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Evaluation of the Dose-Dependent Effects of Malathion and Diazinon on Soil Bacterial Populations

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Abstract: *Organophosphate insecticides remain important tools in crop protection, yet their entry into soil creates a direct interface between pest-control chemistry and non-target microbial communities. This paper presents a journal-style experimental framework for evaluating the dose-dependent effects of malathion and diazinon on culturable soil bacterial populations. Agricultural topsoil is proposed to be exposed to a control and four graded doses of each pesticide, including an agronomically relevant field-rate treatment and higher multiples designed to characterise the response curve. Viable heterotrophic bacteria are quantified as colony-forming units per gram of dry soil (CFU g^{-1}) at multiple incubation times, with parallel measurement of soil pH, moisture and selected physicochemical variables. The principal statistical approach is a factorial mixed-effects model or, where the design is simpler, two-/three-way ANOVA using pesticide identity, dose and time as fixed effects and experimental replicate as a random effect. Dose trends, effect sizes, confidence intervals and post-hoc contrasts are recommended in addition to null-hypothesis testing. The literature indicates that pesticide effects on soil microbiota are strongly dependent on concentration, soil properties, exposure duration and the capacity of indigenous microorganisms to transform xenobiotics. Recent work on diazinon shows both early reductions in bacterial diversity and later enrichment of taxa associated with degradation, while recent malathion studies demonstrate substantial bacterial biodegradation capacity. The study therefore treats bacterial response not as a simple toxic/non-toxic dichotomy but as a dynamic balance among inhibition, selection, adaptation and biodegradation. Because no primary laboratory dataset was supplied, numerical results are intentionally left as data-entry tables rather than fabricated observations. The framework is suitable for conversion into a complete empirical paper once measured CFU and soil-property data are inserted.*

Keywords: malathion; diazinon; organophosphate pesticides; soil bacteria; dose-response; CFU; soil microbiome; ecotoxicology

I. INTRODUCTION

Soil is not merely a physical substrate for crop production; it is a biologically active system in which bacteria, fungi, archaea, protozoa and soil fauna interact with minerals, organic matter, plant roots and water. Bacteria are especially important because they participate in decomposition, mineralisation, nitrogen transformations, phosphorus mobilisation, production and consumption of greenhouse gases, formation of soil aggregates, suppression of some plant pathogens and degradation of anthropogenic chemicals. Experimental evidence that simplification of below-ground communities impairs multiple ecosystem functions has strengthened the argument that soil biodiversity should be treated as an agricultural resource rather than an incidental property of soil (Wagg et al., 2014)[1].

[1] Wang, X. et al. (2024). Actinomycetota, a central constituent microbe during long-term exposure to diazinon, an organophosphorus insecticide. Chemosphere, 354, 141583. https://doi.org/10.1016/j.chemosphere.2024.141583

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Pesticides are introduced into agroecosystems to protect yield and quality, but only part of an application necessarily remains confined to the target organism or target surface. Spray interception, wash-off, drift, incorporation of treated residues and direct soil application can transfer active ingredients or metabolites into soil. Once present, a pesticide may be adsorbed to mineral or organic surfaces, dissolved in soil water, transformed chemically, taken up by organisms, volatilised, leached or degraded biologically. These processes determine both persistence and bioavailability. Accordingly, the microbial effect of a pesticide cannot be inferred from the nominal dose alone; the biologically available fraction is conditioned by soil texture, pH, organic matter, moisture, temperature and previous exposure history.

Malathion and diazinon are organophosphorus insecticides whose primary pesticidal mode of action is associated with inhibition of acetylcholinesterase in susceptible animals. Their environmental behaviour, however, is broader than this toxicological mechanism. Organophosphate residues may become substrates for microorganisms possessing hydrolytic or other catabolic pathways, while susceptible microorganisms may experience direct or indirect stress. Reviews of organophosphate pollution emphasise the dual role of microbes: they are non-target components potentially affected by pesticide exposure and, at the same time, major agents of pesticide detoxification and bioremediation (Mali et al., 2023)[2].

This duality is particularly important in dose-response experiments. At a low concentration, a pesticide may cause no detectable change in total culturable bacteria, may transiently suppress sensitive taxa, or may even coincide with an increase in a subset of organisms able to exploit pesticide-derived carbon, phosphorus or sulfur. At a higher concentration, toxicity may dominate and total viable counts may decline. Repeated exposure can further select for tolerant or degrading populations. Therefore, a single-dose comparison is inadequate for identifying thresholds, non-linearity or differential sensitivity between compounds.[3]

Diazinon provides a recent example of this ecological selection process. A 2024 soil-microcosm study reported that diazinon exposure reduced bacterial diversity particularly at early sampling points, while Actinomycetota became enriched and represented a large proportion of isolated diazinon-degrading strains; diazinon itself declined markedly during long-term incubation. Such findings show why abundance, diversity and degradation should be conceptually separated: a soil may retain or recover total bacterial numbers while its community composition has changed substantially.

Malathion likewise has a substantial microbial-degradation literature. Bacterial isolates and consortia have been reported to transform malathion, and a 2023 soil study demonstrated stronger degradation by a three-member indigenous bacterial consortium than by individual strains or two-member combinations. Earlier work also established that malathion loss can involve both abiotic and microbial processes, making careful controls and interpretation necessary. A modern dose-response study should therefore distinguish a change in bacterial population from pesticide disappearance and, where possible, measure both.

