Applied Environmental Research
- RESEARCH FOR SUSTAINABLE PLANET -

Research Article

Effective Microorganisms (EM) as Bioremediation Agent of Profenofos Pesticide Residue in Vegetable Farm Soil from Benguet Province, Philippines

Maylowen P. Lumayna[1,*] Paulina A. Bawingan[2]

[1] *School of Advanced Studies, School of Natural Sciences, Saint Louis University, Baguio City, 2600, Philippines*
[2] *Visiting Lecturer, School of Advanced Studies, School of Natural Sciences, Saint Louis University, Baguio City, 2600, Philippines*

*Correspondence Email: 2085730@slu.edu.ph

Abstract

Effective microorganisms (EM) have shown remarkable adaptability and have been used in agriculture and environmental management. This study intended to provide insights into the effectiveness of EM technology in rehabilitating soils contaminated with pesticides. Specifically, the study aimed to determine the efficiency of EM in breaking down the profenofos component of Selecron® 500 EC pesticide in a controlled condition. Results show that the initial profenofos (17.2 mg kg^{-1}) degraded in the control and experimental groups by 94.19% and 96.45%, respectively, over a 21-day laboratory experiment. EM-treated soil samples showed a significant difference from untreated samples, as revealed in the Kruskal-Wallis (p=3.55e-12) and Freidman's tests (p=0.20). These findings enhance our understanding of EM's capabilities in pesticide remediation as well as the natural dissipation of pesticides.

ARTICLE HISTORY
Received: 28 Mar. 2024
Accepted: 04 Oct. 2024
Published: 5 Nov. 2024

KEYWORDS
Pesticide degradation;
Pesticide dissipation;
Organophosphates;
Profenofos;
Soil rehabilitation

Introduction

Profenofos, one of the most widely used organophosphates, is classified as moderately toxic, Toxicity Class II, by the World Health Organization [1]. This insecticide is used on cotton, coconut, green chili, fruit, gooseberries, tomato, spring onion, okra, curry leaves, mint leaves, coriander leaves, and fruits and vegetable cultivation to control the tobacco budworm, cotton bollworm, armyworm, cotton aphid, whiteflies, spider mites, plant bugs, and leaf hoppers [2-3].

Despite being slightly less toxic than other organophosphates, its chronic use results in widespread presence, especially in agricultural lands and surrounding areas. Environmental issues associated with profenofos use include pollution of surface and groundwater, soil contamination, increased resistance in insect populations, harm to non-target species, and reproductive toxicity to humans [3-4]. Humans may absorb profenofos through the skin or inhalation, resulting in symptoms such as nausea, diarrhea, and nervous system effects [5]. However, the primary exposure route for profenofos is dietary intake [6].

These concerns led to a global movement for food and environmental safety monitoring in which profenofos were found among the detected pesticides. In Thailand, profenofos was detected in soil 41.8080 mg kg^{-1} in summers and 16.5956 mg kg^{-1} in winters [7]. In Burkina Faso, West Africa, profenofos residue was slightly greater (0.01–0.08 mg kg^{-1}) than other pesticide residues in soil samples collected from the cotton farmland [8]. Head Balloki in the River Ravi, Pakistan, recorded 1.40 ± 0.15 ng L^{-1} profenofos in the water samples collected from the river [9]. A study conducted in Tanzania revealed that 47.5% of locally produced fresh vegetables had pesticide residues, with 74.2% exceeding the maximum residue levels (MRLs) [10]. Further, profenofos residues, together with other pesticides, have been detected in selected fruits and vegetables in a study conducted in Ethiopia [11], Nigeria [12], and Senegal [13].

In the crop-producing areas of the Philippines, reports have shown the presence of profenofos pesticide residue in vegetables, water, and soil. In the Pangasinan Province of the country, eggplant (*Solanum melongena* L.) and soil samples exceeded the MRL for profenofos,

https://doi.org/10.35762/AER.2024049

triazophos, chlorpyrifos, cypermethrin, and malathion [14]. A survey conducted in Benguet Province, a leading vegetable-producing region in the northern Philippines, uses Selecron, a brand name for profenofos, as one of the pesticides for cabbage and potato crops [15]. Profenofos (0.003 mg kg^{-1}) was detected in soil samples and vegetables [16-17], and a very high residue level of 1 mg kg^{-1} was found, significantly exceeding the Acceptable Daily Intake level of about 0.001 mg kg^{-1} [18]. Samples of soil from Kapangan and celery from Buguias, both towns of Benguet Province, showed profenofos content [17]. Pesticide residues of chlorpyrifos, profenofos, cyhalothrin, cypermethrin, and fenvalerate were also found in soil samples from Mankayan in Benguet Province and Sadsadan in Mt. Province [19].

Given the persistent presence of profenofos and the potential issues caused, addressing this problem is extremely important. Bioremediation has gained popularity recently for its effectiveness in breaking down toxic chemicals in aquatic or terrestrial environments [20-21]. This technology can utilize various living organisms, such as plants, fungi, or bacteria. Microbial bioremediation offers advantages, including its ability to detoxify very dilute effluents efficiently and quickly and its capacity for in situ application [22]. This efficiency is attributed to the diverse physical and chemical reactions within microorganisms' metabolic processes, resulting in the degradation and removal of pollutants. Some notable examples used in microbial bioremediation include *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas diminuta* strain Gm, which can degrade organophosphate pesticides. Enterobacter species can break down chlorpyrifos, and *Burkholderia cenocepacia* strain S5-2 can fully degrade methyl parathion (MP). Furthermore, *Pseudomonas fluorescens* and *Bacillus polymyxa* strains are effective at degrading aldrin. Fungi like *Trametes* versicolor and *Lentinus tigrinus* can degrade hydrocarbons in soil [23].

