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A Study on the Response of Soil Microbes Under the Influence of Fertilizers and Pesticides

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Abstract

This research paper investigates the effects of various agricultural pesticides—glyphosate, mancozeb, and diazinon—on specific soil microbial populations. The study considers these impacts across three different soil orders and two soil moisture contents, and their combinations, in a pot experiment. The main findings demonstrated differential effects: glyphosate significantly decreased total actinomycetes while increasing total bacteria, proteolytic bacteria, and fungi; mancozeb significantly decreased proteolytic bacteria and fungi; and diazinon significantly increased total bacteria and proteolytic bacteria. Soil type also played a crucial role, with Agholan soil showing the greatest reduction in proteolytic bacteria, fungi, and actinomycetes, while Girdarasha soil showed a more significant increase in microbial population counts. Furthermore, 100% soil moisture content demonstrated more significant effects on soil microbial

[1] . INTRODUCTION

Soil microorganisms are the biological core of terrestrial systems and carry out several irreplaceable functions in order to maintain the ecological balance and the winter ecological dearth. Microscopic powerhouses that include bacteria, archaea, fungi, protists, and viruses are the most important drivers of critical biogeochemical cycles such as the cycling of carbon, nitrogen, phosphorus, and sulfur (Zaman et al., 2025). Their various metabolic processes contribute to the breakdown of complex organic acids, breaking down the remains of the plant and animal bodies into simpler substances which can be utilised as available nutrients in the plants and others, who live in the soil. In addition to the supply of nutrients, such microbial communities also play proactive roles in the formation of the soil structure through the release of sticky exudates and fungal hyphae, which hold the soil particles together to form stable aggregates, hence increasing the aeration, water infiltration, and soil stability levels (Peele, 1940). This complex microbial action has a direct impact on soil quality, fertility, and resilience, and they are therefore central to the health of an ecosystem. As a result, the existence, variety, and dynamic operations of these microbial communities are seen as important indicators of the health of the soil, which is innately capable of ensuring plant and animal productivity, environmental quality, and human health (Lehmann et al., 2020). Diverse and enriched microbial community with functional activity signals of a healthy and functioning soil that can be self-regulated, hold nutrients, suppress disease, and resist against other environmental disturbances (Xu et al., 2022).

Nevertheless, the current pressure on the agriculture industry to produce more food as the global population continues to scale up has inevitably contributed to the intensification of the farming industry. Out of these approaches, the wide and at times ubiquitous application of synthetic pesticides has become a keystone of contemporary agrochemical control (Thomas et al., 2020). These are carefully engineered chemicals that are used to guard crops against a wide range of biotic attackers in the form of harmful

populations. The interactions between pesticides, soil orders, and moisture contents also showed different significant effects on soil characteristics. This study highlights the complex interactions that govern soil microbial health in agricultural systems, emphasising the need for understanding these dynamics for sustainable pesticide management.

Keywords: *Soil Microbes*, *Fertilizers*, Pesticides, BHC (Benzene Hexachloride), HCH (*Hexachlorocyclohexane), Phorate, Mancozeb*

insects, competitive weeds, along other pathogenic organisms in the form of fungus, hence securing yields and maintaining food security in the world. Although their effectiveness in fulfilling the described shortterm goals cannot be refuted, the usage of these synthetic pesticides, even at a seemingly low or suggested level, presents some alien, xenobiotic chemicals into the intricate and sensitive boundaries of the soil ecosystem (Thomas et al., 2020). It may result in creeping soil contamination and eventual severe chemical and biological-level alteration of this crucial ecosystem, with effects that may well sprawl well beyond the concerned target organisms to the eventual maiming of non-target helpful organisms as well as ecosystem services (Ghannem et al., 2024).

Pesticides are inherently prepared to be biologically active substances, and their effects are, alas, not always limited to the target pests. Being in close contact with the soil particles, widespread and abundant, and having incredibly varying metabolic pathways that encompass the most diverse metabolic pathways, soil microorganisms prove to be among the most susceptible to exposure due to these chemical inputs (Devi et al., 2018). The effects of pesticides on the soil microorganisms may occur in three critical ways: (i) directly through the decrease or reduction of the general population of the microorganisms thus decreasing the size and viability of the microbial population; (ii) indirectly by altering their biochemical responses which may hinder essential enzymatic functions in nutrient cycling and organic matter breakdown; and (iii) fundamentally, by rearranging the complex microbial community composition, preferentially tolerant or adaptive species, effectively inhibiting others (Jiang et al., 202 These are ecologically important changes which potentially cause significant functional diversity to be lost and can substantially impair the delivery of critical ecosystem services that a diverse microbial communities usually delivers, thus resulting in long-term degradation of soil (Zhao et al., 2024).

It is extremely important to note that the outcome of the pesticides on the soil microorganisms is by no means constant; rather, a complex interrelation of several intrinsic and extrinsic factors, this phenomenon is highly diverse and variable. They determine this when taken through the influencing factors which include the intrinsic physicochemical aspects of the pesticide in question such as the chemical composition, solubility in water, persistence (half-life) in the environment, and a specific mode of action (e.g., enzyme inhibition, membrane disruption), and the degradation mechanisms (Perez-Lucas et al., 2019). In that regard, certain pesticides may be easily biodegraded and detoxified by a certain population of certain microbes, or even become a source of carbon or nitrogen to such microbes, whereas longerterm residues may accumulate, triggering chronic toxic effects. Besides, the innate peculiarities of the soil itself are of principal importance in the interaction between pesticides and microbes (Mesquita et al., 2022). Structure and composition of soil (sandy, silty, clayey soil) play a crucial role in determining aeration, the capacity of water retention and the availability of adsorption sites to the pesticide molecules. Pesticide compounds with a large number of compounds are dependent on soil pH to achieve their speciation and bioavailability, which in turn influence their toxicity to microorganisms, their chemical and biological breakdown rates. The presence of high organic matter may hold pesticides and limit the immediate bioavailability to the microbial communities limited but may also extend the time of pesticide in the soil environment (Mishra et al., 2022). Also, the current environmental factors like temperature changes and, more importantly, the soil moisture level influence considerably the mobility of pesticides, their degradation processes, the metabolism and growth rates of other living microbial populations. Microbial growth and enzyme activity can be enhanced by the right amount of moisture, which can be considered to enhance the pace of pesticide degradation, and conditions of drought or waterlogging can seriously hurt these important processes (Gomez et al., 2021).

Microorganisms are found in large numbers in soil, usually between 10^1 and 10^{10} microorganisms/g of soil, with bacteria and fungi being the most prevalent. Given these intricate dynamics and the

demonstrated potential for widely used pesticides, such as the herbicide glyphosate and the insecticide diazinon, to profoundly alter the diversity and population dynamics of microbial communities within diverse soil environments—as evidenced by studies in regions like Telangana and Andhra Pradesh, there is a pressing and continuous need for robust scientific investigation into these effects (Franco et al., 2017). Such research is not merely an academic exercise; it is fundamental to understanding the subtle yet significant ecological consequences of contemporary agricultural practices and to informing future environmental policies. By unravelling these complex interactions and identifying key modulating factors, we can contribute significantly to the development of more sustainable agricultural strategies that effectively safeguard both crop productivity and the invaluable biodiversity, functional integrity, and longterm health of our vital soil ecosystems (Tahat et al., 2020).

Ecology and agro-economics are growing to be interested in the details of how pesticides impact the gut and related microbiomes of soil animals. Earthworms and enchytraeids, and other soil animals facilitate nutrient cycling, soil structure, and soil microbe control by feeding and burrowing (Lu et al., 2020). Its health is partly linked to a balanced intestinal microbiota and stable networks of the microbiome of surrounding soils. Pesticides once taken into the soil system can have a direct impact on the animals, can change the diets of the animals indirectly and rebuild the microbial population that colonises animals through the intestine. In this section, the key insights of the most recent research are summarised, repackaged concerning soil animals, and organised into a thorough experimental framework with made-up data to demonstrate how various pesticide types and concentrations could change the most important microbiome parameters (Franz et. al., 1997). One of the properties that has become a common method in contemporary research uses bacterial community structure as an indicator of sensitive mechanisms of pesticide side-effects. Indicators like the Shannon index of diversity, the relative abundance of supposedly beneficial components (usually those implicated in nutrient cycling or colonisation resistance), and changes toward supposedly opportunistic groups (such as Proteobacteria) are popular. These metrics are important to soil animals since they correlate with host performance: efficiency with which they digest food, resist pathogens, grow, and survive. shifts in these values even as animals exhibit no outward signs of poor health an indicator of future threats to soil fertility and ecosystem integrity (Peng et. al., 2020)

2. MATERIALS AND METHODS

The experimental design for this study was meticulously crafted to investigate the complex interactions between selected pesticides, varying soil characteristics, and different moisture regimes on the dynamics of key soil microbial populations. A controlled pot experiment approach was adopted to allow for precise manipulation of variables and minimise external confounding factors, thereby providing robust insights into these critical environmental interactions.