The present paper develops a rigorous framework to evaluate the dose-dependent effects of malathion and diazinon on soil bacterial populations. The central response variable is viable heterotrophic bacterial abundance expressed as CFU g^{-1} dry soil. The design incorporates graded pesticide doses and repeated sampling so that immediate inhibition, delayed effects and recovery can be examined. The study also proposes statistical methods that explicitly test dose, pesticide identity and time, rather than relying on descriptive comparison of plate counts.

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[2] Mali, H., Shah, C., Raghunandan, B. H., Prajapati, A. S., Patel, D. H., Trivedi, U., & Subramanian, R. B. (2023). Organophosphate pesticides an emerging environmental contaminant: Pollution, toxicity, bioremediation progress, and remaining challenges. Journal of Environmental Sciences, 127,

[3] Konrad, J. G., Chesters, G., & Armstrong, D. E. (1969). Soil degradation of malathion, a phosphorodithioate insecticide. Soil Science Society of America Journal, 33, 259–262. https://doi.org/10.2136/sssaj1969.03615995003300020026x

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The practical importance of the study lies in the relationship between pesticide stewardship and soil health. Recommended pesticide use is based primarily on efficacy, residue, crop-safety and toxicological considerations, but soil microbial endpoints provide complementary information about non-target ecological effects. Demonstrating that bacterial populations are stable at an agronomically relevant dose but inhibited at excessive doses would support adherence to label rates and avoidance of over-application. Conversely, measurable inhibition at realistic exposure levels would justify more detailed field and molecular investigations.

II. LITERATURE REVIEW

2.1 Soil bacterial communities as indicators of soil health

Soil bacterial abundance is an accessible biological endpoint, but it should be interpreted in the context of microbial ecology. Culture-based counts measure only organisms capable of forming colonies under the selected medium and incubation conditions. They therefore do not represent total bacterial richness. Nevertheless, CFU measurements remain useful for controlled comparative experiments because they provide a direct measure of viable culturable organisms and can be repeated economically across many treatments and time points. Their value increases when the same protocol is applied consistently and when results are complemented by functional or molecular indicators.

The broader literature has increasingly shifted from a single microbial endpoint toward multi-indicator assessment. A recent meta-analytic approach to pesticide effects on soil health considered bacterial and fungal CFU alongside respiration, enzymes and functional genes, illustrating that microbial abundance is one component of an ecotoxicological evidence set. Systematic reviews likewise show substantial heterogeneity among pesticide studies because formulations, doses, soils, durations and microbial methods differ. This methodological diversity explains why apparently contradictory pesticide effects can coexist in the literature.

2.2 Mechanisms by which pesticides alter soil bacteria

Pesticides can alter bacterial populations through direct toxicity, substrate effects and indirect ecological pathways. Direct effects may include disruption of membranes, enzyme systems, oxidative balance or energy metabolism. Indirect effects can arise when pesticides alter plant inputs, nutrient availability, fungal competitors or predators. A compound or metabolite may also become a carbon or phosphorus source for specialised degraders, producing enrichment rather than inhibition. Thus, a rise in total CFU after treatment cannot automatically be interpreted as improved soil health; it may represent selective proliferation of a narrow group.

Dose is central to these mechanisms. Toxicological effects commonly strengthen as exposure increases, but microbial systems may display threshold behaviour, adaptation, hormesis-like low-dose stimulation or non-monotonic responses. Soil sorption can also make nominally equal doses biologically unequal across soils. For this reason, experimental reporting should state the active ingredient concentration per mass of dry soil, formulation details, soil moisture and physicochemical characteristics rather than reporting only a volume of commercial product.

2.3 Environmental behaviour of organophosphate pesticides

Organophosphorus pesticides are chemically diverse but share the presence of phosphorus-containing functional groups. Environmental reviews describe hydrolysis, oxidation, photolysis and microbial transformation as important loss pathways. The relative contribution of these processes varies among compounds and matrices. Microbial degradation is especially relevant in biologically active soil, where bacteria and fungi may possess phosphotriesterases, hydrolases, esterases, phosphatases and other enzymes capable of transforming organophosphate structures.

Recent reviews emphasise bioremediation because pesticide-tolerant microbial consortia can combine degradation with plant-growth-promoting functions. However, the existence of degraders does not eliminate ecotoxicological concern. Selection for a degrader can occur simultaneously with loss of sensitive taxa, and metabolites can have different toxicity from the parent compound. Consequently, microbial degradation and microbial community health should be measured as related but distinct outcomes.

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2.4 Malathion: persistence, transformation and bacterial degradation

Malathion is a phosphorodithioate insecticide containing carboxylic ester groups that can undergo hydrolysis. Classical soil work showed rapid degradation in some soils and demonstrated that abiotic processes can be important, while later microbiological studies identified bacteria able to use or transform malathion. This history is methodologically important: a reduction in malathion concentration in a non-sterile soil cannot be attributed solely to bacterial metabolism unless appropriate sterile or abiotic controls are included.