Effective microorganisms (EM) is a Japanese organic and sustainable farming technology. It involves introducing a mixture of beneficial microorganisms into the soil to improve plant growth and health. This mixture includes various naturally occurring microorganisms and beneficial species such as photosynthetic bacteria, lactobacilli, yeasts, and Actinomycetes [24-25]. EM has been shown to suppress plant pathogens, enhance soil productivity, reduce compost odors and flies, and boost crop output [26-27]. EM also has environmental management and restoration applications, such as remediating polluted soil, water bodies, wastewater treatment plants, and municipal solid waste leachate [28-31].

EM's potential in bioremediation is highlighted, especially in removing pollutants like heavy metals and pesticides. It has shown efficient removal of organophosphorus pesticides like dimethoate, herbicides like Velpar K, and chlorpyrifos in drinking water [32-35]. However, there is a lack of documentation on using EM microbial consortia in soil bioremediation of organophosphate pesticides, specifically the profenofos. Organophosphate pesticides pose health risks due to their bioaccumulation in the food chain and harm to both humans and various species. Therefore, developing cost-effective and safe bioremediation techniques for removing organophosphate pesticides from the soil is crucial.

The main objective of this study was to determine whether EM could be used to clean up soil contaminated with pesticides. The study used gas chromatography to analyze soil samples for pesticide residues and assessed the ability of EM to break down profenofos (Selecron® 500 EC) within 21 days in a controlled laboratory environment.

Materials and methods

1) Experimental setup, sampling site, and soil collection

This study employed a controlled experimental research design. In the experimental set-up (Figure 1), both the control and experimental groups received Selecron pesticide, but only the experimental group was treated with effective microorganism activated solution (EMAS). Subsequently, the pesticide content in the control and treated groups were analyzed after each scheduled soil harvest.

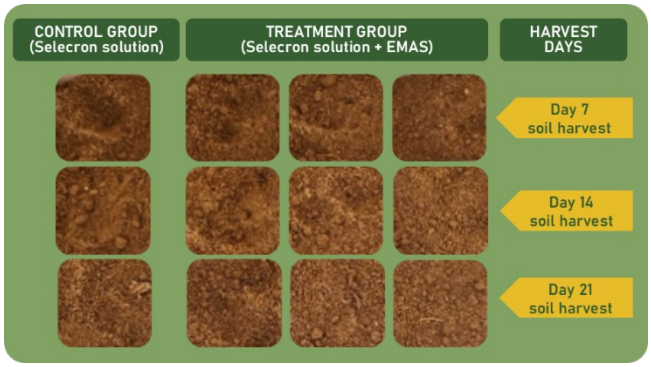

Figure 1 Experimental set-up.

The soil sampling site was a farm (16°49'7.6254" N, 120°49'56.247" E) in Loo, Buguias, Benguet, Philippines (Figure 2), one of the areas where heavy pesticide use occurs, such as organophosphate, carbamate, pyrethroid, etc. The soil sampling was conducted in the second week of April 2022 from 10.00 A.M. to 3.00 P.M. A relative humidity of 84% to 97% and air temperature with a minimum of 17.8 °C and a maximum of 23.7 °C were recorded.

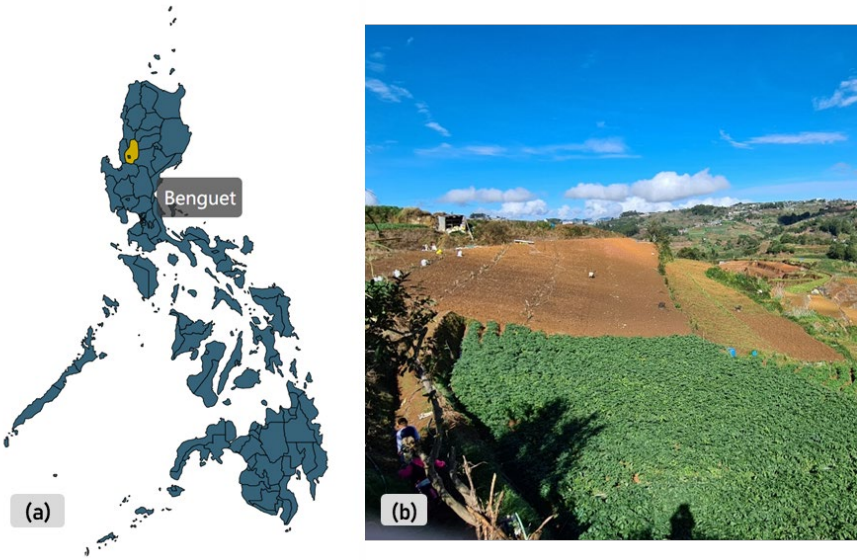

Figure 2 Pictures of (a) Benguet, Philippines as soil sampling site and (b) Farm in Buguias, Benguet, where soil collection was conducted.

