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Effect of Soil pH on Fungal Community Dynamics and Biodegradation of Dimepax and Primextra in Loamy Sand Soil

[1]Eseigbe, D.A., [2]Imade, F.N, [3]Ogie-Odia, E.A., [4]Lawani, M.O,. [5]Igbinosa, N., [6]Ehilen,O.E
[7]Oseremen, M.N and [8]Gbrika,.E.C
[1, 2, 3, 4,6, 7, 8] Department of Plant Science and Biotechnology, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[5]Department of Microbiology, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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Abstract

Soil contamination with herbicides such as Dimepax and Primextra poses a threat to agricultural sustainability and environmental health. Indigenous soil fungi have shown potential for biodegradation of such pollutants, but their activity is often modulated by soil physicochemical factors, notably pH. This study investigates the influence of soil pH on the diversity and dynamics of fungal communities during the biodegradation of Dimepax and Primextra in loamy sand soils. Soil samples of varying pH (6.4, 6.8, 7.7) were collected and analyzed for indigenous microbial composition before herbicide application. Biodegradation experiments spanned 30 days, during which fungal propagule counts were measured at 1, 10, 20, and 30 days following herbicide application. Fungal isolates were identified morphologically and quantified via CFU counts. Initial microbial assessments revealed a diverse fungal community, with *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* spp. present across all soil pH levels. Herbicide application induced dynamic shifts in fungal abundance, with optimal biodegradation activity generally observed at neutral to slightly alkaline pH (6.8–7.7). Over time, key fungal species such as *A. niger*, *A. tamari*, *P. notatum*, and *Fusarium* sp. exhibited substantial increases in propagule counts, suggesting active participation in herbicide degradation. *Actinomycetes* emerged at later stages, indicating potential fungal-bacterial synergy. Soil pH significantly influences fungal community structure and herbicide biodegradation potential. The resilience and activity of indigenous fungi in neutral to slightly alkaline soils highlight their promise for bioremediation of herbicide-contaminated soils, particularly in environments with neutral to slightly alkaline pH conditions. Optimization of soil conditions could further enhance the effectiveness of fungal-based bioremediation strategies.

Corresponding Author: Ogie-Odia, E.A.; efosa.ogieodia@aauekpoma.edu.ng

Introduction

The widespread application of herbicides in modern agriculture has substantially improved crop yields by controlling weeds and enhancing land productivity. However, intensive herbicide use has also raised serious environmental concerns due to the persistence and accumulation of these chemicals in agricultural soils, where they may adversely affect non-target organisms, contaminate water sources, and pose risks to human health (De Corato, 2020; Hussain *et al*., 2022). Among widely used herbicides, Dimepax (a pre-emergence herbicide) and Primextra (a combination of atrazine and metolachlor) are extensively applied in Nigeria for controlling *Rottboellia* and other grass weeds in cereal and vegetable cropping systems. Unfortunately, both herbicides are known to exhibit long persistence in soil due to their slow degradation under natural conditions (Batista *et al*., 2020).

Biodegradation of herbicides by indigenous soil microorganisms represents one of the most promising, sustainable, and environmentally friendly strategies for mitigating herbicide contamination (Megharaj *et al*., 2011; Bhatt *et al*., 2020). Fungi and bacteria can enzymatically degrade a wide range of xenobiotics, including herbicides, converting them into less toxic or non-toxic metabolites (Ramu *et al*., 2020). Microbial biodegradation efficiency, however, is highly dependent on several soil physicochemical factors, notably pH, temperature, and moisture content, which can affect microbial community composition, enzyme activity, and herbicide bioavailability (Das and Adhya, 2015; Zhang *et al*., 2023). Soil pH is a critical determinant of microbial diversity and functionality. Fungal communities tend to tolerate and even thrive under a broader pH range than bacteria, though both are affected by extreme acidity or alkalinity (Rousidou *et al*., 2016). Several studies have reported enhanced herbicide degradation under near-neutral to slightly alkaline pH conditions, where both bacterial and fungal populations are most active (Alvarez *et al*., 2020; Singh & Singh, 2016).