Modern studies provide stronger organism-level evidence. Verma, Singh and Chatterjee (2021)[4] described an Ochrobactrum strain isolated from orchard soil that could utilise malathion and identified degradation metabolites. Ma et al. (2022)[5] isolated efficient malathion-degrading bacteria from hydrothermal sediment, illustrating the wide distribution of catabolic potential. Dar and Kaushik (2023)[6] evaluated indigenous bacterial strains and consortia in amended soil and reported that a three-species consortium achieved more complete removal than individual strains. These studies support the expectation that pesticide exposure can select organisms with specialised degradation capacities.

A 2025 review of biotic and abiotic malathion degradation further highlights the need to integrate microbial and physicochemical mechanisms. From the perspective of the present study, this means that bacterial CFU should not be interpreted as a direct surrogate for malathion persistence. Ideally, residue analysis by chromatographic methods should accompany microbial enumeration, but where resources are limited, the paper should explicitly state that it evaluates bacterial response to nominal applied dose rather than measured internal exposure.

2.5 Diazinon: microbial selection and degradation

Diazinon has been investigated both as an environmental contaminant and as a substrate for microbial remediation. Reviews have identified bacteria and fungi capable of diazinon transformation and have described enzymatic systems implicated in its degradation. Soil moisture, organic matter and porosity can influence persistence, while microbial adaptation may accelerate disappearance after repeated exposure.

Particularly relevant is the 2024 Chemosphere study examining long-term diazinon exposure in soil. The authors observed strong diazinon degradation over time and significant early reductions in bacterial diversity, together with enrichment of Actinomycetota. More than 70% of the diazinon-degrading isolates obtained in that study belonged to this phylum. The result demonstrates that pesticide exposure can function as a selective ecological filter: total community structure changes while organisms with useful catabolic traits become prominent.

Mansouri and Rahnavard (2025) [7]subsequently reported diazinon degradation by bacterial isolates including Pseudomonas oryzihabitans, Enterobacter kobei and Serratia spp., with degradation performance varying by organism, medium and starting concentration. Such concentration-dependent biodegradation reinforces the need for graded-dose experiments rather than binary treated/untreated designs.

2.6 Research gap and rationale

Despite extensive literature on pesticide degradation, many studies focus on isolation of efficient degraders, remediation performance or single pesticide concentrations. Fewer studies are designed specifically to compare

[4] Verma, S., Singh, D., & Chatterjee, S. (2021). Malathion biodegradation by a psychrotolerant bacteria Ochrobactrum sp. M1D and metabolic pathway analysis. Letters in Applied Microbiology, 73(3), 326–335. https://doi.org/10.1111/lam.13517

[5] Ma, L., Dai, X., Ai, G., Zheng, X., Zhang, Y., Pan, C., Hu, M., Jiang, C., Wang, L., & Dong, Z. (2022). Isolation and identification of efficient malathion-degrading bacteria from deep-sea hydrothermal sediment. Microorganisms, 10(9), 1797. https://doi.org/10.3390/microorganisms10091797

[6] Dar, M. A., & Kaushik, G. (2023). Biodegradation of malathion in amended soil by indigenous novel bacterial consortia and analysis of degradation pathway. Soil Systems, 7(4), 81. https://doi.org/10.3390/soilsystems7040081

[7] Mansouri, M., & Rahnavard, A. (2025). Biodegradation of the pesticide diazinon by bacteria isolated from a contaminated soil. CLEAN – Soil, Air, Water, 53(4), e70012. https://doi.org/10.1002/clen.70012

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malathion and diazinon across the same graded-dose series in the same soil while tracking viable bacterial abundance over time. Cross-study comparisons are weak because soil properties, formulations, exposure units and microbial methods differ.[8]

The present framework addresses this gap by holding the soil matrix and incubation conditions constant while varying pesticide identity and dose. Repeated sampling allows separation of acute suppression from recovery or adaptation. A factorial statistical model then tests whether the slope or pattern of the dose response differs between malathion and diazinon. This interaction is scientifically more informative than asking only whether each pesticide differs from control.

III. AIM, OBJECTIVES AND HYPOTHESES

Aim:

- To evaluate and compare the dose-dependent effects of malathion and diazinon on viable soil bacterial populations under controlled soil-microcosm conditions.
- Determine baseline culturable bacterial abundance in untreated agricultural soil.
- Quantify bacterial abundance after exposure to graded malathion doses.
- Quantify bacterial abundance after exposure to graded diazinon doses.
- Determine whether dose-response relationships are linear, threshold-like or otherwise non-linear.
- Compare malathion and diazinon at corresponding dose categories and test pesticide × dose interaction.
- Examine whether bacterial populations recover, remain suppressed or become enriched during incubation.
- Relate microbial responses to measured soil properties and, where available, pesticide-residue data.