Soil collection followed a randomized procedure, with samples taken from various locations within the farm at a depth of 2-6 inches. A total of 15 kg of soil were gathered from the field to create a composite sample. The collected soil was manually cleaned to remove plant components, debris, and rocks. After cleaning, it was sieved, thoroughly mixed, and air-dried for 48 hours. The soil was then reweighed and placed in a clean, sterilized plastic bag, labeled, and taken to the laboratory for the controlled experiment. The soil was divided into 13 sterilized plastic containers containing 1 kg. One of these containers served as a field blank sample containing soil that had not been spiked with pesticides or treated with EMAS. It was analyzed to check for any pesticide residue from standard farming practices. Three containers were designated as the control group, spiked with pesticide solution. Nine containers (three replicates per harvest day) were assigned to the treatment groups, spiked with pesticide solution, and treated with 200 mL of EMAS.

2) Pesticide preparation

Selecron® 500 EC contains the organophosphate profenofos as its active ingredient. It is produced by the global company Syngenta Agrichemical and distributed in the Philippines by Syngenta Philippines, Inc. For this study, Selecron® 500 EC was obtained from a local agricultural store in Baguio City. In the research, a solution was made by mixing 0.3 mL of Selecron® with 100 mL of distilled water, and this pesticide solution was applied to the soil.

3) EMAS preparation

The EM-1® is a product obtained from Harbest Agribusiness Corporation and is distributed by EM Research Philippines, Incorporated. It has a one-year shelf life and requires activation before use. Molasses was added and left to ferment for 7 to 10 days to activate the microorganisms. To create EMAS, 30 mL of molasses was mixed with 1 L of non-chlorinated water, followed by 30 mL of EM-1®. This solution was sealed in a plastic bottle and stored in a dark place for a week, with occasional air release every three days. After 7 to 10 days of fermentation, EMAS was ready for use. In the soil treatment process, 200 mL of EMAS was added to each container.

4) Soil treatment

In the control and treatment groups, 1 kg of soil in a plastic container was spiked with 100 mL pesticide solution. However, the soil in the treatment group was drenched with 200 mL of EMAS. The soil was turned over twice to ensure the pesticide solution, and EMAS were well distributed. Soil sample replicates were treated with the same amount of pesticide solution and EMAS. All treatments were given 100 mL of distilled water every four days to keep the moisture content. The soil was turned over every time water was added to ensure even distribution. Distilled water was used in the experiment to ensure that water was free from impurities, chemicals, pollutants, and potential sources of contamination. The experiment lasted for 21 days in a laboratory room without direct sunlight. The average temperature was 24°C, a pH level of 7.3, and the soil humidity level was 76%.

5) Soil harvest and pesticide analysis

The soil samples were harvested on days 7, 14, and 21 and then submitted for GC analysis to the Satellite Pesticide Analysis Laboratory of the Department of Agriculture - Bureau of Plant Industry (SPAL DA-BPI)

in Guisad, Baguio City, Philippines. The GC system was an Agilent 6890 equipped with a nitrogen-phosphorus detector (NPD)/flame photometric detector (FPD), using helium as the carrier gas. The instrumentation used an autosampler for gas chromatography to analyze pesticide residue from the soil sample.

In the process, a 50g soil sample from the 1 kg profenofos-treated soil underwent two extraction processes: liquid extraction and solid extraction. Liquid extraction used acetone as the solvent. The solid extraction phase was a two-stage clean-up phase that aimed to remove particulates and impurities from the sample. Envi Carb tube was used in the first clean-up stage and the Florisil tube in the second. In gas chromatography instrumentation, two trials were prepared from one sample, and three (3) readings were generated in these two trials. A blank control matrix was used in the laboratory. The data sets generated by the GC in this study followed a linear calibration curve, which indicates good standard calibration and assay performance within a verified analytical range. The raw data produced in the instrumentation were all manually computed following the formula y = mx+b and based on the values given by the calibration graph (Figure 3).

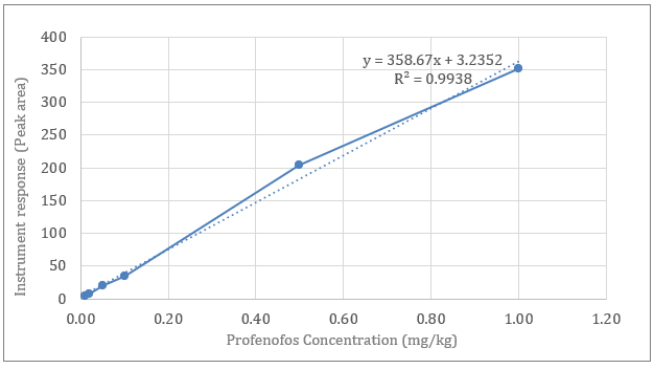

Figure 3 Calibration graph used in quantifying the profenofos.