2.1. Soil Sample Collection and Preparation

Soil samples were judiciously collected from distinct agricultural sites to represent a range of inherent edaphic properties. Specifically, the study utilised soils classified under two major soil orders: Entisols and Vertisols. The chosen representative soil types were from Telangana and Andhra Pradesh. This strategic selection of diverse soil orders was paramount, as it provided a foundational basis for analysing varying edaphic influences—such as texture, pH, organic matter content, and mineralogy—on how microbial communities respond to the stress induced by pesticide exposure (USAID, 2008). Entisols, often characterised by their minimal soil profile development, and Vertisols, known for their high clay content and prominent shrink-swell properties, offer markedly different physicochemical environments that can profoundly affect pesticide fate and microbial activity.

For collection, undisturbed topsoil (0-15 cm depth) samples were ideally collected aseptically from multiple random points within each designated site to ensure representative sampling and minimise spatial heterogeneity. Following collection, the soil samples were immediately transported to the laboratory, where they were air-dried at ambient temperature (typically 20-25°C) to a constant weight to facilitate homogenization and removal of large debris (e.g., plant roots, stones) without significantly altering their microbial community structure. The air-dried samples were then gently sieved through a 2mm mesh to obtain a fine, uniform soil matrix suitable for experimental treatments and subsequent

analyses. This preparation ensures consistency across experimental units and provides a standardised substrate for pesticide application and microbial assessment. Before treatment, baseline physicochemical properties of each soil type (e.g., pH, electrical conductivity, organic carbon content, and texture analysis) were determined to characterise their inherent differences fully, as these properties are known to influence microbial survival and pesticide degradation pathways.

2.2. Pesticides Preparation

The study systematically investigated the effects of three specific agricultural pesticides, each representing a different class and mode of action, on the indigenous soil microbial communities. The most abundant and usable pesticides in Telangana and Andhra Pradesh Region including BHC (Benzene Hexachloride), also known as HCH (Hexachlorocyclohexane) (organochlorine insecticide), Phorate (organochlorine insecticide), Mancozeb (fungicide). These pesticides were prepared at their commercial recommended doses according to their active ingredients (a.i.). Each pesticide was placed in a sprayer (the amount of spraying water for each pesticide was 60 liters/1,000 square meters) and was ready for application (Hill, 2008).

2.3. Soil treatment by pesticides

The experimental design incorporated a factorial arrangement, where each soil type was subjected to individual applications of these pesticides, as well as specific combinations thereof, at environmentally relevant concentrations. Pesticide solutions were prepared using analytical-grade reagents to ensure purity and accuracy. Application to the soil samples was performed uniformly, typically by thoroughly mixing the calculated amount of pesticide solution with the sieved soil to achieve the desired treatment concentrations. Untreated control soil samples (receiving only distilled water) were included for each soil type to serve as a baseline for comparison, allowing for the isolation of pesticide-induced effects from natural microbial fluctuations. All treatments were replicated (e.g., three to five times) to ensure statistical validity and reproducibility of the results. The treated soil samples were then carefully transferred to sterile pots or containers, prepared for the next stage of the experiment.

2.4. Experimental procedure

A factorial experiment (4 × 3 × 2) was carried out in the green house facility present at Government College (Autonomous) Rajahmundry, employing a Completely Randomized Design (CRD) with three replications, resulting in 24 treatment combinations (Table 1). A total of 72 uniform, pre-labelled plastic pots (15 cm diameter × 17 cm height) were used, each filled with 4 kg of pesticide-treated soil. Baseline soil samples were collected from each pot before treatment. Each pot was placed above a collection tray to capture leachate, which was returned to the pot to prevent nutrient and microbial loss. To minimise external contamination, pots were covered with sterile filter paper. Irrigation was carried out daily using tap water at two moisture levels: 50% and 100% field capacity. Daily water loss was monitored by weighing each pot and replenishing the lost water accordingly. The experiment was conducted over two months, with five sampling intervals at biweekly periods, under variable greenhouse climatic conditions (Table 2). The design was optimised to assess microbial and physicochemical responses under controlled but dynamic environmental conditions. **Table 1: experimental design.**

No. of treatments		Pesticide	Soil order	Moisture Content (w)
1	Bw50-R1	BHC	Region 1	50%
2	Bw100-R1			100%
3	Bw50-R2		Region 2	50%
4	Bw100-R2			100%
5	Bw50-R3		Region 3	50%
6	Bw100-R3			100%
7	Pw50-R1	Phorate	Region 1	50%
8	Pw100-R1			100%
9	Pw50-R2		Region 2	50%

10	Pw100-R2			100%
11	Pw50-R3		Region 3	50%
12	Pw100-R3			100%
13	Mw50-R1	Mancozeb	Region 1	50%
14	Mw100-R1			100%
15	Mw50-R2		Region 2	50%
16	Mw100-R2			100%
17	Mw50-R3		Region 3	50%
18	Mw100-R3			100%
19	Cw50-R1	Control (no pesticide)	Region 1	50%
20	Cw100-R1			100%
21	Cw50-R2		Region 2	50%
22	Cw100-R2			100%
23	Cw50-R3		Region 3	50%
24	Cw100-R3			100%

Table 2: Climatic Conditions During the Study

Parameters	1st Sampling	2nd Sampling	3rd Sampling	Mean
Maximum temperature (°C)	36.00	35.80	36.00	35.93
Minimum temperature (°C)	23.5	25.5	27.5	25.50
Dry temperature (°C)	30.1	30.5	32.3	30.97
Humidity (%)	28	30	22	26.67
Wind velocity ($m \cdot s^{-1}$)	1	1.3	2.4	1.57
Wind direction (°)	290	220	200	236.67
Maximum wind velocity ($m \cdot s^{-1}$)	3	3	6	4.00

2.5. Culture media preparation

The study focused on quantifying the populations of four key microbial groups, selected for their ecological significance and their diverse metabolic roles within the soil ecosystem (Atlas, 2005; Cheesbrough, 1992). These groups serve as robust indicators of the overall health and functional capacity of the soil microbiome:

a) Total Bacterial Count:

Nutrient agar (NA) was prepared by dissolving 28 g of commercially available powder in 1 L of distilled water. The pH was adjusted to 7.0 ± 0.2, followed by thorough mixing and gentle heating until boiling. The medium was then sterilised by autoclaving at 121°C and 15 psi for 15 minutes (Harley and Prescott, 1996).

b) Proteolytic Bacteria:

For isolating proteolytic bacteria, nutrient gelatin agar was used. The medium was formulated by dissolving the following constituents in 1 L of distilled water: gelatin (15 g), agar (15 g), peptone (4 g), and yeast extract (1 g). The pH was adjusted to 7.0 ± 0.2, followed by mixing, boiling, and autoclaving under the same conditions as above. **c) Fungal Enumeration:**

Potato Dextrose Agar (PDA) was prepared by dissolving 39 g of PDA powder in 1 L of distilled water. After heating to boiling and autoclaving, the medium was supplemented with 0.2 mg of chloramphenicol to inhibit bacterial growth before pouring it into Petri dishes. **d) Actinomycetes Count:**

Starch Casein Agar (SCA) was used for isolating actinomycetes. It was prepared by adding and thorough mixing of the following components [starch 10 g; casein 3 g; KNO_3 2 g; NaCl 2 g; K_2HPO_4 2 g; $MgSO_4.7H_2O$ 0.05 g; $CaCO_3$ 0.02 g; $FeSO_4.7H_2O$ 0.01 g and agar 15 g] with 1L distilled water and the pH of the complete medium was adjusted to 7.0±0.2.

2.6. Microbiological Analysis

Microbial populations in the soil samples were quantified using the standard plate count method. The total bacterial count and total proteolytic bacterial population were determined from 1 gram of ovendried soil by plating appropriate dilutions onto respective media, followed by incubation at 30–35°C for 24–48 hours (Harley and Prescott, 1996). Only plates yielding 30 to 300 colony-forming units (CFUs) were considered valid for enumeration, ensuring statistical reliability of counts. For fungal enumeration, plates were incubated at 23 ± 2°C for a period of 5 to 7 days to allow for adequate mycelial growth and sporulation. The actinomycete population was assessed via the spread plate technique, with incubation carried out at 30°C for 14 days, by established protocols (Aneja, 2003).

2.7. Statistical Analysis

All experimental data were statistically analysed using SPSS software version 11.5 and Microsoft Excel 2010. Treatment means were compared using the Revised Least Significant Difference (R-LSD) test at a significance level of $p \leq 0.05$, ensuring rigorous evaluation of treatment effects across microbial groups.

3. RESULT AND DISCUSSION

3.1. Effect of treatments on the total bacterial population

The study investigated the effect of various fertilisers and pesticides on the total bacterial population in soil ($\times 10^5$ g^{-1} dry soil) over three distinct sampling periods: 24 hours, 2 weeks, and 4 weeks after application. The treatments included chemical pesticides (BHC, Phorate, Mancozeb), untreated control, region-specific samples, different soil moisture regimes, and combined treatment interactions.

