptococcus, and Micrococcus, as documented in Table 1 and Figure 3.

Table 2. Biochemical tests of indigenous bacteria.

No	Indicators	BL 1	BL 2	BL 3	BL 4	BL 5	BL 6	BL 7	BL 8
1	Carbohydrate Fermentation	+	+	+	-	-	-	+	-
2	Methyl red	-	-	-	-	+	-	+	-
3	Voges-Proskauer	-	-	-	-	-	-	+	-
4	Citrate test	+	+	+	-	+	-	+	-
5	Indole production	-	-	-	+	-	+	-	+
6	Urea hydrolysis	+	+	+	-	-	-	-	-
7	Nitrate reduction	-	-	-	-	+	-	+	-
8	Starch hydrolysis	+	+	+	-	+	-	+	-
9	Casein hydrolysis	-	-	-	-	+	-	+	-
10	Gelatin hydrolysis	+	+	+	-	-	-	+	-
11	Catalase	+	+	+	-	-	-	+	-
12	Sulfide Production	+	+	+	+	+	+	-	+

Table 3. Mold morphology in the analyzed samples.

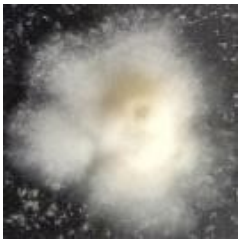
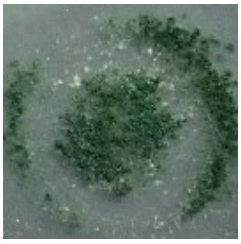
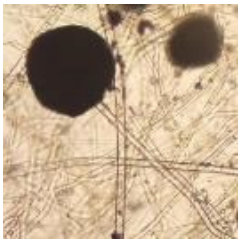
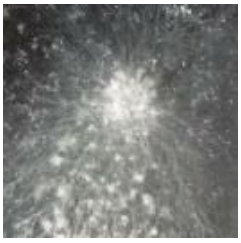
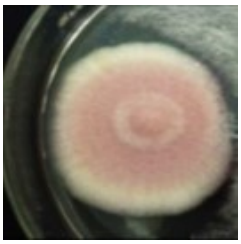
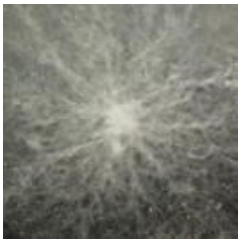
Codes	Macroscopic images	Conidia	Spores	Genera characteristic
FL1				Penicillium Powdery, Blackish ash (top), Black (bottom), Septat hypae
FL2				Monilia Velvety, Yellowish white (top), Yellow (bottom), Septat hyphae.
FL3				Monilia Velvety White (top), White center brown (bottom), Septat hyphae
FL4				Aspergillus Powdery, Brown (top), Brownish white (bottom), Aseptat hyphae.
FL5				Trichoderma Powdery, Dark green (top), Light green (bottom), Aseptat hyphae
FL6				Monilia Cottony, White (top and bottom), Aseptat hyphae.
FL7				Aspergillus Cottony, Brownish green (top), White (bottom), Septat hyphae

Table 3. *Cont.*

Codes	Macroscopic Images	Conidia	Spores	Genera Characteristic
FL8				Fusarium Velvety, Pink and yellowish (top), Pink and yellowish (bottom), Septat hyphae.
FL9				Penicillium Cottony, white ash (top), White (bottom), Septat hyphae
FL10				Penicillium Velvety, Yellowish white (top), White (bottom), Septat hyphae.

Notes: Indigenous fungi from Puntukdoro Village, Magetan, Indonesia. FLx : potential fungi x.

3.2.2. N, P, K, and pH Levels

The N, P, K, and pH levels were tested weekly on day-7, -14, -21, and -28. Research data indicate that control soil (untreated) exhibited trace amounts of N, as confirmed by NPK kit tests, which showed a white solution indicating trace levels (Table 4). Treated soil (P1 to P7) showed a weekly increase in N levels, except during the first week, as evidenced by the solution change in color from white to increasingly intense pink. This suggests that treatment, especially with liquid bio-slurry, affects soil N levels. Microorganisms are essential in increasing the levels of nitrogen in treated soil through several processes. One such process is nitrogen fixation, which involves the conversion of atmospheric N_2 into ammonia through the use of the nitrogenase enzyme. Nitrification, carried out by Nitrosomonas and Nitrobacter, transforms ammonia into nitrate. Additionally, denitrification converts nitrates and nitrites back into ammonia. The decay of N-organic compounds through ammonification also produces ammonia ions. These microbial activities make nitrogen available for plant uptake, and biological nitrogen fixation contributes 30–50 kg N/ha/year to the soil. Moreover, microbial mineralization of organic matter, particularly crop residues, significantly contributes to soil nitrogen enrichment [46][48].