Primary null hypothesis (H_0): bacterial abundance does not differ among pesticide doses after accounting for pesticide identity and sampling time. Primary alternative hypothesis (H_1): bacterial abundance changes significantly with dose. A second null hypothesis states that the dose-response pattern is the same for malathion and diazinon; its alternative predicts a significant pesticide × dose interaction. For repeated sampling, a third hypothesis tests whether treatment effects vary with time.[9]

IV. MATERIALS AND METHODS

4.1 Study design

A controlled soil-microcosm experiment is recommended. One homogenised agricultural soil batch should be divided among experimental units to minimise background variability. The minimum design comprises an untreated control plus four graded doses for each pesticide. A scientifically defensible dose series is 0, 0.5×, 1×, 2× and 4× the field-equivalent rate, converted to mg active ingredient kg^{-1} dry soil. The exact conversion must be calculated from the product label, application rate, assumed soil incorporation depth and soil bulk density; these values should be reported rather than guessed. At least three independent microcosm replicates per treatment are recommended, with four or five preferred where resources permit.[10]

[8] Cycoń, M., Mrozik, A., & Piotrowska-Seget, Z. (2017). Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. Chemosphere, 172, 52–71. https://doi.org/10.1016/j.chemosphere.2016.12.129

[9] Fenner, K., Canonica, S., Wackett, L. P., & Elsner, M. (2013). Evaluating pesticide degradation in the environment: Blind spots and emerging opportunities. Science, 341, 752–758. https://doi.org/10.1126/science.1236281

[10] Imfeld, G., & Vuilleumier, S. (2012). Measuring the effects of pesticides on bacterial communities in soil: A critical review. European Journal of Soil Biology, 49, 22–30. https://doi.org/10.1016/j.ejsobi.2011.11.010

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4.2 Soil collection and characterisation

Collect surface soil (approximately 0–15 cm) from a representative agricultural site with documented cropping and pesticide history. Obtain multiple subsamples using a zig-zag or stratified pattern and combine them into a composite sample. Remove stones and coarse plant residues without sterilising the soil. Determine moisture content so that pesticide dose and CFU can be expressed on a dry-mass basis. Record pH, electrical conductivity, organic carbon or organic matter, texture and, where feasible, available N, P and K. Prior pesticide exposure should be documented because adapted microbial communities may respond differently from naïve soils.[11]

4.3 Preparation of pesticide treatments

Use analytical-grade active ingredients where the objective is toxicological comparison, or clearly identified commercial formulations where the objective is agricultural realism. Do not mix these interpretations. Prepare stock solutions using a solvent compatible with the compound and microbiological endpoint. If a carrier solvent is required, include a solvent control at the same final concentration. Apply treatment uniformly to soil and mix thoroughly while avoiding excessive disturbance. Adjust all microcosms to the same target moisture content, preferably a defined percentage of water-holding capacity.[12]

4.4 Incubation and sampling

Incubate microcosms under controlled temperature and moisture. A practical sampling schedule is day 0 (after equilibration/application), 7, 14, 21 and 28; a longer endpoint such as day 56 can be added to examine recovery. If destructive sampling is used, prepare separate microcosms for each time point. If repeated subsampling is used, remove only a small, standardised mass and account for disturbance. Randomise microcosm positions during incubation to minimise positional effects.[13]

4.5 Enumeration of viable heterotrophic bacteria

For each sample, aseptically suspend a known mass of soil in sterile diluent and prepare serial ten-fold dilutions. Plate appropriate dilutions on a general-purpose medium suitable for heterotrophic soil bacteria. Use replicate plates and count only plates within the laboratory's validated countable range. Express abundance as CFU g^{-1} dry soil. The calculation is based on colony count, dilution, plated volume and dry-mass correction. Because microbial counts are typically right-skewed and span orders of magnitude, analyse $\log_{10}$(CFU g^{-1}) unless model diagnostics support analysis on the original scale.[14]

4.6 Optional complementary endpoints

Where facilities permit, strengthen the study by measuring soil dehydrogenase activity, microbial respiration, microbial biomass carbon and selected nutrient-cycling enzymes. Molecular analysis using 16S rRNA gene amplicon sequencing can reveal changes in community composition that CFU cannot detect. Pesticide residues can be quantified by validated chromatographic methods to estimate degradation kinetics and distinguish nominal dose from actual exposure.

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[11] Johnsen, K., Jacobsen, C. S., Torsvik, V., & Sørensen, J. (2001). Pesticide effects on bacterial diversity in agricultural soils—A review. Biology and Fertility of Soils, 33, 443–453. https://doi.org/10.1007/s003740100351

[12] Kumar, S. S., Ghosh, P., Malyan, S. K., Sharma, J., & Kumar, V. (2019). A comprehensive review on enzymatic degradation of the organophosphate pesticide malathion in the environment. Journal of Environmental Science and Health, Part C, 37(4), 288–329. https://doi.org/10.1080/10590501.2019.1654809

[13] Konrad, J. G., Chesters, G., & Armstrong, D. E. (1969). Soil degradation of malathion, a phosphorodithioate insecticide. Soil Science Society of America Journal, 33, 259–262. https://doi.org/10.2136/sssaj1969.03615995003300020026x

[14] Riah, W., Laval, K., Laroche-Ajzenberg, E., Mougin, C., Latour, X., & Trinsoutrot-Gattin, I. (2014). Effects of pesticides on soil enzymes: A review. Environmental Chemistry Letters, 12, 257–273. https://doi.org/10.1007/s10311-014-0458-2

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4.7 Quality assurance

Include media sterility controls, dilution blanks, duplicate or triplicate plating, calibrated pipettes and documented incubation conditions. Randomise plating order where possible. Record colonies independently or verify a subset of counts by a second observer. For residue analysis, include recovery spikes, blanks and calibration standards. Predefine exclusion criteria, for example contamination or uncountable plates, before examining treatment effects.[15]