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However, the optimal pH for microbial degradation can vary depending on the specific herbicide and local soil microbiome composition.

Temperature is another key environmental factor influencing microbial growth rates and enzymatic activity. Generally, higher temperatures up to an optimum range (~30–35°C) accelerate microbial metabolism and biodegradation processes (Lone *et al*., 2023). For example, *Aspergillus* spp. and *Bacillus* spp. are known to exhibit enhanced herbicide degradation potential at elevated temperatures (Das & Adhya, 2015; Alvarenga *et al*., 2019). Conversely, sub-optimal or fluctuating temperatures can slow degradation or lead to incomplete herbicide transformation.

Soil moisture also plays a vital role by affecting microbial mobility, substrate diffusion, and oxygen availability (Gavrilescu, 2005). In excessively dry soils, microbial activity is constrained due to desiccation stress, while saturated conditions may favour anaerobic pathways that are less efficient for herbicide degradation (Bhatt *et al*., 2020). Maintaining optimal moisture (field capacity) generally promotes the highest microbial activity and biodegradation rates.

In Nigeria's humid forest agroecosystems, where Primextra and Dimepax are routinely used, the influence of soil pH, temperature, and moisture on herbicide biodegradation remains poorly characterized. Furthermore, little is known about the dynamics of native fungal and bacterial populations involved in the degradation of these herbicides under varying environmental conditions. Several studies have focused primarily on bacterial degraders (Shushkova *et al*., 2018), while the role of fungi and fungal-bacterial interactions in herbicide biodegradation in tropical soils is still underexplored.

Recent reports highlight that fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* can play critical roles in degrading herbicides, often outperforming bacterial degraders under certain conditions (Alvarenga *et al*., 2019; Batista *et al*., 2020). Actinomycetes are also emerging as important contributors to herbicide biodegradation, producing diverse extracellular enzymes capable of breaking down complex chemical structures (Das and Adhya, 2015). Understanding how indigenous microbial communities respond to environmental gradients in pH, temperature, and moisture during herbicide degradation is therefore essential for developing effective bioremediation strategies tailored to local soil conditions. Such knowledge can inform practices to optimize natural attenuation processes or support bioaugmentation and biostimulation approaches (Hussain *et al*., 2022).

The objective of this study was to investigate the influence of key soil environmental factors namely pH, temperature, and moisture on the microbial biodegradation of two commonly used herbicides, Dimepax and Primextra, in loamy sand soils from Ekpoma, Nigeria. The study aimed to characterize fungal and bacterial community dynamics during herbicide degradation under varying soil pH conditions (6.4, 6.8, and 7.7), evaluate the effects of temperature (25°C and 35°C) on microbial degradation efficiency in both air-dried and moist soils, and assess the temporal progression of fungal, bacterial, and actinomycete populations over a 30-day incubation period. By addressing these factors, the research sought to enhance understanding of microbial processes that influence herbicide persistence and degradation in tropical agricultural soils.

Materials and Methods

Soil Collection and Sterilization

Soils for this study were collected from the top 15cm from three different locations in Ekpoma with the coordinates of 6° 44' 34.8" N and 6° 8' 25.044 E respectively. Ekpoma experiences a humid tropical climate. The annual rainfall in the area exceeds 2000mm with a bimodal distribution. The first peak occurs in July with monthly precipitation of 344.7mm and the second in September with 457.2mm. The highest mean monthly temperature of 291.1oC is recorded in March and the lowest of 24.4oC in June. The topography is undulating which has given the advantage to easy construction and connectivity of roads. These soils were tested for their pH by using a pH metre. Soil collected from the valley along Ukhun Road had a pH of 7.7, those collected from the College of Medicine had a pH of 6.8 while the soil collected from the Ihunmudumu area had a pH of 6.4.