6) Preparation of sterilized soil

In a separate experiment, 1 kg of soil was sterilized at 121°C for 2 hours using an autoclave, Hirayama Hiclave HV-50 Upright Autoclave Sterilizer, and then oven-dried in a Binder E28 Sterilization Oven at 180°C for 30 min. Another 1 kg of soil was left unsterilized. This procedure took place at the Natural Sciences Research Unit (NSRU) of Saint Louis University (SLU) in Baguio City. Both the sterilized and unsterilized soil samples were treated with the Selecron, EMAS, and analyzed using GC to compare profenofos and EM concentrations between them.

7) Statistical analysis

Descriptive statistics, mean, and standard deviation were used to analyze the GC results. The mean was used to get the average pesticide concentration of the three GC readings per replicate and the overall mean of the three replicates per harvest day. On the other hand, standard deviation (SD) was used to get the variability of the data around the average. Kruskal-Wallis Test and Friedman's Test were the main nonparametric statistical tools used, and the IBM SPSS v.25 statistical program was used. Alternatively, the Statistics Kingdom online statistics calculator was also used to analyze the data.

Ethical considerations

The researcher complied with all Saint Louis University-Research Ethics Committee (SLU-REC), Saint Louis University, Baguio City, Philippines requirements and approvals before performing the experimental setup and data gathering. The proper and safe disposal of microbiological agents, pesticides, and other hazardous wastes prescribed by the Environmental Management Bureau-Department of Environment and Natural Resources (EMB-DENR) was undertaken.

Results and discussion

1) Profenofos content among the soil samples

Table 1 summarizes the results of the 21-day experiment. To ensure the absence of pesticide residue from farm inputs, a blank sample, acting as a negative control, was initially analyzed, and the results showed less than 0.005 mg kg^{-1}, the level of quantification (LOQ). On Day 0, Selecron solution was added to both the control and treatment group soils, resulting in an initial profenofos concentration of 17.2 mg kg^{-1} as determined by gas chromatographic analysis. The profenofos concentration decreased during all the harvest days (Day 7, 14, and 21); however, the decrease occurred not only in the EM-treated soil but also in the untreated (control) soil.

Table 1 Profenofos concentration (mg kg^{-1}) detected in soil with a 7-day soil sampling interval

	Day 0	Day 7	Day 14	Day 21
Blank sample	<0.005	<0.005	<0.005	<0.005
Control group	17.2	4.33	7.02	1.00
Treatment group		*R1 3.86*	*5.47*	*0.65*
		R2 5.72	*8.24*	*0.60*
		R3 6.40	*5.86*	*0.60*
	Mean	5.33	6.52	0.61

Note: R1, R2, R3 refer to replicates 1, 2, and 3; values for R1, R2, and R3 are the means of six GC readings (three readings for two samples of each replicate soil)

1.1) Day 7 harvest

The mean concentration of profenofos after seven days had decreased significantly: 76.4% for R1, 66.7% for R2, and 62.8% for R3. However, there was also a 74.8% decrease in the control group, which was higher than in R2 and R3. The Kruskal-Wallis H test indicated that there is a significant difference in the profenofos concentration between the different groups, $\chi^2(3)= 21.17$, $p<0.001$ with a mean rank score of 9.5 for the control group, 3.5 for R1, 15.83 for R2, 21.17 for R3. The post-hoc Dunn's test using a Bonferroni corrected alpha of 0.0083 indicated that the mean ranks of the following pairs are significantly different; control-R3, R1-R2, R1-R3. This indicates that the concentration of profenofos in R1 is significantly lower than that of R2 and R3 soil samples but not significantly lower than the control group. On the other hand, the concentration of profenofos R3 soil harvested on Day 7 is significantly higher than the control group.

1.2) Day 14 harvest

The other soil samples harvested on Day 14 also showed a remarkable decrease in profenofos concentration: 68.2% for R1, 52.1% for R2, and 65.9% for R3, and the control soil sample showed 59.2%. R1 again showed the highest decrease in profenofos concentration, while R2 had a lower profenofos concentration than the control. The Kruskal-Wallis test indicated a significant difference in the dependent variable between the different groups, $\chi^2(3)=10.18$, $p=0.017$, with a mean rank score of 16.83 for the control group, 7.33 for R1, 17.33 for R2, and 8.5 for R3. This means that the concentration of profenofos in all groups varies significantly.

Comparing the results between Day 7 and 14, except for R3, there was a higher profenofos concentration in Day 14 harvest than in Day 7 for the different groups. The Kruskal-Wallis test indicated that there is a significant difference in the profenofos concentration between the different groups, $\chi^2(7)=35.75$, $p<0.001$, with a mean rank score of 11 for the control-Day 7, 39.67 for control-Day 14, 3.5 for R1-Day 7, 22.5 for R1-Day 14, 21.67 for R2-Day 7, 39.17 for R2-Day 14, 33.83 for R3-Day 7, 24.67 for R3-Day 14. The post-hoc Dunn's test using a Bonferroni corrected alpha of 0.0018 indicated that the mean ranks of the following pairs are significantly different: control-Day 7 and control-Day14; control-Day 7 and R2-Day 14; control-Day 14 and R1-Day 7; R1-Day 7 and R2-Day 14; R1-Day 7 and R3-Day 7. Among the EM-treated groups, R1-Day 7 shows a significantly lower profenofos concentration than the other groups.