4.8 Experimental treatment matrix

Code	Pesticide	Dose category	Recommended expression	Replicates
C	None	Control	0 mg a.i. kg^{-1} dry soil	≥3
M0.5	Malathion	Low	0.5× field-equivalent	≥3
M1	Malathion	Field rate	1× field-equivalent	≥3
M2	Malathion	High	2× field-equivalent	≥3
M4	Malathion	Very high	4× field-equivalent	≥3
D0.5	Diazinon	Low	0.5× field-equivalent	≥3
D1	Diazinon	Field rate	1× field-equivalent	≥3
D2	Diazinon	High	2× field-equivalent	≥3
D4	Diazinon	Very high	4× field-equivalent	≥3

V. STATISTICAL ANALYSIS FRAMEWORK

The experimental unit, not the individual agar plate, is the unit of inference. Technical replicate plates should first be combined to obtain one bacterial-abundance estimate for each independent soil microcosm at each time point. Treating replicate plates as independent biological observations would constitute pseudoreplication and artificially inflate statistical power.[16]

The preferred response variable is $\log_{10}$ CFU g^{-1} dry soil. For a design with repeated observations from the same microcosm, fit a linear mixed-effects model with pesticide, dose, time and their interactions as fixed effects and microcosm identity as a random intercept. If each time point uses independent destructive microcosms, a factorial ANOVA/general linear model can be used. The model should explicitly test pesticide × dose, dose × time and pesticide × dose × time interactions. A significant pesticide × dose interaction indicates that the dose-response relationship differs between malathion and diazinon.[17]

Model assumptions should be assessed using residual plots, $Q-Q$ plots and tests or diagnostics for variance heterogeneity. Transformation should be justified by distributional behaviour rather than applied mechanically. If normal-model assumptions remain poor, a generalised model appropriate for count-derived data or robust/non-parametric alternatives may be considered, although CFU values after log transformation commonly permit linear modelling.[18]

Post-hoc comparisons should be limited to scientifically meaningful contrasts: each dose versus control within pesticide and time, malathion versus diazinon at corresponding dose categories, and trend contrasts across dose. Tukey

[15] Singh, B. K., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. FEMS Microbiology Reviews, 30(3), 428–471. https://doi.org/10.1111/j.1574-6976.2006.00018.x

[16] Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences, 111(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

[17] Wołejko, E., Jabłońska-Trypuć, A., Wydro, U., Butarewicz, A., & Łozowicka, B. (2020). Soil biological activity as an indicator of soil pollution with pesticides—A review. Applied Soil Ecology, 147, 103356. https://doi.org/10.1016/j.apsoil.2019.09.006

[18] El-Gendy, A. O. et al. (2023). Biodegradation of malathion and chlorpyrifos by microorganisms isolated from agricultural drainage water in Egypt. Water Science, 37, 426–438. https://doi.org/10.1080/23570008.2023.2283665

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adjustment is appropriate for all-pair comparisons; Dunnett-type contrasts are preferable when the principal question is treatment versus control. Report adjusted p-values, estimated mean differences and 95% confidence intervals.

Effect sizes are essential. For each treatment, report the difference in $\log_{10}$ CFU relative to control and optionally convert this to fold change. Percentage inhibition can be presented descriptively as $[(C - T)/C] \times 100$, but inference should be performed on replicate-level data rather than on percentages calculated from group means. Where a monotonic response is plausible, regression using actual mg a.i. kg^{-1} is preferable to treating dose only as a category. Polynomial or segmented models may be compared if the response is non-linear.

If pesticide residues are measured, first-order or other validated kinetic models can estimate dissipation rate constants and half-lives. Associations between residue concentration and bacterial abundance can then be examined using regression or mixed models. This approach is stronger than correlating bacterial counts with nominal dose because it incorporates actual exposure over time.[19]

Statistical significance should be set a priori, commonly $\alpha = 0.05$. However, interpretation should not rely solely on whether p is below 0.05. Confidence intervals, magnitude, biological plausibility, consistency across time and the environmental relevance of the dose should determine the conclusion. [20]

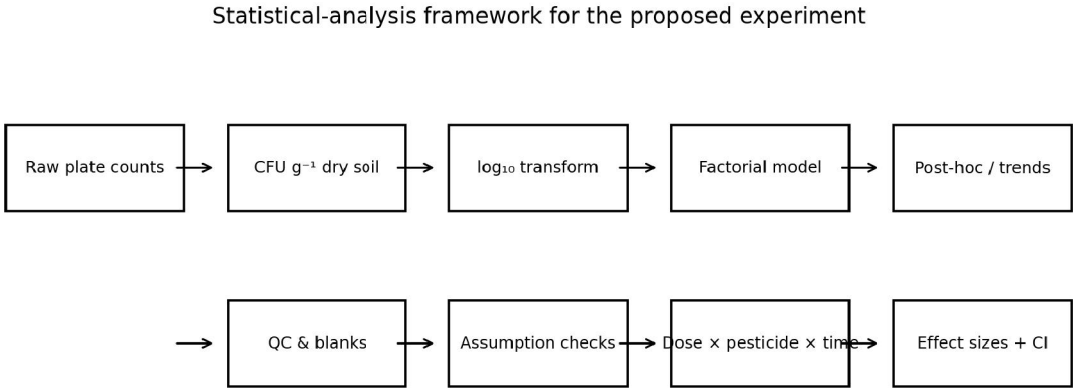

Figure 1 Statistical-analysis framework for the proposed experiment.