Fungi have a significant impact on soil nitrogen levels, with key genera such as Aspergillus, Penicillium, and Trichoderma playing crucial roles. Aspergillus species, including *Aspergillus flavus* and *Aspergillus niger*, are essential in solubilizing phosphate, which indirectly aids nitrogen fixation by enhancing soil fertility and promoting plant growth. Additionally, these species contribute to the nitrogen cycle by decomposing organic matter. Penicillium species, such as *Penicillium citrinum*, are also vital in improving soil health by solubilizing phosphates and producing organic acids, which increase nutrient availability, including nitrogen. Pseudomonas, Bacillus, Aspergillus, Penicillium, and Trichoderma are microbes capable of increasing the nitrogen content in the soil [49]-[51]. These microbial isolates were identified and examined in this study. Soil conditions—physical, chemical, and biological—affect the conversion of N_2 into ammonia. Based on previous studies, the

total N content in bio-slurry amounts to 2.34%. The N concentration in liquid bio-slurry is higher than that of solid bio-slurry, standing at 2.92% for liquid bio-slurry and 1.47% for solid bio-slurry [52]. Consequently, applying liquid bio-slurry to the soil can enhance N levels. Among the treatments, P7 experienced the most rapid increase in N levels, reaching high levels at W3 and W4. The P7W4 treatment is considered optimal, as it exhibited the most intense color compared to the other treatments. The P7 treatment comprises 10% bioslurry, 10% biofostikc, and 10% indigenous microorganisms. Bio-slurry contains N-fixing bacteria and probiotic bacteria.

According to the data presented in Table 5, the starting level of phosphate is quite high. A range of factors contribute to the rise in soil phosphate levels, both naturally and as a result of human activities. The application of P fertilizers, such as NaH_2PO_4, triple superphosphate, rock phosphate, and magnesium thermophosphate, significantly increases soil P levels by facilitating the conversion of organic P to inorganic P, which is more readily available for plant absorption. Additionally, the mineral composition of the soil, including the presence of elements such as calcium, iron, and aluminum, plays a critical role in determining P availability, as these elements can bind P, making it less accessible to plants. However, the use of P-solubilizing microbes can enhance P availability by dissolving these bound phosphates [53]-[56].

Phosphate-solubilizing microorganisms hold great significance in improving soil fertility by dissolving bound phosphorus, enabling plants to absorb it. These beneficial microorganisms, including bacteria and fungi such as Bacillus, Pseudomonas, Penicillium, and Aspergillus, employ various mechanisms to solubilize both inorganic and organic P compounds. They produce organic acids, including citric, oxalic, and lactic acids, as well as inorganic acids like HCl and H_2SO_4, which decrease soil pH and release P from insoluble compounds [57]-[59]. These microorganisms were also successfully isolated in this study, as demonstrated in Figure 3 and Tables 2 and 3.

The rise in K levels in treated soil can be attributed to the activity of microorganisms in dissolving K. Several studies have reported that microbes can break down silicate minerals like feldspar and mica to release K, a process known as transforming unavailable potassium [60]. Microbial mechanisms for dissolving potassium include acidolysis, chelation, exchange reactions, complexolysis, and the production of organic acids such as malic acid, oxalic acid, citric acid, and formic acid [61]. The rate of dissolution of potassium ions from soil minerals can be influenced by these organic acids. K-solubilizing bacteria

Table 4. N, P, K, and pH levels during bioremediation studies.