1.3) Day 21 harvest

Results on Day 21 show a very notable decrease in the profenofos concentration compared to Day 0: R1 had a 96.2% decrease, 96.5% in both R2 and R3. The control soil sample showed a 94.2% decrease. The Kruskal-Wallis test indicated that there is a significant difference in the profenofos concentration between the different groups, $\chi^2(3)=17.99$, $p<0.001$, with a mean rank score of 21.5 for the control, 14.42 for R1, 5.5 for R2, 8.58 for R3.

The post-hoc Dunn's test using a Bonferroni corrected alpha of 0.0083 indicated that the mean ranks of the following pairs are significantly different: control and R2; control and R3. This implies that the profenofos concentration of R2 and R3 is considerably lower than that of the control soil sample.

Comparing the values in all three harvest days, the Kruskal-Wallis test indicated that there is a significant difference in the profenofos concentration between the different groups, $\chi^2(11)=65.39$, $p<0.001$, with a mean rank score of 35 for control-Day 7, 63.67 for control-Day 14, 21.5 for control-Day 21, 27.5 for R1-Day 7, 46.5 for R1-Day 14, 14.42 for R1-Day 21, 45.67 for R2-Day 7, 63.17 for R2-Day 14, 5.5 for R2-Day 21, 57.83 for R3-Day 7, 48.67 for R3-Day 14, and 8.58 for R3-Day 21. The post-hoc Dunn's test using a Bonferroni corrected alpha of 0.00076 indicated that the mean ranks of the following pairs are significantly different: control-Day 14 and control-Day 21, control-Day 14 and R1-Day 21, control-Day 14 and R2-Day 21 R3-Day 21; R1-Day 14 and R2-Day 21; R2-Day 14 and control-Day 2, R2-Day 14 and R1-Day 21, R2-Day 14 and R2-Day 21, R2-Day 14 and R3-Day 21; R3-Day 7 and all replicates of Day 21; R4-Day 14 and R2-Day 21. Notably, the significant difference in the profenofos concentration occurs between the readings on Day 21 and some of the readings on Days 7 and 14, both in the EM-treated and the control groups.

Overall, results show a decrease in profenofos concentration during the three harvest days in the EM-treated groups compared to Day 0. Profenofos degraded substantially the longer the exposure to effective micro-organisms as indicated by the results in the Day 21 harvest compared to Day 7 and Day 14. The higher readings obtained on Day 14 than on Day 7 may be attributed to unequal distribution or activation of the EMs in the soil and the quantity of pesticide available for the pesticide-degrading microorganisms. Sampling was done on the same soil replicate, and the Kruskal-Wallis test result did not show a significant difference in the readings between soil harvest on Day 7 and Day 14 of the same soil replicate, i.e. profenofos concentration of R1-Day 7 did not vary significantly from the profenofos concentration of R1-Day 14, R2-Day 14 with R2-Day 14, and R3-Day 7 with R3-Day 14. Additionally, this fluctuation

could be influenced by the physiological status of the microorganisms, the survival and proliferation of pesticide-degrading microorganisms, and the sustainable population of these microorganisms [41].

The other consideration, however, is the considerable reduction in profenofos concentration in the control group, although some treated groups showed a significantly higher decline in the pesticide concentration. To some degree, this can be attributed to the natural dissipation process of pesticides. For instance, it was found that profenofos has a half-life of 3.75 days in paddy soil in an open field over 21 days [36]. Another report states that profenofos residues persist in soil for 10 to 15 days and degrade with a half-life of 2.2 to 5.4 days in the field [37]. This natural dissipation could explain the considerable lowering in profenofos concentration observed in the control including the EM-treated soils, particularly on day 21. Although no specific literature regarding the half-life of profenofos in laboratory settings is available, this factor should still be considered in the present study. It is important to note that the experiment was set up in a shaded laboratory area, minimizing dissipation through photolysis and wash-off. Furthermore, the dissipation of profenofos in this study appears to be influenced by temperature-related volatilization. Profenofos dissipation rates can vary widely due to environmental conditions and factors such as sunlight, temperature, pH, hydrolysis, and wash-off. Research has shown that a humid tropical climate, like the one where this study was conducted, can lead to a faster pesticide dissipation rate compared to regions with lower temperatures, such as temperate and subtropical areas. The optimal temperature observed was 34.59 °C to achieve maximal degradation of profenofos (93.39%) [38] and 32.94 °C [39]. This study's recorded average temperature was 24°C, suggesting a slower dissipation rate.

Furthermore, a biphasic degradation pattern was observed, where the most rapid degradation occurred in the first seven days in both the control and treatment groups (74.83% and 69.01%). Subsequently, the degradation rate slowed from days 8 to 14 (45.45% and 45.07%), and the slowest degradation rate was seen in days 15 to 21 (37.68% and 39.93%). A report noted the same biphasic dissipation pattern for pesticides, specifically, a faster dissipation occurred in phase I (0–3 days), followed by a slower dissipation rate in phase II (3–22 days) [40].

2) Profenofos content between sterilized and unsterilized soil

A separate experiment was conducted to determine whether natural soil microorganisms played a role in profenofos degradation. One soil group was sterilized sufficiently to eliminate microorganisms present in them; another was not sterilized. Both soil samples were then treated with identical EM and pesticide solution concentrations. The profenofos concentrations in both sterilized and unsterilized soil samples are presented in Table 2. The results from the gas chromatography analysis revealed that the unsterilized soil had a lower profenofos concentration at 12.44 mg kg^{-1} compared to the sterilized soil with a concentration of 12.74 mg kg^{-1}; however, there was no significant difference in the profenofos concentration between the sterilized and unsterilized soil samples.