VI. RESULTS: JOURNAL-READY DATA PRESENTATION

No primary laboratory observations were supplied with this request. To preserve research integrity, the following tables and graph are data-entry structures and analytical templates; they do not contain invented experimental results.[21]

6.1 Descriptive results

Pesticide	Dose	Day	n	Mean CFU g^{-1}	Mean $\log_{10}$ CFU	SD
Control	0	0	—	—	—	—
Control	0	7	—	—	—	—
Control	0	14	—	—	—	—

[19] Mishra, S. et al. (2023). Pesticide-tolerant microbial consortia: Potential candidates for remediation/clean-up of pesticide-contaminated agricultural soil. Environmental Research, 236, 116724. https://doi.org/10.1016/j.envres.2023.116724

[20] Bhatt, P. et al. (2023). Bacterial remediation of pesticide polluted soils: Exploring the feasibility of site restoration. Journal of Hazardous Materials, 441, 129906. https://doi.org/10.1016/j.jhazmat.2022.129906

[21] Yadav, S. L., Birla, D., Inwati, D. K., Yadav, M., Yadav, I. R., Makwana, S. N., Lakshman, & Papnai, N. (2023). Impact of agrochemicals on soil biota and ways to mitigate it: A review. International Journal of Environment and Climate Change, 13(5), 366–375. https://doi.org/10.9734/ijecc/2023/v13i51779

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Control	0	21	—	—	—	—
Control	0	28	—	—	—	—
Malathion	0.5×	0	—	—	—	—
Malathion	0.5×	7	—	—	—	—
Malathion	0.5×	14	—	—	—	—
Malathion	0.5×	21	—	—	—	—
Malathion	0.5×	28	—	—	—	—
Malathion	1×	0	—	—	—	—
Malathion	1×	7	—	—	—	—
Malathion	1×	14	—	—	—	—
Malathion	1×	21	—	—	—	—
Malathion	1×	28	—	—	—	—
Malathion	2×	0	—	—	—	—
Malathion	2×	7	—	—	—	—
Malathion	2×	14	—	—	—	—
Malathion	2×	21	—	—	—	—
Malathion	2×	28	—	—	—	—
Malathion	4×	0	—	—	—	—
Malathion	4×	7	—	—	—	—
Malathion	4×	14	—	—	—	—
Malathion	4×	21	—	—	—	—
Malathion	4×	28	—	—	—	—
Diazinon	0.5×	0	—	—	—	—
Diazinon	0.5×	7	—	—	—	—
Diazinon	0.5×	14	—	—	—	—
Diazinon	0.5×	21	—	—	—	—
Diazinon	0.5×	28	—	—	—	—
Diazinon	1×	0	—	—	—	—
Diazinon	1×	7	—	—	—	—
Diazinon	1×	14	—	—	—	—
Diazinon	1×	21	—	—	—	—
Diazinon	1×	28	—	—	—	—
Diazinon	2×	0	—	—	—	—
Diazinon	2×	7	—	—	—	—
Diazinon	2×	14	—	—	—	—
Diazinon	2×	21	—	—	—	—
Diazinon	2×	28	—	—	—	—
Diazinon	4×	0	—	—	—	—
Diazinon	4×	7	—	—	—	—
Diazinon	4×	14	—	—	—	—
Diazinon	4×	21	—	—	—	—
Diazinon	4×	28	—	—	—	—

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6.2 Inferential-statistics table

Effect	Df	Test statistic	p value	Interpretation
Pesticide	—	—	—	Insert after analysis
Dose	—	—	—	Insert after analysis
Time	—	—	—	Insert after analysis
Pesticide × Dose	—	—	—	Insert after analysis
Pesticide × Time	—	—	—	Insert after analysis
Dose × Time	—	—	—	Insert after analysis
Pesticide × Dose × Time	—	—	—	Insert after analysis

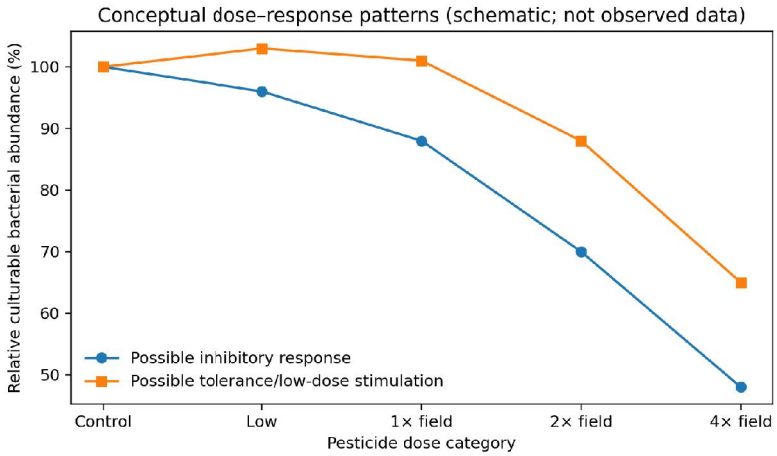

Figure 2 Conceptual dose–response patterns. This is a schematic illustration, not measured study data; replace with observed treatment means and 95% confidence intervals after laboratory analysis.