Treatments	Incubation time															
	W1				W2				W3				W4			
	N	P	K	pH	N	P	K	pH	N	P	K	pH	N	P	K	pH
P0	T	H	T	5	T	H	T	5	T	H	T	5	T	H	T	5
P1	T	H	L	5	M	H	L	6	M	H	L	6	H	H	M	6
P2	T	H	T	5	L	L	L	5	L	H	M	6	L	H	M	6
P3	T	H	L	5	T	H	L	6	L	H	M	6	M	H	M	6
P4	T	H	L	6	M	H	L	6	M	M	M	6	H	H	M	6
P5	T	H	L	6	M	H	L	6	M	H	M	6	H	H	M	6
P6	T	H	T	6	L	H	L	6	M	H	M	6	M	H	M	6
P7	T	H	L	6	M	H	L	6	H	H	M	6	H	H	H	6

Notes: P0 = control treatment (soil+pesticide only), P1 = treatment with 30% bio-slurry, P2 = treatment with 30% biofostik, P3 = treatment with 30% indigenous microorganisms, P4 = treatment with 15% bio-slurry and 15% biofostik, P5 = treatment with 15% bio-slurry and 15% indigenous microorganisms, P6 = treatment with 15% biofostik and 15% indigenous microorganisms, P7 = treatment with 10% bio-slurry, 10% biofostik, and 10% indigenous microorganisms. NPK levels: T = trace, L = low, M = medium, H = high.

produce and secrete organic acids, either by directly releasing K into rocks or silicate ions, which can make K soluble, or by dissolving K in the soil in the form of soluble rocks and silicate minerals (mica, illite, and orthoclase) [62].

The elevation in pH levels in treated soil to neutrality is attributed to the activities of microorganisms striving to restore optimal environmental conditions. The decomposition of proteins by deceased bacterial cells or the degradation of specific groups can generate basic compounds or ions, raising pH levels. Within their adaptive tolerance limits, bacteria can maintain homeostasis against environmental acidity [63]. This process entails exchanging K^+ cations from within the cell with the abundant H^+ in the environment, thus mitigating environmental acidity. Basic cations, such as Ca, Mg, K, and Na, which are the result of the degradation of organic materials, contribute to an increase in pH levels. Consequently, the soil becomes saturated with cations, leading to an increase in soil pH.

3.2.3. Profenofos Levels during Bioremediation

The most significant reduction in profenofos pesticide residue levels was observed in treatment P7W4, attributed to the microbial degradation process. This treatment involved the addition of bioslurry derived from biogas waste, as well as exogenous (Cellulolytic molds formula/Biofostik) and indigenous microorganisms. Both the bioslurry and exogenous microorganisms comprised potential microorganisms essential for soil health. Bioslurry manure, particularly cow dung slurry, has been demonstrated to improve pesticide biodegradation by increasing microbial growth and activity in contaminated soils [64]-[67]. Indigenous microorganisms have shown considerable potential in reducing profenofos contamination in various environments. Numerous studies have isolated and identified bacteria capable of degrading profenofos, including the genera Pseudomonas and Bacillus, which utilize profenofos as a carbon source and achieve degradation rates of over 90% [68][69]. Previous research has shown that Bacillus altitudinis PF1 was capable of degrading 93% of profenofos within a 30-day period [70]. Additionally, the combination of *Bacillus cereus* with *Aneurinibacillus migulanus* has been reported

Table 5. Tests of between-subjects effects dependent variable Profenofos levels.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	373.406[a]	31	12.045	6.533	0.000	0.760
Intercept	5719.594	1	5719.594	3102.153	0.000	0.980
Formula	248.156	7	35.451	19.228	0.000	0.678
Treatment Time	63.281	3	21.094	11.441	0.000	0.349
Formula * Treatment Time	61,969	21	2.951	1.600	0.077	0.344
Error	118.000	64	1.844			
Total	6211.000	96				
Corrected Total	491.406	95				

to significantly enhance the degradation rate of profenofos [71]. In the current study, complete degradation of profenofos was achieved by the fourth week, aligning with earlier findings and providing further evidence that utilizing a consortium of microorganisms can effectively expedite the bioremediation process. The results of the statistical analysis (Table 5), indicate significant effects of the formula and time on the profenofos levels during bioremediation. The partial eta squared values suggest that the formula had a stronger influence on the profenofos concentration during bioremediation compared to time. Cellulolytic mold, when added as an exogenous microorganism, has demonstrated its capacity to degrade lignocellulosic biomass. These microorganisms possess enzymatic capabilities that enable them to degrade various organic compounds, such as pesticides like profenofos. Moreover, their potential for pesticide degradation suggests a broader range of applications [72]. Soil microbes in pesticide-contaminated environments can utilize these pesticide compounds as both a carbon and energy source for growth [73].