Aside from the natural dissipation of pesticide, the lack of a significant difference in the profenofos concentration in this test suggests that the microbial content of EM may have contributed to the observed results. Accordingly, there are 80 species of microorganisms in the EM, including some of the named species of bacteria and fungi: *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Saccharomyces cerevisiae*, *Candida utilis*, *Actinomycetes*, *Streptomyces albus*, *Streptomyces griseus*, *Aspergillus oryzae* and *Mucor hiemalis* [41].

Lactic acid bacteria such as *L. plantarum* [42] was found to degrade organophosphorus pesticides significantly in a short time. *R. palustris* strain has shown a capacity to effectively degrade pyrethroids [43], while complete degradation of cyhalofop-butyl after 5 days [44]. Removal of organophosphorus using *R. sphaeroides* in the treated wastewater and soil was reported, the removal reached 100% after 5 days (1,500 mg L^{-1}) [45], and strains of Aspergillus as organophosphate degrader [46]. Further, EM cultures increased the number of fermentative bacteria, Enterobacter, and the starch-digesting bacteria, *Azotobacter* and *Clostridia*, in soil [47]. A number of these bacteria are fermentative. The different bacterial strains possibly collaborate to resist stressful conditions caused by a harmful pollutant and its byproducts while using profenofos as a nutrient source.

Table 2 Profenofos Concentration (mg kg^{-1}) in Sterilized and Unsterilized Soil

Soil type	Profenofos concentration	Mean	Standard deviation	Standard error of mean
Sterilized soil	12.74 mg kg^{-1}	11.0683	3.92904	1.60402
Unsterilized soil	12. 44 mg kg^{-1}	12.4433	1.00889	0.41188

Note: F-test: p=0.057412, no significant difference

These bacteria possess specific genes, such as opd, mpd, and phn operon, that code for enzymes involved in pesticide degradation. These enzymes, such as organophosphate hydrolase (OPH), methyl parathion hydrolase (MPH), and C-P lyases, are responsible for breaking down organophosphates into non-toxic components [48]. The C-P lyase enzyme primarily facilitates the C-P bond cleavage, a critical step in organophosphate degradation. This enzyme enables the breakdown of carbon-phosphorus (C-P) bonds in organophosphorus compounds, a process often challenging to achieve through conventional degradation methods.

Conclusion

This study demonstrates the degradation of profenofos in the soil. After a 21-day experimental duration of applying EM, there was a significant difference in profenofos concentration between the control and treatment groups; the profenofos concentration between the sterilized and unsterilized soil samples showed no significant difference. This study suggests that microorganisms present in the EM contributed to the degradation of profenofos pesticide in the soil. However, the pesticide's half-life may have contributed largely to the results since the pesticide also decreased even in the untreated soil.

Recommendations

Subsequent studies need to be done to confirm the results of the study. The authors suggest conducting a study on the natural dissipation of pesticides used in Buguias, Benguet, Philippines under farm conditions, either in situ or under controlled and actual farm conditions for comparison.

Financial constraints prevented the researcher from conducting more trials. However, the study provides initial data on the possible use of EMs to degrade pesticides applied in agricultural soil. The importance of natural soil microbiota also should not be taken lightly since some natural soil microbes can degrade pesticides; hence, farmers should adhere to good practices that maintain soil health and microbiota. More importantly, if pesticide use cannot be avoided, farmers should follow recommended pesticide application intervals allowing time for pesticide natural dissipation before the next application.

Acknowledgment

My heartfelt thanks to the laboratory technicians of SPAL, Department of Agriculture, NSRU, Saint Louis University, Department of Biology, Benguet State University, and Mr. Wilson Laranang, MSMT of the IRTMC, San Fernando, for their technical assistance with the laboratory work needed in this research. I would also like to thank Ms. Merlyn Contic and Ms. Rosana Tuguinay for assisting with the laboratory work and statistical analysis.

Statement of Conflict of Interest

The researchers declare no conflict of interest in the conduct of this study.

References

[1] WHO. The WHO recommended classification of pesticides by hazard and guidelines to classification. World Health Organization, Geneva, 2009. ISBN: 9789240005662.

[2] USEPA. Profenofos facts. USEPA, Office of Prevention, Pesticides, and Toxic Substances. 2000. Washington, DC.

[3] Kushwaha, M., Verma, S., Chatterjee, S. Profenofos, An acetylcholinesterase-inhibiting organophosphorus pesticide: A short review of its usage, toxicity, and biodegradation. Journal of Environmental Quality, 2016, 45, 1478–1489.

[4] Kaur, H., Garg, H. Pesticides: Environmental impacts and management strategies. Pesticides - Toxic Aspects, IntechOpen, 2014.

[5] Phopin, K., Ruankham, W., Prachayasittikul, S., Prachayasittikul, V., Tantimongcolwat, T. Revealing the mechanistic interactions of profenofos and captan pesticides with serum protein via biophysical and computational investigations. Scientific Reports, 2024, 14, 1788.