All the apparatus employed in the medium preparation and isolation were thoroughly washed with detergent and sterilized in the oven at 150°C temperature for six (6) hours and allowed to cool before use. The apparatus used include Petri dishes, beakers, inoculating loop. The incubators were cleaned and sterilized with 80% acetone before use.

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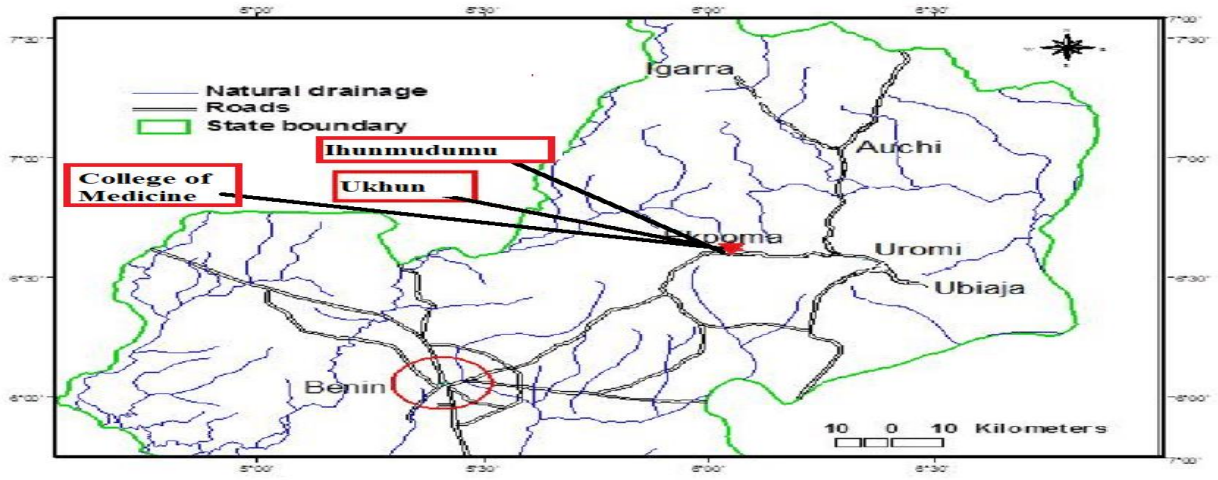

Fig. 1: Sketched map of Edo State showing highlighted locations under study in Ekpoma.

Culture medium

The mediums utilized for this work were potato dextrose agar (PDA) and yeast extract agar (YEA). PDA was prepared by using 250g of healthy Irish potato tubers which were peeled, sliced and boiled to a soft state. After being allowed to cool, the supernatant was carefully filtered through a sterile laboratory test. A sieve of 100um aperture, 10g of dextrose sugar and 20g of agar was added and made up to 1 litre with distilled water. The mixture was dispensed into 250ml conical flasks which were tightly covered with non-absorbent cotton wool and sealed with aluminium foil. The flask was autoclaved at 1.1kg/cm^2 (15Lb/sq inch) pressure for 15 minutes. The medium was allowed to cool to 50^{o}C before pouring into previously sterilized petri dishes and allowed to solidify.

YEA was prepared by using 250g of yeast extract which was put inside a beaker and 20g of agar was added and made up to 1 litre with distilled water. The mixture was dispensed into 250ml conical flasks. They were tightly covered with non-absorbent cotton wool and sealed with aluminium foil before sterilization in an autoclave at 1.1kg/cm^2 (15Lb/sq inch).