The bacterial population showed pronounced variability depending on the treatment applied. Notably, BR1 (61.53±3.39^{a}), Bw100 (61.97±10.68^{a}), and Bw100-R1 (94.92±14.24^{a}) recorded the highest microbial counts, indicating that some treatments or environmental conditions initially stimulated bacterial proliferation. Conversely, very low populations were observed in Mw50-R1 (0.47±0.00^{h}) and Bw100-R2 (0.66±0.06^{gh}), suggesting an immediate inhibitory effect, potentially due to high pesticide toxicity or unfavourable soil chemical changes. Interestingly, the BHC treatment (39.69±7.14^{a}) also supported relatively high bacterial numbers at this stage, implying selective tolerance by certain bacterial groups.

Two weeks after application, marked reductions in bacterial counts were seen in most pesticide-treated soils, with several treatments (Bw50-R1, Bw100-R1) showing negative or near-zero values, indicating severe suppression or measurement approaching detection limits. This suggests acute pesticide toxicity impacting overall microbial biomass. In contrast, the control (C) maintained a high count (19.20±3.76^{a}), highlighting the absence of chemical stress. Some treatments, such as Cw100-R1 (57.61±5.86^{a}) and Pw50R2 (21.68±0.00^{b}), exhibited unexpectedly high populations, possibly due to microbial adaptation, growth of resistant species, or nutrient enrichment from treatment breakdown products.

Table 3: Effect of treatments on the total bacterial population ($\times 10^5$ CFU·g^{-1} dry soil).

Treatments	1st sampling (24h)	2nd sampling (2 weeks)	3rd sampling (4 weeks)
B (BHC)	39.69±7.14^{a}	3.69±1.10^{c}	9.56±2.78^{b}
P (Phorate)	6.15±1.42^{b}	6.40±2.24^{b}	7.30±1.77^{c}
M (Mancozeb)	9.43±2.38^{b}	7.62±1.12^{b}	12.28±2.61^{a}
C (Control)	6.05±1.25^{b}	19.20±3.76^{a}	12.49±0.81^{a}
R1 (Region1)	21.53±1.19^{a}	10.82±3.88^{a}	7.00±1.19^{c}
R2 (Region1)	8.59±1.90^{c}	9.03±2.58^{b}	10.72±2.11^{b}
R3 (Region1)	15.88±4.69^{b}	7.84±1.21^{b}	13.50±1.69^{a}
w50	7.61±2.30^{b}	8.74±1.82^{b}	11.47±1.84^{a}
w100	23.05±4.60^{a}	9.72±4.52^{b}	9.34±1.19^{b}
BR1	61.53±3.39^{a}	6.04±0.00^{fg}	7.38±0.32^{fg}
BR2	8.07±2.41^{cde}	2.03±1.05^{h}	4.65±0.35^{hi}
PR3	49.46±40.85^{b}	10.04±0.16^{cd}	16.66±3.07^{a}

PR1	4.98±3.99^{de}	1.05±0.71^{h}	3.51±1.10^{i}
PR2	7.37±1.69^{cde}	12.49±3.19^{bc}	8.42±2.83^{ef}
PR3	6.10±2.94^{de}	5.65±1.51^{g}	9.96±3.44^{de}
MR1	13.55±14.08^{c}	6.46±1.38^{g}	5.75±0.12^{gh}
MR2	9.60±3.18^{cd}	8.96±2.02^{de}	17.16±3.46^{a}
MR3	5.14±1.14^{de}	7.43±2.15^{g}	13.93±1.78^{b}
CR1	6.04±0.26^{de}	36.75±9.86^{a}	11.38±1.42^{cd}
CR2	9.31±1.45^{cd}	12.62±4.27^{b}	12.67±0.46^{bc}
CR3	2.80±0.33^{e}	8.23±2.47^{def}	13.43±2.34^{b}
Bw50	17.41±5.72^{b}	3.99±1.17^{de}	11.58±2.66^{a}
Bw100	61.97±10.68^{a}	3.39±1.45^{e}	7.55±1.63^{b}
Pw50	5.24±2.33^{c}	10.53±3.87^{bc}	8.20±2.60^{b}
Pw100	7.06±1.95^{c}	2.26±0.96^{e}	6.39±2.84^{b}
Mw50	1.30±1.14^{c}	7.78±1.05^{c}	13.47±4.95^{a}
Mw100	17.56±5.37^{b}	7.45±2.26^{cd}	11.09±2.85^{a}
Cw50	6.51±2.39^{c}	12.64±2.47^{b}	12.64±1.69^{a}
Cw100	5.59±1.36^{c}	25.75±5.88^{a}	12.34±0.63^{a}
R1w50	8.73±3.64^{c}	6.37±2.96^{c}	7.22±1.03^{c}
R1w100	34.32±10.76^{a}	15.26±13.84^{a}	6.79±2.34^{c}
R2w50	8.83±2.69^{c}	9.76±2.11^{b}	11.18±2.19^{b}
R2w100	8.34±2.09^{c}	8.29±2.71^{b}	10.27±1.80^{b}
R3w50	5.28±1.07^{c}	10.08±0.94^{b}	16.02±2.36^{a}
R3w100	26.47±7.33^{b}	5.60±1.60^{c}	11.93±1.87^{b}
Bw50-R1	28.14±4.36^{b}	-0.99±0.00^{n}	7.70±0.25^{h}
Bw100-R1	94.92±14.24^{a}	-0.99±0.00^{n}	7.06±0.45^{hi}
Bw50-R2	15.48±0.31^{c}	3.08±0.00^{kl}	4.30±0.30^{k}
Bw100-R2	0.66±0.06^{gh}	0.08±0.06^{m}	5.00±0.00^{jk}
Bw50-R3	8.61±1.06^{d}	9.88±0.06^{fg}	22.74±0.29^{a}
Bw100-R3	90.32±8.25^{a}	10.20±0.00^{ef}	11.53±0.37^{fg}
Pw50-R1	1.99±0.00^{gh}	2.76±0.00^{lm}	6.60±0.24^{ijk}
Pw100-R1	8.97±2.64^{d}	1.34±0.00^{m}	2.41±0.00^{l}
Pw50-R2	5.68±0.96^{defg}	21.68±0.00^{b}	6.59±1.27^{ijk}
Pw100-R2	9.07±0.98^{d}	3.31±0.00^{jkl}	11.25±0.68^{defg}
Pw50-R3	9.04±0.00^{d}	9.16±0.00^{fg}	14.41±0.66^{c}
Pw100-R3	3.15±0.00^{defg}	4.15±0.00^{kl}	7.52±0.06^{hij}
Mw50-R1	0.47±0.00^{h}	8.84±1.70^{gh}	6.63±0.18^{ijk}
Mw100-R1	27.63±2.54^{b}	6.08±0.00^{ijk}	6.87±0.13^{hijk}
Mw50-R2	4.41±0.00^{defg}	6.94±0.00^{hi}	23.62±0.66^{a}
Mw100-R2	16.78±0.00^{c}	12.99±3.16^{de}	12.69±0.31^{cdefg}
Mw50-R3	2.00±0.00^{gh}	10.58±1.95^{fg}	13.15±0.54^{cdef}
Mw100-R3	10.28±2.42^{d}	6.29±0.00^{ij}	16.70±3.43^{b}
Cw50-R1	7.30±0.00^{def}	17.88±0.63^{c}	10.95±0.50^{g}
Cw100-R1	6.78±0.00^{defg}	57.61±5.86^{a}	13.81±0.87^{cde}
Cw50-R2	11.76±0.00^{cd}	9.35±0.12^{fg}	13.21±0.72^{cdef}
Cw100-R2	8.86±0.00^{de}	17.89±0.12^{c}	14.13±0.31^{cd}

Cw50-R3	3.48±0.00^{fgh}	13.69±0.24^{d}	16.77±0.60^{b}
Cw100-R3	4.13±0.00^{defg}	4.76±0.00^{jkl}	12.09±3.45^{efg}

Values represent the mean ± standard error (S.E.).

By the fourth week, bacterial populations in several treatments recovered substantially, indicating resilience and adaptive capacity of the soil microbiome. Notably, Bw50-R3 (22.74±0.29^{a}), Mw50-R2 (23.62±0.66^{a}), and PR3 (16.66±3.07^{a}) recorded high counts, reflecting possible degradation of pesticide residues and subsequent proliferation of degrading bacteria. Mancozeb (12.28±2.61^{a}) and the control (12.49±0.81^{a}) sustained stable, relatively high populations, suggesting lower long-term toxicity compared to organochlorine and organophosphate pesticides.

BHC, although initially supporting high bacterial populations, showed a sharp decline at 2 weeks, followed by partial recovery at 4 weeks. Phorate consistently maintained moderate bacterial counts, suggesting a balanced inhibitory and selective effect. Mancozeb showed relatively less detrimental influence, with gradual increases over time, indicating possible utilisation of its breakdown products as a carbon or nutrient source by specific microbes.

Moisture content also played a significant role. Treatments at 50% and 100% moisture (e.g., R3w50, R3w100) displayed variable responses, with certain combinations (Mw50-R2, Cw100-R1) promoting significant bacterial recovery by the final sampling. This underscores the interplay between moisture regimes and pesticide degradation rates, influencing microbial recovery patterns.