VII. DISCUSSION

The primary question in this study is whether viable soil bacterial abundance changes systematically as malathion or diazinon dose increases. The answer should be based on the full dose × pesticide × time structure rather than on isolated pairwise differences. If counts remain similar to control at 0.5× and 1× but decline at 2× and 4×, the most defensible interpretation would be a concentration threshold above which microbial stress becomes evident. Such a pattern would support the practical distinction between agronomically relevant exposure and excessive application.[22]

If bacterial counts decline immediately after treatment but approach control values later, the response would suggest resilience or adaptation. Recovery could result from pesticide dissipation, microbial acclimation, proliferation of tolerant organisms or replacement of sensitive populations. It would not necessarily mean that the community returned to its original composition. The 2024 diazinon study is instructive because it documented early diversity reduction together with enrichment of Actinomycetota and extensive degradation over longer exposure. A CFU-based experiment might detect recovery in viable abundance while missing this compositional turnover.[23]

Conversely, persistent suppression across later sampling points would indicate a more durable disturbance. Persistence would be particularly important if it occurred at the field-equivalent rate. Such a result should prompt investigation of soil enzyme activity, nitrification, microbial biomass and community composition, because sustained reduction in

[22] Hassaan, M. A., & El Nemr, A. (2020). Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. Egyptian Journal of Aquatic Research, 46(3), 207–220. https://doi.org/10.1016/j.ejar.2020.08.007

[23] Abraham, J., & Srujana, A. (2014). Microbial utilization of malathion isolated from contaminated soil of sugarcane fields in Vellore. Agricultural Research, 3, 339–345. https://doi.org/10.1007/s40003-014-0129-6

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viable bacteria could have functional consequences. Soil biodiversity experiments have shown that reductions in below-ground diversity and community complexity can impair nutrient cycling, decomposition and plant-related ecosystem functions.[24]

An apparent increase in CFU at low or intermediate pesticide doses requires cautious interpretation. It may reflect utilisation of pesticide or metabolites by adapted bacteria, release from competition, or a general substrate effect. Literature on malathion biodegradation demonstrates that several bacteria can transform the compound and that consortia may outperform single isolates. Therefore, stimulation of culturable abundance is biologically plausible. Yet total abundance alone cannot establish that the pesticide is beneficial; selective enrichment can coexist with loss of sensitive taxa.

Differences between malathion and diazinon should be interpreted through the interaction term. If both pesticides reduce abundance but diazinon produces a steeper decline, the conclusion is not simply that both are 'toxic'; rather, diazinon would show greater inhibitory potency under the tested soil and exposure conditions. If the compounds cross over across doses or times, the interaction may be non-linear, and compound ranking should not be reduced to a single overall mean.[25]

Soil properties are likely to modify these effects. Organic matter can sorb hydrophobic compounds and reduce immediate bioavailability, but it can also support larger microbial biomass and provide co-substrates for cometabolism. Moisture controls diffusion and microbial activity. pH influences chemical hydrolysis, sorption and enzyme activity. Recent field modelling of diazinon degradation reported relationships between degradation and moisture, organic matter and porosity, illustrating why environmental covariates should be measured and reported rather than treated as background detail.[26]

The formulation used is another interpretive issue. Commercial pesticide products contain co-formulants that may alter dispersion, bioavailability or microbial response. A study using analytical-grade active ingredient estimates compound-specific effects more cleanly, whereas a study using commercial formulation better approximates farm exposure. Both are legitimate, but the paper must identify which question is being answered. If possible, parallel active-ingredient and formulation treatments can separate these effects.

The use of CFU as the principal endpoint has strengths and limitations. It is inexpensive, transparent and directly measures viable colony-forming organisms under defined conditions. It is therefore suitable for detecting large treatment effects and for laboratories without sequencing infrastructure. Its limitation is that most soil bacteria are not recovered by a single culture medium. Modern ecotoxicological assessment increasingly combines culture-based counts with 16S rRNA sequencing, functional genes, respiration and enzyme assays. A strong follow-up study would use CFU as one layer in a multi-endpoint design.

The environmental significance of a statistically significant result depends on effect size and exposure realism. A small reduction at 4× or 10× the recommended rate may be statistically detectable yet have limited relevance to normal use, while a substantial and persistent reduction at 1× would be more concerning. Accordingly, the discussion should

[24] Abo-Amer, A. E. (2007). Involvement of chromosomally-encoded genes in malathion utilization by Pseudomonas aeruginosa AA112. Acta Microbiologica et Immunologica Hungarica, 54(3), 261–277. https://doi.org/10.1556/AMicr.54.2007.3.5

[25] Ali, N. et al. (2016). Biodegradation of chlorpyrifos, malathion, and dimethoate by three strains of bacteria isolated from pesticide-polluted soils in Sudan. Journal of Agricultural and Food Chemistry, 64(45), 8491–8498. https://doi.org/10.1021/acs.jafc.6b03334

[26] Geed, S. R., Kureel, M. K., Giri, B. S., Singh, R. S., & Rai, B. N. (2016). Biodegradation of malathion and evaluation of kinetic parameters using three bacterial species. Resource-Efficient Technologies, 2(S1), S3–S11. https://doi.org/10.1016/j.reffit.2016.09.005

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always identify which dose corresponds to the realistic field-equivalent exposure and distinguish hazard characterisation from likely field risk.[27]

The literature also supports examining pesticide degradation alongside microbial response. Organophosphate-degrading bacteria are candidates for bioremediation, and recent reviews describe microbial consortia, enzymes and engineered approaches as promising tools. In a soil-health study, however, degradation should not overshadow non-target effects. The ideal outcome is rapid detoxification without major or persistent disruption of beneficial microbial functions.