Effect of pH on Herbicide Degradation

A range of soil pH was selected to represent variation in soil pH across all the ecologies of the forest humid region of Edo state where Primextra and Dimepax may be used for control of *Rottboellia* and other grasses. The pH of these soils tested were 6.4, 6.8 and 7.7 in a box 1m x 1m and 15cm deep. Primextra and Dimepax were sprayed onto each of the soil samples at a rate of 3.0kg a.i per hectare before sampling. Each herbicide-treated soil was thoroughly mixed. 1g of soil sample was taken from each herbicide-treated soil and mixed with 9 ml of distilled water. From this dilution series of 10^{-2}, 10^{-3} and 10^{-4} were inoculated into the media of PDA and YEA in petri dishes using a standard loop of 0.02 ml and incubated at 28^{o}C±2 for 48-72 hours. These soil samples were platted for days 1, 10, 20 and 30 respectively and the results were compared to those of the control soil samples without herbicide application.

Each treatment was replicated three times (n = 3). Untreated control plots (without herbicide application) were included for comparison.

Effect of Temperature and Moisture on Herbicide Degradation

To evaluate the influence of temperature and moisture on the degradation of herbicides, soil samples were categorized into two conditions: air-dried (for 21 days) and fresh moist soil at field capacity. Each soil condition was treated with Primextra and Dimepax at a concentration of 3.0 kg active ingredient per hectare, equivalent to approximately 1.54 mg a.i./kg of soil, assuming a bulk density of 1.3 g/cm³. The treated soils were thoroughly mixed to ensure even distribution of the herbicides. The experiment was conducted under two temperature regimes namely 25°C and 35°C within controlled-environment incubators. Each combination of temperature, moisture level, and herbicide type was replicated three times (n = 3). Soil samples were collected at 1, 10, 20, and 30 days after treatment. For microbial analysis, 1 g of soil from each replicate was suspended in 9 mL of sterile distilled water and serially diluted to 10^{-2}, 10^{-3}, and 10^{-4}. A 0.02 mL aliquot from each dilution was inoculated onto PDA and YEA media and incubated at 28 ± 2°C for 48–72 hours. Microbial colony growth was recorded and compared to that of untreated control soils, which did not receive any herbicide application.

Statistical Analysis

All experiments were conducted using a completely randomized design (CRD) with three replicates (n = 3)

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per treatment. Data on microbial colony counts (CFU/mL) obtained from PDA and YEA plates were log-transformed to normalize the distribution before analysis. Analysis of Variance (ANOVA) is used where appropriate. All statistical analyses were performed using SPSS version 20

Results

Table 1: Microbial isolates from the three soil samples used for the experiment before herbicide application

Microbial groups	**Isolates**	**Soil pH**			**Propagules per gram soil x 10^6**		
		6.4	6.8	7.7	pH 6.4	pH 6.8	pH 7.7)
Fungal Isolates	*A. tamari*	+	+	+	6.5	4.5	6.5
	A. niger	+	+	+	5.5	4.5	6.5
	A. flavus	+	+	+	5.0	6.0	8.0
	Rhizopus sp.	+	+	+	5.0	5.0	6.0
	P.notatum	+	+	+	7.5	8.0	9.5
	Fusarium sp.	+	+	+	9.0	9.5	10.5
	Cladosporium sp.	-	+	+	-	3.5	5.0
					CFU/g Soil x 10^6		
Bacterial Isolates	*Bacillus* sp.	+	+	+	10.5	9.5	7.7
	E. helicola	+	+	+	5.0	5.5	7.5
	Xanthomonas sp.	+	+	+	3.0	4.5	6.0

CFU – Colony Forming Unit

Table 2. Effects of soil pH on fungal biodegradation of Dimepax in loamy sand soil (Propagules per gram soil × 10^6) over time.