The findings indicate that pesticide application exerts a pronounced but often transient stress on soil bacterial communities. While some compounds cause immediate suppression, microbial populations often recover within weeks, likely due to the emergence of resistant strains or biodegraders. The data also suggest that moisture optimisation can mitigate pesticide stress, enhancing bacterial resilience. This highlights the importance of integrated soil management practices that balance pest control with microbial conservation, ensuring long-term soil health and fertility.

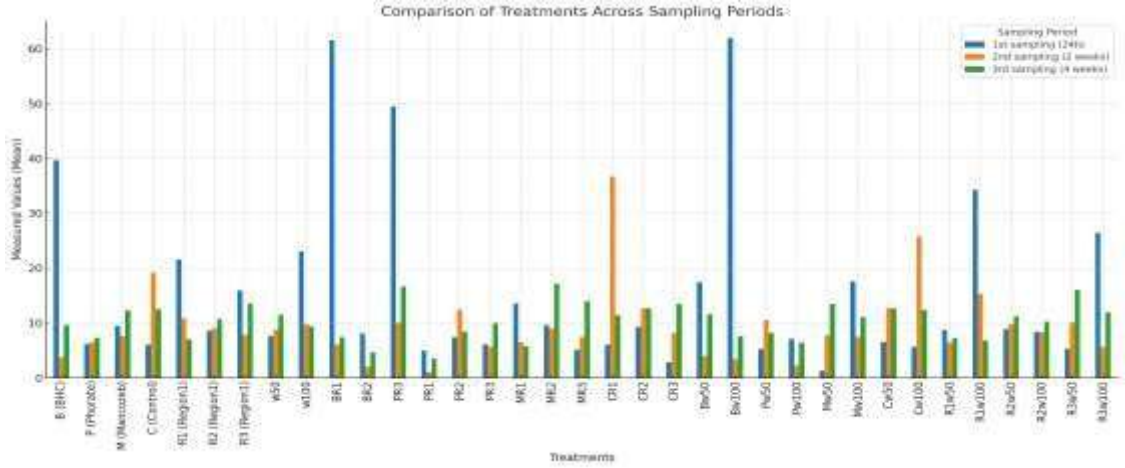

Figure 1: Graph showing the effect of treatments on the total bacterial population

3.2. Effect of treatments on the soil total proteolytic bacterial population

The results presented in Table 4 provide a comprehensive analysis of the impact of different fertilisers and pesticides on the total proteolytic bacterial population in soil over three key sampling periods: 24 hours, 2 weeks, and 4 weeks after application. These findings are critical to understanding how agrochemical treatments influence soil microbial ecology, especially proteolytic bacteria that play an essential role in organic matter decomposition and nitrogen cycling.

Table 4: Effect of various treatments on soil total proteolytic bacterial population (×10^5 CFU·g^{-1} dry soil).

Treatments	1st sampling (24h)	2nd sampling (2 weeks)	3rd sampling (4 weeks)
B (BHC)	5.31±2.11^{a}	1.72±0.33^{b}	4.52±1.66^{a}
P (Phorate)	2.51±0.76^{c}	0.65±0.37^{b}	3.51±1.78^{b}

M (Mancozeb)	3.33±1.91^{b}	4.95±1.22^{b}	3.90±1.73^{ab}
C (Control)	1.53±0.45^{d}	8.86±2.29^{a}	1.47±0.52^{c}
R1 (Region1)	2.85±1.52	6.16±2.60	1.35±0.68^{c}
R2 (Region1)	3.12±0.69	2.76±1.08	3.61±1.33^{b}
R3 (Region1)	3.48±1.64	3.27±1.14	5.15±1.51^{a}
w50	2.59±0.79^{b}	2.68±0.84^{b}	4.59±1.26^{a}
w100	3.77±1.29^{a}	5.45±1.71	2.14±0.74^{b}
BR1	3.60±0.26^{c}	-0.91±0.00^{b}	1.96±0.01^{cd}
BR2	3.21±1.08^{c}	1.27±0.09^{b}	2.43±1.06^{cd}
PR3	9.22±2.33^{a}	8.80±1.67^{b}	9.19±2.39^{a}
PR1	0.58±1.05^{fg}	0.36±0.81^{b}	-0.71±0.06^{e}
PR2	3.70±0.73^{c}	0.17±0.52^{b}	5.16±1.38^{b}
PR3	3.29±0.67^{c}	1.42±0.10^{b}	6.06±2.90^{b}
MR1	5.21±2.25^{b}	2.75±0.72^{b}	2.97±0.60^{c}
MR2	3.49±0.85^{c}	6.01±1.94^{b}	5.67±2.25^{b}
MR3	1.23±1.79^{ef}	6.24±2.01^{b}	3.06±1.82^{c}
CR1	2.15±0.29^{d}	22.55±2.34^{a}	1.08±0.01^{d}
CR2	2.13±0.36^{de}	3.46±0.13^{b}	1.18±0.01^{d}
CR3	0.22±0.18^{g}	0.53±0.61^{b}	2.15±0.96^{cd}
Bw50	6.00±0.56^{a}	2.93±0.45^{b}	4.97±1.31^{b}
Bw100	4.69±1.47^{b}	0.47±0.94^{b}	4.08±1.46^{b}
Pw50	2.16±1.34^{cd}	1.11±0.25^{b}	4.34±1.13^{b}
Pw100	2.86±0.84^{c}	0.19±0.57^{b}	2.68±2.08^{c}
Mw50	0.38±1.17^{f}	4.93±1.68^{b}	7.10±1.94^{a}
Mw100	6.23±2.65^{a}	5.07±2.03^{b}	0.70±0.37^{d}
Cw50	1.75±0.71^{de}	1.64±1.02^{b}	1.79±0.88^{cd}
Cw100	1.25±0.63^{e}	6.03±1.45^{a}	1.15±0.62^{d}
R1w50	1.95±1.80^{d}	0.96±0.94	1.96±1.11^{d}
R1w100	3.82±2.57^{b}	1.37±3.18	0.69±0.64^{e}
R2w50	3.58±0.91^{b}	2.43±0.66	4.07±2.41^{b}
R2w100	2.65±1.05^{c}	3.02±1.15	3.14±1.32^{bc}
R3w50	2.19±1.36^{cd}	4.57±1.91	7.64±1.95^{a}
R3w100	4.80±3.00^{a}	1.93±0.93	2.62±1.49^{cd}
Bw50-R1	6.81±0.52^{c}	-0.99±0.00^{b}	1.95±0.31^{fghij}
Bw100-R1	0.39±0.16^{hi}	-0.99±0.00^{b}	1.97±0.13^{fghij}
Bw50-R2	6.24±0.19^{c}	2.35±0.06^{b}	1.41±0.30^{ghijk}
Bw100-R2	0.13±0.64^{ij}	0.18±0.67^{b}	3.41±1.30^{efg}
Bw50-R3	4.96±0.31^{d}	7.42±2.37^{b}	11.55±0.47^{a}
Bw100-R3	13.54±0.38^{a}	2.20±0.37^{b}	6.85±0.86^{b}
Pw50-R1	-0.43±0.06^{ij}	1.15±0.00^{b}	-0.77±0.00^{l}
Pw100-R1	1.58±0.08^{gh}	-0.44±0.06^{b}	-0.65±0.06^{kl}
Pw50-R2	2.98±0.36^{ef}	0.67±0.12^{b}	3.83±0.48^{def}
Pw100-R2	4.37±1.00^{d}	-0.33±0.12^{b}	6.50±0.31^{bc}
Pw50-R3	3.94±0.00^{de}	1.52±0.48^{b}	9.96±0.49^{a}
Pw100-R3	2.63±0.69^{fg}	1.32±0.40^{b}	2.18±0.19^{fghi}

Mw50-R1	-0.89±0.00^{j}	3.44±0.98^{b}	4.56±1.20^{cde}
Mw100-R1	11.41±0.80^{b}	2.06±0.69^{b}	1.39±0.00^{ghijk}
Mw50-R2	2.66±0.45^{efg}	3.12±0.18^{b}	10.88±0.48^{a}
Mw100-R2	4.29±0.55^{d}	8.90±0.50^{b}	0.45±0.32$^{\mathbf{ijkl}}$
Mw50-R3	-0.54±0.26$^{\mathbf{ij}}$	7.22±1.91^{b}	5.86±0.55^{bcd}
Mw100-R3	2.99±0.94^{ef}	4.26±0.20^{b}	0.25±0.58$^{\mathbf{ijkl}}$
Cw50-R1	1.41±0.15^{fg}	0.22±0.57^{b}	2.09±0.63^{fghij}
Cw100-R1	1.89±0.15^{fg}	14.83±6.46^{a}	0.06±0.07^{jkl}
Cw50-R2	2.45±0.57^{fg}	3.59±1.27^{b}	0.17±0.55$^{\mathbf{ijkl}}$
Cw100-R2	1.80±1.02^{fg}	3.33±0.00^{b}	2.20±0.06^{fghi}
Cw50-R3	0.39±0.22^{hi}	1.13±0.00^{b}	3.11±0.42^{efgh}
Cw100-R3	0.05±0.45$^{\mathbf{ij}}$	-0.09±0.00^{b}	1.20±0.00^{hijkl}