[6] Greish, S., Ismail, S. M., Mosleh, Y., Loutfy, N., Dessouki, A.A., Ahmed, M.T. Human risk assessment of profenofos: A case study in Ismailia, Egypt. Polycyclic Aromatic Compounds, 2011, 31, 1, 28–47.

[7] Harnpicharnchai, K., Chaiear, N., Charerntanyarak, L. Residues of organophos-phate pesticides used in vegetable cultivation in ambient air, surface water and soil in Bueng Niam Subdistrict, Khon-Kaen, Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health, 2013, 44, 1088–1097.

[8] Nillos, M.G., Rodriguez-Fuentes, G., Gan, J., Schlenk, D. Enantioselective acetyl-cholinesterase inhibition of the organophosphorous insecticides profenofos, fonofos, and crotoxyphos. Environmental Toxicology and Chemistry, 2007, 26, 1949–1954.

[9] Mahboob, S., Niazi, F., Sultana, S., Ahmad, Z. Assessment of pesticide residues in water, sediments and muscles of Cyprinus carpio from

Head Balloki in the River Ravi. Life Science Journal, 2013, 10, 32–38.

[10] Kapeleka, J.A., Sauli, E., Sadik, O., Ndakidemi, P.A. Co-exposure risks of pesticides residues and bacterial contamination in fresh fruits and vegetables under smallholder horticultural production systems in Tanzania. PLoS ONE, 2020, 15, 7.

[11] Loha, K.M., Lamoree, M., de Boer, J. Pesticide residue levels in vegetables and surface waters at the Central Rift Valley (CRV) of Ethiopia. Environmental Monitoring and Assessment, 2020, 192, 546.

[12] Omeje, J.S., Asegbeloyin, J.N., Ihedioha, J.N. Ekere, N.R., Ochonogor, A.E., Abugu, H.O., Alum, O.L. Monitoring of pesticide residues in fresh fruits and vegetables available in Nigerian markets and assessment of their associated health risks. Environmental Monitoring and Assessment, 20220, 194, 516.

[13] Diop, A., Diop, Y.M., Thiare, D.D., Cazier, F., Sarr, S.O., Kasprowiak, A., Landy, D., Delattre, F. Monitoring survey of the use patterns and pesticide residues on vegetables in the Niayes zone, Senegal. Chemosphere, 2016, 144, 1715–1721.

[14] Del Prado-Lu, J.L. Insecticide residues in soil, water, and eggplant fruits and farmers' health effects due to exposure to pesticides. Environmental Health and Preventive Medicine, 2015, 20, 1, 53–62.

[15] Lu, J.L. Analysis of trends of the types of pesticide used, residues and related factors among farmers in the largest vegetable producing area in the Philippines. Journal of Rural Medicine, 2010, 5, 2, 184–189.

[16] Lu, J.L.D.P. Multipesticide Residue assessment of agricultural soil and water in major farming areas in Benguet, Philippines. Archives of Environmental Contamination and Toxicology, 2010, 59, 175–181.

[17] Reyes, G.A., Laurean, C.P. A comprehensive assessment of pesticide residues in two vegetable-farming municipalities in Benguet. Northern Luzon Research Journal, 2007, 1, 39–54.

[18] Lu J.L. Occupational safety of farmers in the vegetable industry. International Journal of Occupational Safety and Ergonomics, 2011, 17, 4, 445–453.

[19] Ngidlo, R.T. Impacts of pesticides and fertilizers on soil, tail water and groundwater in three vegetable producing areas in the Cordillera Region, Northern Philippines. American Journal of Experimental Agriculture, 2013, 3(4), 780–793.

[20] Sayed, K., Baloo, L., Sharma, N.K. Bioremediation of total petroleum hydrocarbons (TPH) by bio-augmentation and biostimulation in water with floating oil spill containment booms as bioreactor Basin. International Journal of Environmental Research and Public Health, 2021, 18, 5, 2226.

[21] Wuana, R.A., Okieimen, F.E. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Network Ecology, 2011.

[22] Shamim, S. Biosorption of heavy metals. IntechOpen, 2018. [Online] Available from: https://www.intechopen.com/chapters/58112.

[23] Deshmukh, R., Khardenavis, A.A., Purohit, H.J. Diverse metabolic capacities of fungi for bioremediation. Indian Journal of Microbiology, 2016, 56, 3, 247–264.

[24] Javaid, A. Beneficial microorganisms for sustainable agriculture. Sustainable Agriculture Reviews, 2010, 347–369.

[25] Safwat, S.M., Matta, M.E. Environmental applications of effective microorganisms: A review of current knowledge and recommendations for future directions. Journal of Engineering and Applied Science 2021, 68, 48.

[26] Higa, T., Wididana, G.N. The concept and theories of effective microorganisms. University of Ryukyus, Okinawa, 2004.

[27] Iriti, M., Scarafoni, A., Pierce, S., Castorina, G., Vitalini, S. Soil application of effective microorganisms (EM) maintains leaf photosynthetic efficiency, increases seed yield and quality traits of bean (*Phaseolus vulgaris* L.) Plants Grown on Different Substrates. International Journal of Molecular Sciences, 2019, 20, 2327.