Time After Treatment (days)	Fungal Isolate	Propagules per gram soil x 10^6		
		Soil pH 6.4	Soil pH 6.8	Soil pH 7.7
1	*A. flavus*	0.5	1.0	—
	A. tamari	1.0	0.5	—
	Fusarium sp.	0.5	—	1.0
	Rhizopus sp.	0.5	1.0	0.5
10	*A. niger*	1.5	—	0.5
	A. flavus	—	2.0	1.5
	A. tamari	1.5	0.5	—
	P. notatum	—	1.5	1.5
	Rhizopus sp.	1.0	1.5	1.0
20	*A. niger*	0.5	2.5	1.5
	A. tamari	2.0	1.5	2.0
	P. notatum	—	2.0	2.5
	Rhizopus sp.	1.5	2.0	1.5
	A. flavus	0.5	—	—
	Actinomycetes	0.5	—	—
30	*A. niger*	2.5	3.0	2.5
	A. tamari	2.5	2.0	2.5
	P. notatum	—	2.5	3.0
	Rhizopus sp.	2.0	2.0	2.0
	A. flavus	1.5	—	—
	Actinomycetes	2.0	1.0	0.5

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Table 3. Effects of soil pH on fungal biodegradation of Primextra in loamy sand soil (Propagules per gram soil × 10^6) over time.

Time After Treatment (days)	Fungal Isolate	Soil pH 6.4	Soil pH 6.8	Soil pH 7.7
1	*Aspergillus niger*	1.0	—	—
	Aspergillus flavus	—	1.0	1.0
	Aspergillus tamari	0.5	1.0	0.5
	Rhizopus sp.	1.0	0.5	0.5
10	*Aspergillus niger*	2.0	—	—
	Aspergillus flavus	—	1.5	1.5
	Aspergillus tamari	1.5	1.0	1.5
	Penicillium notatum	—	1.5	—
	Fusarium sp.	—	—	0.5
	Rhizopus sp.	1.0	1.5	1.0
20	*Aspergillus niger*	2.5	—	—
	Aspergillus flavus	—	2.0	2.0
	Aspergillus tamari	2.0	2.5	2.0
	Penicillium notatum	—	1.5	—
	Fusarium sp.	—	—	2.5
	Rhizopus sp.	1.5	2.0	1.5
	Aspergillus flavus	1.0	—	—
	Actinomycetes	1.5	2.0	0.5
30	*Aspergillus niger*	3.0	—	—
	Aspergillus flavus	—	2.5	2.5
	Aspergillus tamari	2.5	3.0	2.5
	Penicillium notatum	—	2.5	—
	Fusarium sp.	—	—	3.0
	Rhizopus sp.	2.0	2.5	2.0
	Aspergillus flavus	2.0	—	—
	Actinomycetes	2.5	2.0	1.5

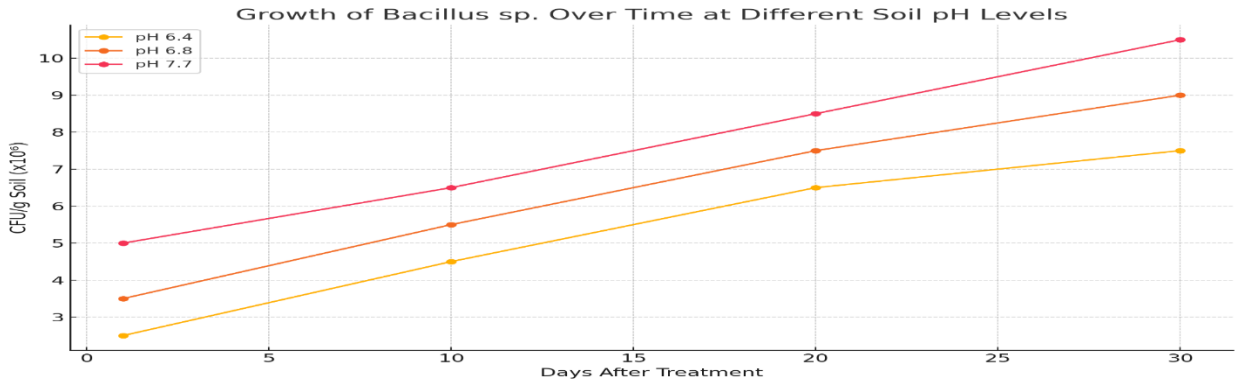

Fig 2: Effects of soil pH on bacterial biodegradation of Dimepax in loamy sand soil