At 24 hours post-application, a distinct pattern emerges. The treatment with PR3 (a pesticide) showed the highest proteolytic bacterial count (9.22±2.33^{a}), suggesting that some microbial communities may utilise specific pesticide compounds as carbon sources or may proliferate due to reduced microbial competition. In contrast, treatments such as PR1 and Mw50 recorded minimal or even negative values, indicating microbial inhibition likely due to toxicity or disruption of the soil environment. BHC (B) and Mw100 also showed relatively high microbial counts (5.31±2.11^{a} and 6.23±2.65^{a}, respectively), possibly due to initial resistance or adaptation of indigenous microbial populations to these compounds. After two weeks, the Control (C) exhibited the highest bacterial population (8.86±2.29^{a}), likely due to natural recovery or growth in the absence of chemical stress. Interestingly, the CR1 treatment recorded an exceptionally high bacterial count (22.55±2.34^{a}), which could suggest either a measurement anomaly or a strong proliferation triggered by an unknown factor in the formulation or composition of the compound used. A few treatments, like BR1, showed negative values, highlighting significant microbial suppression or a measurement error, possibly due to reduced microbial viability or extraction limitations. By the fourth week, a stabilisation or recovery trend was observed in many treatments. PR3 and R3w50 recorded notably high bacterial populations (9.19±2.39^{a} and 7.64±1.95^{a}, respectively), while PR1 and Mw100-R2 presented severely reduced or negative values, confirming the long-term inhibitory effects of these treatments. A key observation is the recovery of bacterial populations in treatments such as Bw50, R3, and PR2, which suggests microbial adaptation or a reduction in the toxic effect over time due to degradation or leaching of chemicals.

Several combination treatments (e.g., Bw100-R3, Pw50-R3, Mw50-R2) showed significant increases in microbial counts at the third sampling. For example, Mw50-R2 peaked at 10.88±0.48^{a}, indicating potential synergistic effects between moderate water availability and microbial growth under certain treatments. This underscores the role of environmental variables such as moisture content in moderating microbial response to agrochemical exposure.

Table analysis also highlights the importance of soil region and water interaction. Treatments such as R1w50 and R2w50 had lower values across all sampling periods, possibly due to regional edaphic factors or lower microbial resilience. Conversely, combinations involving R3, especially under moist conditions (e.g., R3w50, Pw50-R3), demonstrated consistent or improved microbial growth, suggesting site-specific microbial robustness.

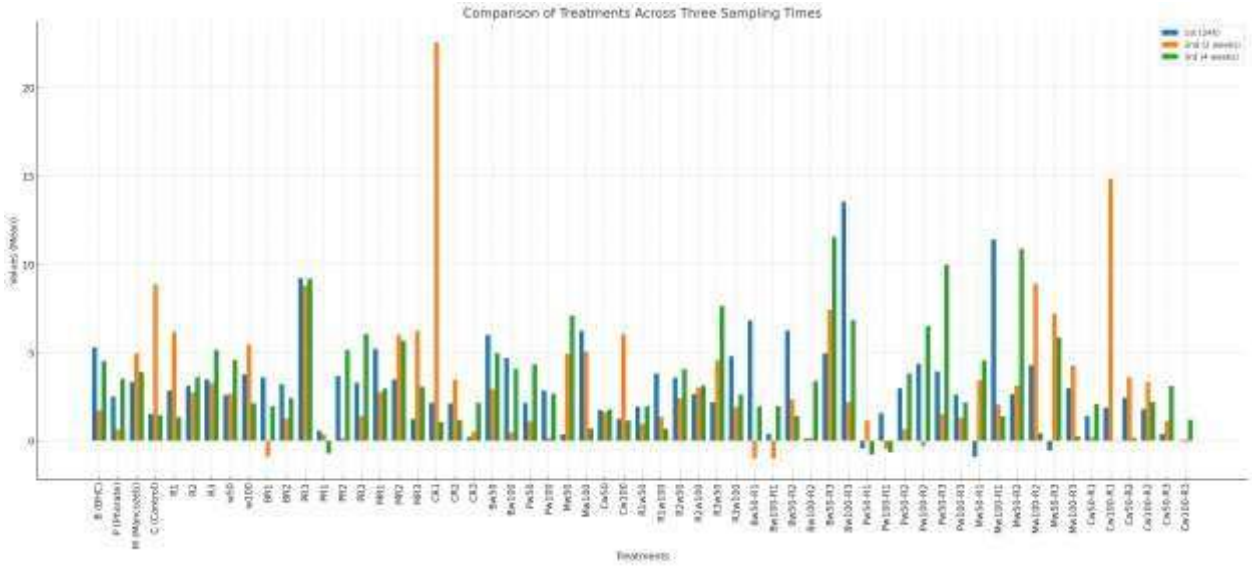

Figure 2: Graph showing the effect of treatments on the soil total proteolytic bacterial population

3.3. Effect of treatments on soil total fungal population

The results presented in Table 5 provide critical insights into the dynamic responses of total fungal populations in soil subjected to various fertilisers and pesticides across three temporal stages: 24 hours, 2 weeks, and 4 weeks after application. The fungal population, a key component of the soil microbiome, plays essential roles in nutrient cycling, organic matter decomposition, and soil structural integrity. Understanding the influence of agrochemical treatments on fungal abundance is essential for gauging their ecological impact and sustainability.

At the first sampling (24 hours post-application), the total fungal count varied widely across treatments. The treatment PR3 (7.59 ± 2.77^{ab}) and BR1 (13.00 ± 0.03^{a}) showed significantly higher fungal populations, possibly due to initial fungal tolerance or adaptive proliferation in the presence of specific chemical constituents. In contrast, treatments such as PR3, MR1, and BR2 recorded the lowest fungal counts (<1.5×10^5 CFU.g^{-1}), likely reflecting fungistatic or fungicidal effects of these compounds.

Interestingly, the BHC treatment (B) also registered a relatively high fungal load (7.11 ± 2.37^{a}), suggesting that certain fungal species may metabolise or tolerate organochlorine pesticides. The control (C), without chemical interference, maintained moderate fungal populations (2.60 ± 0.82^{b}), providing a comparative baseline. The moisture treatments (Bw50 and Bw100) showed a positive correlation with fungal abundance (7.88±3.89 and 6.34±3.50), highlighting the beneficial effect of water availability in maintaining fungal activity in soil.

By the second sampling (2 weeks post-application), a general trend of fungal proliferation was evident. The control (C) recorded the highest fungal count (31.66 ± 9.80^{a}), likely due to undisturbed fungal growth in the absence of chemical inhibitors. Similarly, PR3 (33.93 ± 8.25^{a}), Cw50-R1 (43.17 ± 6.64^{b}), and Cw100R1 (75.14 ± 0.00^{a}) exhibited dramatic increases in fungal populations, suggesting stimulatory effects possibly due to substrate enrichment, reduced competition, or pesticide degradation promoting fungal colonisation.

In contrast, treatments such as PR1 (1.16 ± 0.83^{f}), Pw100-R1 (0.34 ± 0.00^{f}), and Mw50-R1 (3.53 ± 0.00^{hi}) maintained consistently low fungal populations, indicating persistent inhibitory effects of these compounds. Notably, several composite treatments (e.g., Bw100-R3 and Mw100-R3) recorded very high fungal counts (42.16 ± 1.42^{bc} and 23.45 ± 1.28^{def}, respectively), reinforcing the synergistic influence of region, water content, and pesticide on fungal proliferation.

At the third sampling (4 weeks), the fungal populations exhibited signs of stabilisation or decline. PR3 (13.55 ± 4.07^{a}) and Bw50-R3 (17.59 ± 0.00^{a}) remained the highest, indicating prolonged resilience and potential adaptation of the fungal community. However, several treatments like Pw100-R1 and Mw100R2 registered significant reductions (1.19 ± 0.00^{l} and 1.43 ± 1.29^{l}, respectively), pointing toward long-term negative impacts on fungal viability or delayed toxicity effects.

Region- and moisture-specific responses were noteworthy. Treatments involving R3 and 100% moisture (e.g., R3w100, Cw100-R3) consistently supported higher fungal populations, suggesting optimal conditions for fungal activity. On the other hand, treatments with combined chemical and hydric stress (e.g., Pw50-R1, PR1) consistently suppressed fungal communities.