If the experimental results show a significant pesticide × dose × time interaction, the study would provide evidence that microbial sensitivity is dynamic rather than fixed. Such an interaction might occur, for example, if diazinon initially suppresses bacteria more strongly but populations recover as degrading taxa become enriched, while malathion produces a smaller initial effect and faster return to baseline. These trajectories can be biologically more informative than a single final-day comparison.[28]

Finally, conclusions should be bounded by the experimental design. Laboratory microcosms improve control but simplify field reality. Plants, rhizodeposition, rainfall, temperature fluctuations, ultraviolet exposure, repeated applications and spatial heterogeneity can alter both pesticide fate and microbial response. Laboratory evidence should therefore be viewed as mechanistic and comparative, with field validation needed before broad agronomic generalisation.[29]

VIII. ENVIRONMENTAL AND AGRICULTURAL IMPLICATIONS

The study can inform pesticide stewardship by identifying dose ranges at which measurable bacterial disturbance begins. If field-equivalent doses have little persistent effect but supra-recommended doses suppress bacteria, the result reinforces accurate calibration, avoidance of unnecessary repeat applications and adherence to integrated pest-management principles.

Where inhibition occurs at realistic doses, soil-health monitoring becomes more important. Organic amendments, crop rotations, reduced pesticide frequency and biologically based pest-management strategies may help maintain microbial resilience, although mitigation practices should themselves be tested rather than assumed effective.

The work also has bioremediation relevance. Enrichment of malathion- or diazinon-tolerant bacteria can provide candidates for isolation and characterisation. However, any proposed degrader or consortium should be evaluated for ecological safety, survival, degradation products and performance under field conditions before application.[30]

IX. LIMITATIONS

The principal limitation is the reliance on culture-dependent enumeration, which captures only the culturable fraction under the chosen conditions. A second limitation is that nominal pesticide dose may differ from actual bioavailable concentration because of sorption and degradation. Laboratory incubation also excludes many field processes. Finally, soil-specific findings may not generalise to soils with different texture, pH, organic matter or pesticide-use history.

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[27] Kumar, S. et al. (2021). Environmental occurrence, toxicity concerns, and degradation of diazinon using a microbial system. Frontiers in Microbiology, 12, 717286. https://doi.org/10.3389/fmicb.2021.717286

[28] van der Heijden, M. G. A., Bardgett, R. D., & van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11(3), 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

[29]. Akter, S., Hulugalle, N. R., & Jasonsmith, J. (2023). Changes in soil microbial communities after exposure to neonicotinoids: A systematic review. Environmental Microbiology Reports, 15(6), 431–444. https://doi.org/10.1111/1758-2229.13193

[30] Cycoń, M., Mrozik, A., & Piotrowska-Seget, Z. (2017). Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. Chemosphere, 172, 52–71. https://doi.org/10.1016/j.chemosphere.2016.12.129

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These limitations should be addressed through residue analysis, molecular community profiling, functional assays and multi-soil field validation.

X. CONCLUSION

A rigorous evaluation of malathion and diazinon requires more than a treated-versus-control comparison. The proposed graded-dose, repeated-time design allows the investigator to determine whether soil bacterial populations show tolerance, threshold inhibition, persistent suppression, recovery or selective enrichment. Factorial statistical analysis directly tests whether the two pesticides differ in their dose-response behaviour and whether effects change over time. The contemporary literature supports this approach: organophosphate exposure can disturb microbial communities while simultaneously selecting organisms capable of pesticide degradation. Consequently, abundance, community structure and pesticide dissipation should be interpreted as complementary dimensions of the soil response. The present paper provides a complete empirical framework without fabricating results. Once measured CFU values and soil characteristics are inserted, the tables and statistical plan can be used to generate a defensible Results section and evidence-based conclusions relevant to soil health and responsible pesticide use.

XI. RECOMMENDED REPORTING CHECKLIST

- State pesticide active ingredient, purity/formulation and manufacturer.
- Report doses as mg active ingredient kg^{-1} dry soil and explain field-rate conversion.
- Report soil source, depth, texture, pH, organic matter and pesticide history.
- Distinguish biological replicates from technical plate replicates.
- Report raw/summary CFU and $\log_{10}$-transformed values.
- Provide effect sizes and 95% confidence intervals, not p-values alone.
- Identify the field-equivalent dose in every dose-response figure.
- Do not describe unmeasured pesticide disappearance as microbial degradation.
- State clearly whether figures contain observed data, literature data or conceptual schematics.

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