Cellulolytic molds can degrade organophosphate compounds through various biochemical pathways. Aspergillus degrades organophosphate pesticide by using it as a sole carbon and phosphorus source, forming metabolic products through hydrolysis and deoxidation reactions. This involves C–P bond cleavage, producing dimethyl hydrogen phosphate and chloral hydrate, which are further metabolized into less harmful compounds [74]. Trichoderma degrades organophosphates through P–O bond hydrolysis, facilitated by enzymes like paraoxonase -like hydrolases [75]. These enzymatic activities are part of a bioremediation strategy using microbial consortia and cell-free enzyme systems to enhance organophosphate degradation in contaminated environments [76][77]. Degradation pathways often involve hydroxylation, hydrolysis, and dealkylation reactions, as seen in organophosphate flame retardant degradation by white-rot fungi [78].

Profenofos degradation is a complex process that relies on several key enzymes to break down this compound into less harmful substances. One of the main enzymes involved in this process is organophosphorus hydrolase (OPH), encoded by the mpd and opd genes, essential for the hydrolysis of organophosphates such as profenofos [67]. OPH is an enzyme that can degrade a wide range of organophosphates, including pesticides and nerve agents, by cleaving phosphorus-ester bonds, which are responsible for their toxicity OPH enzymes have demonstrated efficacy in hydrolyzing organophosphate esters, thereby converting them into less harmful substances. The activity of these enzymes is influenced by various factors, including the microbial community and environmental conditions. Furthermore, the optimization of microbial culture conditions can markedly enhance OPH production, as evidenced by the use of specific carbon and nitrogen sources [78]. Additionally, the α- and β-subunit of terminal deoxygenase and naphthalene dioxygenase genes have been identified in Pseudomonas CN44, suggesting their role in the degradation process. Similarly, Pseudoxanthomonas suwonensis strain HNM degrades profenofos by hydrolyzing it to yield 4-bromo-2-chlorophenol, which is used as a carbon source for growth [67]. The presence of dioxygenase genes in these bacteria suggests their role in the oxidative cleavage of profenofos. In conclusion, hydrolases, dioxygenases, and other oxidative enzymes play a crucial role in the microbial degradation of profenofos, contributing to its bioremediation in various environmental matrices.

Indigenous microbes respond to pesticide contamination by incorporating it into their metabolic processes as a carbon source. The end products of the mineralization process during pesticide residue degradation are directly utilized by microbial cells. Bacteria possess functional enzymes that aid in the pesticide degradation process. Soil microbes produce hydrolysis enzymes, such as phosphatase and esterase, capable of breaking down the chemical composition of pesticides with unstable chain structures. These enzymes can carry out the oxidation process at the extracellular level in soil contaminated with pesticide residues and at the intracellular level in bacterial cells, resulting in increased solubility of the residue into organic components that plants can absorb. During bioremediation, various types of microbes play a role, facilitating co-metabolism in the degradation of profenofos residues. The higher the microbial activity, the faster the decline in

profenofos residue levels. Elevated microbial activity influences the microbes' ability to produce enzymes involved in degrading profenofos. As bacteria commence to use profenofos as a source of carbon and energy, the production of these enzymes increases, leading to a decrease in profenofos residues.

4. CONCLUSIONS

This study examined the bioremediation of soil contaminated with profenofos through the application of bio-slurry, exogenous, and indigenous microorganisms sourced from Puntukdoro farmland. The appropriate mixture of bioslurry, exogenous, and indigenous microorganisms can significantly influence soil quality during the bioremediation of pesticide-contaminated soil. The optimal combination is found in the P7 treatment, which comprises 10% bioslurry, 10% biofostik and 10% indigenous microorganisms. In the P7 treatment, N levels reached optimal (high) in W3, K levels reached optimal (high) in W4, and K levels also reached optimal (high) in W4. Additionally, pH levels reached 6 in W1, pesticide residue levels were reduced by 4.718 mg/kg in W4, while the lowest result formula in the P2 treatment, consisting of 30% biofostic, was not optimal. In the P2 treatment, N levels only reached low in W4, P levels decreased to low in W2, K levels reached low in W3, pH levels remained at 5 in W2, and pesticide residue levels were reduced by only 0.293 mg/kg in W1. Future studies should compare these findings with those utilizing standard pesticides analyzed by a laboratory, as the current study exclusively employed commercial pesticides readily available on the market.

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P. P. conceptualized and designed the study. O. H. S. and S. U. executed the experiments and collected data. F. F. and R. R. contributed to data interpretation and manuscript preparation. N. N. provided project supervision and critical revisions. All authors contributed to the manuscript writing, reviewed the final version, and approved it for submission.

Conflicts of Interest

The authors declare that there is no conflict of interest. The funding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the

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