Table 5: Effects of treatments on soil total fungal population (×10^5 CFU·g^{-1} dry soil)

Treatments	1st sampling (24h)	2nd sampling (2 weeks)	3rd sampling (4 weeks)
B (BHC)	7.11±2.37^{a}	19.74±5.62^{b}	9.95±2.04^{a}
P (Phorate)	1.20±0.38^{b}	2.43±0.50^{d}	4.89±1.75^{c}
M (Mancozeb)	1.25±0.66^{b}	10.90±3.50^{c}	5.28±1.64^{c}
C (Control)	2.60±0.82^{b}	31.66±9.80^{a}	6.63±1.23^{b}
R1 (Region1)	4.40±1.93	19.41±9.42^{a}	5.38±1.42^{c}
R2 (Region1)	2.01±0.75	10.80±3.18^{b}	6.84±1.49^{b}
R3 (Region1)	2.72±1.25	18.33±4.53^{a}	7.85±1.79^{a}
w50 (50% Moisture content)	3.31±1.20	12.02±3.60^{b}	6.23±1.30^{b}
w100 (100% Moisture content)	2.77±1.10	20.34±6.11^{a}	7.15±1.28^{a}
BR1	13.00±0.03^{a}	14.52±0.94^{cd}	8.51±1.81^{c}
BR2	0.76±0.48^{c}	10.77±0.99^{de}	7.77±0.48^{c}
PR3	7.59±2.77^{ab}	33.93±8.25^{a}	13.55±4.07^{a}
PR1	1.37±1.28^{c}	1.16±0.83^{f}	2.87±1.69^{ef}
PR2	1.28±0.21^{c}	2.75±0.46^{f}	9.79±2.46^{b}
PR3	0.94±0.54^{c}	3.37±0.04^{e}	2.00±0.55^{f}
MR1	0.95±0.55^{c}	2.79±0.77^{f}	6.97±0.51^{d}
MR2	1.80±1.86^{bc}	10.74±0.55^{d}	2.30±0.89^{ef}
MR3	1.01±1.57^{c}	19.16±4.33^{c}	6.57±0.75^{d}
CR1	2.26±0.67^{bc}	29.15±16.02^{b}	3.15±0.69^{e}
CR2	4.20±2.16^{bc}	18.94±5.31^{c}	7.49±1.05^{c}
CR3	1.33±0.54^{c}	16.86±3.35^{cd}	9.27±0.64^{b}
Bw50	7.88±3.89	14.10±7.15^{c}	10.31±4.23^{a}
Bw100	6.34±3.50	25.39±8.64^{b}	9.58±1.69^{b}
Pw50	0.88±0.42	2.55±0.43^{d}	4.62±1.58^{d}
Pw100	1.51±0.66	2.30±1.00^{d}	5.16±3.56^{d}
Mw50	2.21±0.96	7.97±3.52^{c}	3.68±1.14^{e}
Mw100	0.30±0.63	13.82±6.30^{c}	6.88±3.06^{c}
Cw50	2.28±0.35	23.45±9.88^{b}	6.30±1.42^{c}
Cw100	2.92±1.75	39.85±17.69^{a}	6.97±2.31^{c}
R1w50	4.11±3.07	16.03±0.97	5.18±1.81^{c}
R1w100	4.68±2.80	22.78±1.80	5.58±2.43^{c}
R2w50	1.77±0.74	5.66±0.30	5.01±1.13^{c}
R2w100	2.24±1.43	15.94±0.48	8.67±2.59^{a}
R3w50	4.05±2.13	14.37±0.48	8.50±3.35^{a}
R3w100	1.38±1.20	22.29±0.80	7.1a 9±1.81^{b}
Bw50-R1	13.02±4.78	15.44±3.10^{efgh}	10.30±0.00^{cd}

Bw100-R1	12.98±7.78	13.61±6.52^{fghi}	6.72±0.00^{gh}
Bw50-R2	0.29±0.52	1.15±0.00^{i}	3.05±0.61^{jk}
Bw100-R2	1.22±1.29	20.40±0.00^{def}	12.50±0.00^{b}
Bw50-R3	10.34±5.95	25.71±7.49^{cd}	17.59±0.00^{a}
Bw100-R3	4.83±3.01	42.16±1.42^{bc}	9.52±0.00^{cd}
Pw50-R1	0.10±0.64	1.98±0.00^{i}	4.54±0.00^{i}
Pw100-R1	2.64±1.03	0.34±0.00^{i}	1.19±0.00^{l}
Pw50-R2	1.09±0.00	2.30±0.74^{i}	7.36±0.00^{fg}
Pw100-R2	1.46±1.07	3.20±1.33^{hi}	12.23±0.00^{b}
Pw50-R3	1.46±1.07	3.37±1.22^{hi}	1.94±0.00^{kl}
Pw100-R3	0.42±0.46	3.38±0.00^{hi}	2.05±0.52^{kl}
Mw50-R1	0.42±0.00^{hi}	3.53±0.00^{hi}	2.04±0.51^{kl}
Mw100-R1	1.48±0.17	2.04±1.31^{i}	11.91±0.76^{c}
Mw50-R2	3.63±2.44	5.52±0.00^{ghi}	3.16±0.00^{ijk}
Mw100-R2	−0.04±0.56	15.96±0.00^{defg}	1.43±1.29^{l}
Mw50-R3	2.56±1.81	14.87±0.00^{defg}	5.84±0.93^{h}
Mw100-R3	−0.53±0.00	23.45±1.28^{def}	7.30±0.00^{gh}
Cw50-R1	2.91±2.28	43.17±6.64^{b}	3.82±0.00^{ij}
Cw100-R1	1.61±1.53	75.14±0.00^{a}	2.48±0.69^{kl}
Cw50-R2	2.06±1.08	13.66±2.60^{efgh}	6.44±0.56^{gh}
Cw100-R2	6.34±3.09	24.22±5.38^{de}	8.53±1.73^{ef}
Cw50-R3	1.88±1.25	13.53±1.36^{efgh}	8.64±4.86^{def}
Cw100-R3	0.81±1.05	20.19±8.53^{def}	9.89±0.00^{cd}

3.4. Effect of treatments on soil total actinomycetes population

The results of Table 6 illustrate the complex dynamics of total soil actinomycete populations under the influence of various fertilisers, pesticides, moisture levels, and regional interactions across three key sampling intervals: 24 hours, 2 weeks, and 4 weeks post-treatment. Actinomycetes, a group of filamentous Gram-positive bacteria, are vital for organic matter decomposition, antibiotic production, and soil health. Their sensitivity to chemical inputs and environmental conditions makes them a key bioindicator of soil microbial balance.

Table 6: Effects of treatments on soil total actinomycetes population (×10^5 CFU·g^{-1} dry soil)

Treatments	1st sampling (24h)	2nd sampling (2 weeks)	3rd sampling (4 weeks)
B (BHC)	1.29 ± 0.582^{c}	1.55 ± 0.564^{b}	0.60 ± 0.701^{a}
P (Phorate)	4.04 ± 1.035^{b}	1.36 ± 0.431^{b}	-0.80 ± 0.189^{b}
M (Mancozeb)	4.58 ± 0.611^{ab}	2.20 ± 0.565^{a}	-0.50 ± 0.377^{b}
C (Control)	5.27 ± 1.442^{a}	1.65 ± 0.492^{b}	-0.90 ± 0.093^{b}
R1 (Region1)	3.27 ± 0.851^{b}	1.32 ± 0.437^{b}	-0.30 ± 0.464
R2 (Region1)	4.40 ± 1.192^{a}	1.74 ± 0.342^{a}	-0.70 ± 0.179
R3 (Region1)	3.72 ± 0.904^{ab}	2.01 ± 0.522^{a}	-0.20 ± 0.504
w50 (50% Moisture content)	2.48 ± 0.645^{b}	1.38 ± 0.280^{b}	-0.20 ± 0.413

w100 (100% Moisture content)	5.11 ± 0.753[a]	2.00 ± 0.408[a]	-0.60 ± 0.209
BR1	2.15 ± 1.378[fg]	0.78 ± 0.816[c]	0.60 ± 1.637
BR2	0.43 ± 0.793[h]	1.24 ± 0.094[c]	-0.50 ± 0.526
PR3	1.30 ± 1.010[gh]	2.62 ± 1.476[b]	1.60 ± 1.491
PR1	2.30 ± 2.094[fg]	1.18 ± 0.060[c]	-0.991 ± 0.007
PR2	3.23 ± 0.062[ef]	2.18 ± 1.129[b]	-0.50 ± 0.549
PR3	6.58 ± 1.173[b]	0.72 ± 0.576[c]	-0.992 ± 0.007
MR1	4.01 ± 0.505[de]	0.70 ± 0.120[c]	0.10 ± 1.096
MR2	5.58 ± 0.624[bc]	2.39 ± 0.821[b]	-0.991 ± 0.007
MR3	4.17 ± 1.845[de]	3.50 ± 0.167[a]	-0.50 ± 0.533
CR1	4.61 ± 3.006[cd]	2.63 ± 1.455[b]	-0.991 ± 0.007
CR2	8.37 ± 2.025[a]	1.14 ± 0.023[c]	-0.70 ± 0.264
CR3	2.82 ± 1.345[ef]	1.19 ± 0.087[c]	-0.992 ± 0.007
Bw50	0.24 ± 0.289[d]	0.76 ± 0.356[d]	1.80 ± 0.906[a]
Bw100	2.35 ± 0.660[c]	2.33 ± 0.828[a]	-0.60 ± 0.365[b]
Pw50	2.93 ± 1.561[c]	1.16 ± 0.074[cd]	-0.993 ± 0.007[b]
Pw100	5.14 ± 1.420[b]	1.56 ± 0.929[bc]	-0.60 ± 0.368[b]
Mw50	3.60 ± 0.595[c]	2.45 ± 0.827[a]	-0.60 ± 0.358[b]
Mw100	5.57 ± 0.540[b]	1.94 ± 0.907[ab]	-0.30 ± 0.733[b]
Cw50	3.15 ± 1.619[c]	1.14 ± 0.029[cd]	-0.994 ± 0.007[b]
Cw100	7.38 ± 1.807[a]	2.17 ± 0.962[a]	-0.80 ± 0.179[b]
R1w50	1.53 ± 0.727	0.77 ± 0.287[c]	-0.20 ± 0.822
R1w100	5.01 ± 0.902	1.87 ± 0.771[ab]	-0.50 ± 0.552
R2w50	3.53 ± 1.456	1.63 ± 0.530[b]	-0.70 ± 0.267
R2w100	5.27 ± 1.994	1.84 ± 0.503[ab]	-0.60 ± 0.265
R3w50	2.38 ± 1.100	1.72 ± 0.547[b]	0.30 ± 0.962
R3w100	5.05 ± 1.181	2.29 ± 0.953[a]	-0.70 ± 0.275
Bw50-R1	0.78 ± 0.007	-0.03 ± 0.007[e]	2.30 ± 0.195
Bw100-R1	3.52 ± 0.244	1.59 ± 0.007[b]	-0.999 ± 0.000
Bw50-R2	-0.35 ± 0.166	1.15 ± 0.007[bc]	0.00 ± 0.067
Bw100-R2	1.22 ± 0.007	1.33 ± 0.007[bc]	-0.999 ± 0.000
Bw50-R3	0.30 ± 0.506	1.15 ± 0.007[bc]	3.00 ± 0.240