[28] Ngilangil, L.E., Vilar, D.A. Effective microorganisms as remediation for marginal soil in the Philippines. Chemical Engineering Transactions, 2020, 78, 253–258.

[29] Kannan, D., Kumar, S.V. Effective microorganisms are used in the domestic effluent treatment system. Balkan Water Observation and Information System, 2012.

[30] Lavanya, V., Kannan, D. Effective microorganisms used in domestic effluent treatment system. Journal of Applied Sciences, 2019, 19, 3, 188–198.

[31] Sree, K.D.S., Dash, S.N., Leelavathi, A. Biological remediation of the municipal solid waste leachate - A case study of Hyderabad integrated MSW limited. Nature Environment and Pollution Technology, 2020, 19, 1, 379–383.

[32] Abdel-Megeed, A., El-Nakieb, F.A. Bioremediation of pesticides in soil will significantly help reduce pollutants and remove residues that vegetables can absorb. Terrestrial and Aquatic Environmental Toxicology, 2008, 2, 1, 1–4.

[33] Ramos, M.A.G., Yoshioka, S.A. Bioremediation of herbicide velpar K® in vitro in aqueous solution with application of EM-4 (effective microorganisms). Brazilian Archives of Biology and Technology, 2012, 55, 1, 145–149.

[34] Fernandez-Lopez, M. G., Popoca-Ursino, C., Sanchez-Salinas, E., Tinoco-Valencia, R., Folch-Mallol, J.L., Dantan-Gonzalez, E., Laura Ortiz-Hernandez, M. Enhancing methyl parathion degradation by the immobilization of Burkholderia sp. isolated from agricultural soils. Microbiology Open, 2017, 6, 5.

[35] Ali, D., Nagpure, N.S., Kumar, S., Kumar, R., Kushwaha, B., Lakra, W.S. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish Channa punctatus (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food and Chemical Toxicology, 2009, 47, 3, 650–656.

[36] He, J., Fan, M., Liu, X. Environmental behavior of profenofos under paddy field conditions. Bulletin of Environmental Contamination and Toxicology, 2010, 84, 6, 771–774.

[37] Gupta, S., Gajbhiye, V.T., Sharma, R.K., Gupta, R.K. Dissipation of cypermethrin, chlorpyriphos, and profenofos in tomato fruits and soil following application of pre-mix formulations. Environmental Monitoring and Assessment, 2010, 174, 1–4.

[38] Jabeen, H., Iqbal, S., Anwar, S., Parales, R.E. Optimization of profenofos degradation by a novel bacterial consortium PBAC using response surface methodology. International Biodeterioration & Biodegradation, 2015, 100, 89–97.

[39] Ploykuhanl, C., Vangnai, A.S., Wantala, K., Siripattanakul-Ratpukdi, S. Characterization of profenofos degradation by Pseudomonas plecoglossicida strain PF1 using surface response methodology. Desalination and Water Treatment, 2017, 89, 142–149.

[40] Henry, M.C. The dissipation of profenofos, λ-cyhalothrin, and chlorothalonil in vegetables and soil under humid tropical conditions. Masters thesis, Universiti Malaysia Sarawak, (UNIMAS), 2015. [Online] Available from: https://ir.unimas.my/id/eprint/10945/

[41] Xu, H.L. Effects of a microbial inoculant and organic fertilizers on the growth, photosynthesis and yield of sweet corn. Journal of Crop Production, 2001, 3, 1, 183–214.

[42] Yuan, S., Li, C., Yu, H., Xie, Y., Guo, Y., Yao, W. Screening of lactic acid bacteria for degrading organophosphorus pesticides and their potential protective effects against pesticide toxicity. LWT, 2021, 147, 111672.

[43] Zhang, S., Luo, X., Peng, J., Zhang, D., Liu, Y. Genome sequence of pyrethroid-degrading bacterium Rhodopseudomonas palustris strain JSC-3b. Genome Announcements, 2014, 2, 1.

[44] Li, M., Ning, P., Sun, Y., Luo, J., Yang, J. Characteristics and application of Rhodopseudomonas palustris as a microbial cell factory. Frontiers in Bioengineering and Biotechnology, 2022, 10, 897003.

[45] Wu, P., Zhang, Y., Chen, Z., Wang, Y., Zhu, F., Cao, B., …, Li, N. The organophosphorus pesticides in soil was degradated by Rhodobacter sphaeroides after wastewater treatment. Biochemical Engineering Journal, 2019, 141, 247–251.

[46] Kumar, S., Kaushik, G., Dar, M. A., Nimesh, S., Lypez-Chuken, U.J., Villarreal-Chiu, J.F. Microbial Degradation of organophosphate pesticides: A review. Pedosphere, 2018, 28(2), 190–208.

[47] Xu, Y., Liu, C., Bao, J., Zhu, H, Chen, Y., Luo, Y., Zhang, L. Microbial diversity and physico-chemical properties in farmland soils amended by effective microorganisms and fulvic acid for cropping Asian ginseng. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2020, 50, 1, 12563.

[48] Sirajuddin, S., Khan, M.A., Qader, S.A.U., Iqbal, S., Sattar, H., Ansari, A. A comparative study on degradation of complex malathion organophosphate using Escherichia coli IES-02 and a novel carboxylesterase. International Journal of Biological Macromolecules, 2020, 145, 445–455.