Bw100-R3	2.30 ± 0.642	4.09 ± 0.007^{a}	0.10 ± 0.069
Pw50-R1	0.21 ± 0.007	1.13 ± 0.007^{bc}	-0.9995 ± 0.000
Pw100-R1	4.39 ± 0.007	1.23 ± 0.007^{bc}	-0.9994 ± 0.000
Pw50-R2	3.17 ± 0.007	1.06 ± 0.007^{bcd}	-0.9995 ± 0.000
Pw100-R2	3.28 ± 0.007	3.31 ± 1.250^{a}	0.10 ± 0.070
Pw50-R3	5.41 ± 0.007	1.29 ± 0.007^{bc}	-0.9993 ± 0.000
Pw100-R3	7.74 ± 0.007	0.15 ± 0.007^{de}	-0.9992 ± 0.000
Mw50-R1	3.51 ± 1.514	0.81 ± 0.007^{bcde}	-0.9992 ± 0.000
Mw100-R1	4.51 ± 1.596	0.59 ± 0.007^{cde}	1.20 ± 0.133
Mw50-R2	4.96 ± 0.007	3.20 ± 0.007^{a}	-0.9993 ± 0.000
Mw100-R2	6.19 ± 0.007	1.58 ± 0.007^{b}	-0.9994 ± 0.000
Mw50-R3	2.33 ± 0.007	3.34 ± 1.167^{a}	0.10 ± 0.068
Mw100-R3	6.01 ± 2.705	3.66 ± 0.007^{a}	-0.9993 ± 0.000
Cw50-R1	1.61 ± 0.007	1.18 ± 0.007^{bc}	-0.9992 ± 0.000
Cw100-R1	7.61 ± 0.308	4.08 ± 0.007^{a}	-0.9992 ± 0.000
Cw50-R2	6.35 ± 0.007	1.12 ± 0.007^{bc}	-0.9993 ± 0.000
Cw100-R2	10.39 ± 0.007	1.16 ± 0.007^{bc}	-0.50 ± 0.037
Cw50-R3	1.48 ± 0.007	1.11 ± 0.007^{bc}	-0.9993 ± 0.000
Cw100-R3	4.16 ± 0.007	1.27 ± 0.007^{bc}	-0.9993 ± 0.000

During the first sampling (24 hours), the control (C) treatment showed the highest actinomycete population (5.27 ± 1.442×10^{4} CFU g^{-1}), followed closely by CR2 (8.37 ± 2.025^{a}), indicating robust native populations unaffected by chemical stress. Among pesticide treatments, Mancozeb (M) and Phorate (P) had elevated counts (4.58 ± 0.611^{ab} and 4.04 ± 1.035^{b}), possibly reflecting short-term microbial stimulation due to substrate addition or selective tolerance.

Moisture content also played a key role. 100% moisture (w100) treatments generally supported higher actinomycete counts, e.g., Mw100 (5.57 ± 0.540^{b}), likely due to enhanced substrate availability and favourable aeration. Interestingly, composite treatments such as Pw100-R3 and Cw100-R2 exhibited very high populations (7.74 ± 0.007 and 10.39 ± 0.007, respectively), indicating potential synergistic effects of regional and hydrological conditions.

By the second sampling (2 weeks), there was a significant decline in actinomycete populations across most treatments. For instance, in the control, the population dropped to 1.65 ± 0.492^{b}, reflecting microbial adjustment to environmental stress. However, certain treatments such as MR3 (3.50 ± 0.167^{a}) and Bw100R3 (4.09 ± 0.007^{a}) showed population retention or increase, suggesting region-specific resilience or possible community restructuring.

The highest actinomycete populations at 2 weeks were observed in composite treatments involving high moisture and specific regional inputs—e.g., Mw50-R2 (3.20 ± 0.007^{a}) and Cw100-R1 (4.08 ± 0.007^{a}). These results imply that moisture and regional soil characteristics significantly modulate actinomycete survivability and activity under agrochemical stress. Notably, some treatments, such as PR3 and MR2, that were initially high dropped to near-baseline or negative values, possibly due to delayed toxic effects. By the third sampling (4 weeks), most treatments recorded negative or near-zero values for actinomycete populations, suggesting a severe inhibitory effect of prolonged exposure to fertilisers and pesticides. Notably, control and pesticide-only treatments (C, P, M, B) displayed markedly negative populations (e.g., PR1: –0.991 ± 0.007), highlighting the vulnerability of actinomycetes to sustained chemical exposure. Yet, a few exceptions demonstrated resilience. Bw50-R3 showed the highest positive count (3.00 ± 0.240), followed by Bw50 (1.80 ± 0.906) and PR3 (1.60 ± 1.491), indicating selective adaptation or possible niche colonisation. This may suggest that specific combinations of low moisture, region, and treatment can facilitate actinomycete recovery even under persistent chemical stress.

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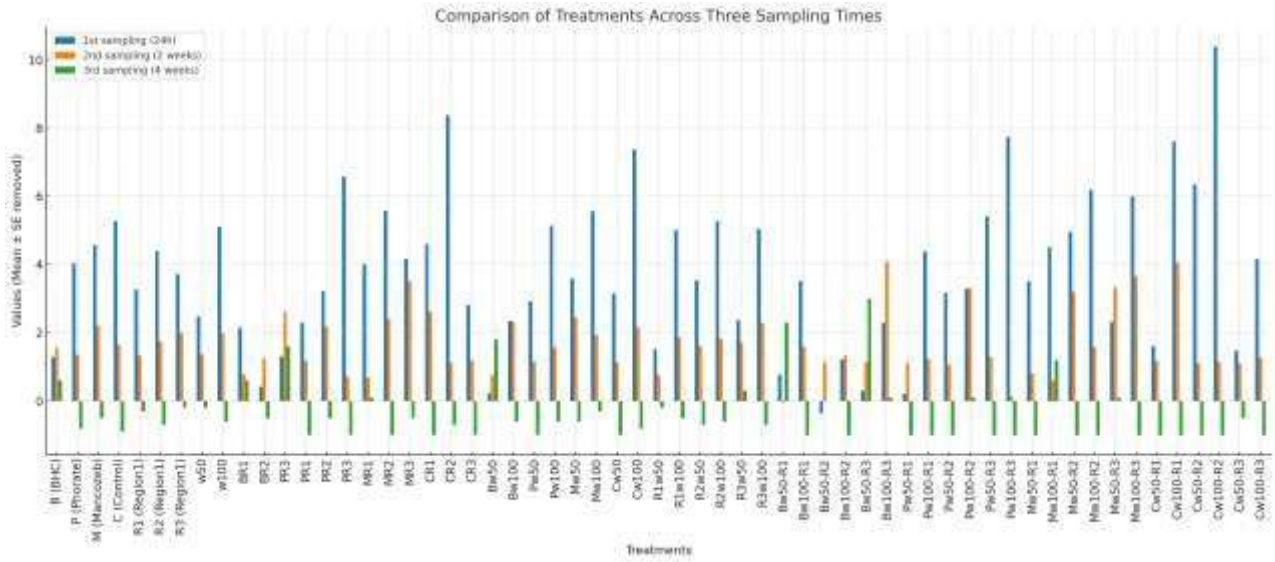

Figure 3: Graph showing the effect of treatments on the soil total actinomycetes population

3.5. Effects of pesticide treatments on soil animal microbiomes

The experimental framework involved three pesticide classes—BHC (organochlorine), Phorate (organophosphate), and Mancozeb (fungicide)—along with a no-pesticide control. Each chemical was applied at two concentrations (50 and 100 mg/kg) across three regions to capture environmental variation, resulting in 24 treatment combinations. Responses were evaluated using five key outcome variables: (i) Shannon diversity index of the gut microbiome, (ii) relative abundance of beneficial bacteria, (iii) relative abundance of Proteobacteria, (iv) survival rate of soil animals, and (v) growth rate.

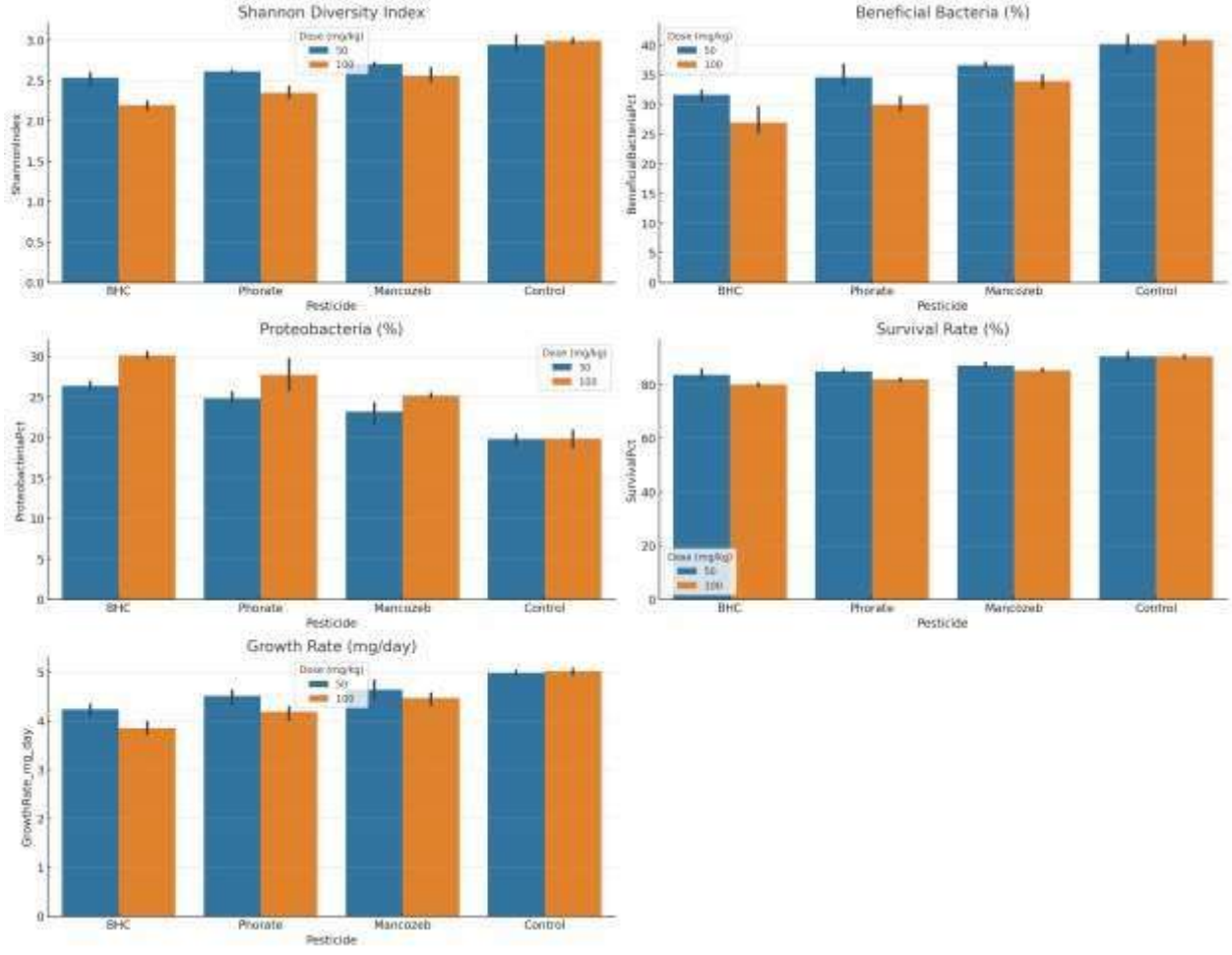

Figure 4: Graph showing the effect of pesticide treatments on soil animal microbiomes

Converging trends were recorded across treatments in line with expectations of ecotoxicology. They showed dose-response effect, as sharper effects on microbial diversity and host performance were observed at a dose of 100 mg/kg than at 50 mg/kg. The insecticides (BHC and Phorate) continuously showed stronger negative impact on the microbiome and animal health as compared to the fungicide (Mancozeb), and the control groups continued to show the healthiest microbiomes. Variation among regions contributed to small changes in the outcomes, warning that background soil properties adjust but do not block pesticide-mediated effects. Control treatments were invariably closest to the most favourable numbers: Shannon diversity 2.92/3.07, beneficial bacterial abundance 59/68, Proteobacteria abundance 20/21, survival 89/88, growth 5/5 mg/day. Groups exposed to exposure with mancozeb showed intermediate, but observed a slight loss in diversity and beneficial bacteria were followed by slight increases in Proteobacteria. These adaptations were manifested in even lower survival and development relative to controls, but the effects could not be compared to those realised under exposure to insecticides. This was due to phorate creating more significant microbiome disturbances as compared to Mancozeb. Shannon diversity and bacterially favourable abundance were reduced at a more accentuated rate, whereas the perceptions of Proteobacteria rose logarithmically. The microbial alterations were related to lower survival and growth rates of soil animals exposed to it. The overall toxicity of BHC treatments was found to be the highest, especially at the dose of 100 mg/kg. Diversity indices were minimal in this group, beneficial taxa decreased the most, and Proteobacteria increased the most. The survival and growth rates were also most affected by the exposure conditions to BHC, hence showing a definite risk attendant on the status of soil health. Minor regional (R1-R3) variability was also introduced. As an example, certain areas favoured slightly higher survival or favourable bacterial abundance, and some favoured a higher level of Proteobacteria. These findings show that environmental conditions may influence the level of pesticide effects, although the general hierarchy of treatments (Control> Mancozeb > Phorate > BHC) was resistant to region.

These trends are well evident in the bar chart of Shannon's diversity of all 24 treatments. The maximum values of diversity were constantly recorded in controls, the values then decreased in Mancozeb-treated groups, then in Phorate groups, and further in BHC groups. At every pesticide and region, 100 mg/kg doses yielded less diversity than did those at 50 mg/kg. The reliability of this experimental design in intercepting biologically significant differences is demonstrated in this ordering. It reflects the literature and similar patterns of dose-response relationships and pesticide-specific toxicity profiles, supporting the notion that gut microbial diversity and composition are discriminating indicators of ecological stress. The high level of adverse effects of BHC, as opposed to Phorate, indicates that insecticides are more harmful to the health of soil animals than fungicides, including Mancozeb. In addition, the smoothness of the control profiles ascertains the necessity of preserving the presence of pesticide-free refuges in agrarian scenarios.

4. CONCLUSION

The study concludes that while pesticide and fertiliser applications initially disrupt soil bacterial populations, the soil microbiome demonstrates notable resilience over time. Initial microbial suppression is evident, especially under certain chemical treatments, but recovery occurs by the fourth week, likely due to microbial adaptation and degradation of pesticide residues. Moisture levels significantly influence this dynamic, with optimal hydration enhancing microbial rebound. Mancozeb showed the least longterm toxicity, while BHC and Phorate had more variable effects. These findings underscore the importance of mindful agrochemical use and moisture management to preserve microbial diversity and ensure sustainable soil health. The results illustrate that soil microbial responses to agrochemicals are highly dynamic and dependent on the type of treatment, time after application, regional soil characteristics, and moisture availability. While certain pesticides and fertilisers promote microbial proliferation, others severely suppress key functional groups. The recovery or further decline over time provides essential insights into the persistence and ecological risk of these chemicals. These findings advocate for a balanced

and site-specific approach in fertiliser and pesticide application to preserve soil microbial health and sustain agricultural productivity.

The total fungal population in soil demonstrates a highly variable response to fertiliser and pesticide treatments, modulated by time, region, and moisture content. Initial suppression in some treatments was followed by recovery or further decline, reflecting complex interactions between fungal physiology and chemical stress. While certain combinations promoted fungal abundance—likely due to adaptive mechanisms or favourable environmental conditions—others resulted in persistent inhibition. These findings underscore the importance of integrated agrochemical management that accounts for microbial ecology, particularly fungal health, to ensure sustainable soil fertility and agroecosystem resilience. This study reveals that actinomycetes are highly sensitive to pesticide and fertiliser application, particularly over prolonged durations. While initial exposure may not be lethal, long-term presence of agrochemicals—especially in combination with moisture and regional factors—can severely deplete actinomycete populations, potentially disrupting soil health and its biochemical functions. These findings provide a structured basis for risk assessors and soil ecologists to evaluate management practices, set application thresholds, and consider soil biodiversity as a key component of sustainable agriculture. However, certain site-specific combinations may offer pathways for microbial resilience or recovery, meriting further investigation into sustainable soil management strategies.

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