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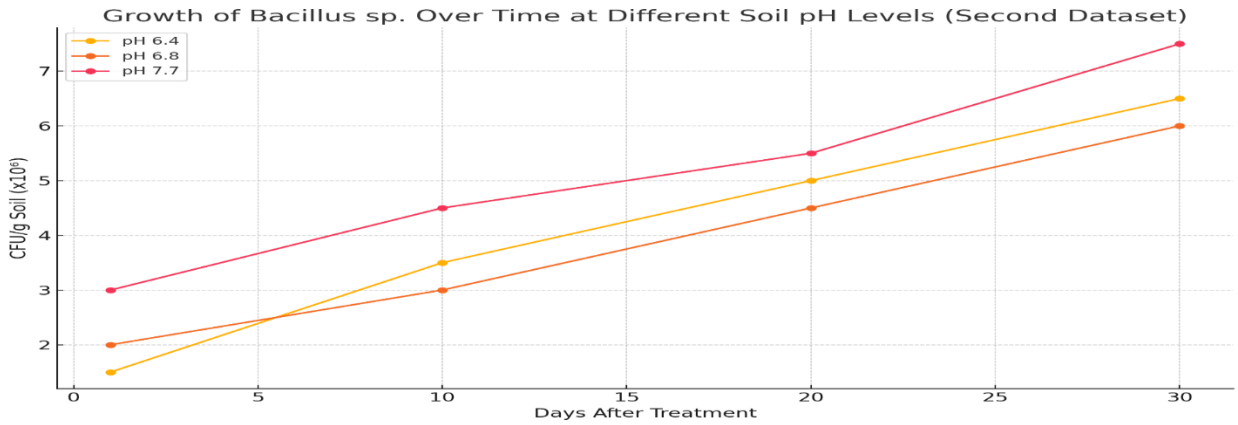

Fig 3: Effects of soil pH on bacterial biodegradation of Primextra in loamy sand soil

Table 4: Effects of temperature on fungal biodegradation of Dimepax in dry loamy sand soil

Time(days) After treatment	**Fungal Isolates**	**Propagules per gram soil x 10^6**	
		Temperature (°C)	
1	*Aspergillus niger*	25	35
	Aspergillus flavus	1.0	0.5
	Rhizopus sp.	0.5	0.5
10	*Aspergillus niger*	1.5	1.0
	Aspergillus flavus	1.5	1.5
	Rhizopus sp.	1.0	1.0
	Fusarium sp.	1.0	-
20	*Aspergillus niger*	2.0	2.5
	Aspergillus flavus	3.0	2.0
	Rhizopus sp.	1.5	0.5
	Fusarium sp.	1.5	1.5
30	*Aspergillus niger*	3.0	3.5
	Aspergillus flavus	3.5	4.0
	Rhizopus sp.	2.0	2.0
	Fusarium sp.	2.0	3.5
	Actinomycetes	1.0	1.5

Table 5: Effects of temperature on fungal biodegradation of Primextra in dry loamy sand soil

Time (days) After treatment	**Fungal Isolates**	**Propagules per gram soil x 10^6**	
		Temperature (°C)	
1	*Aspergillus flavus*	25	35
	Aspergillus niger	0.5	0.5
	Aspergillus tamarri	1.0	0.5
	Penicillium notatum	0.5	-
10	*Aspergillus flavus*	1.5	1.5
	Aspergillus niger	1.0	2.0

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	Aspergillus tamarri	1.5	-
	Penicillium notatum	1.0	-
	Rhizopus sp.	0.5	1.0
20	*Aspergillus flavus*	2.0	2.5
	Aspergillus niger	2.0	2.5
	Aspergillus tamarri	2.5	-
	Rhizopus sp.	1.0	1.5
	Fusarium	-	1.0
	Actinomycetes	-	0.5
30	*Aspergillus flavus*	2.5	3.5
	Aspergillus niger	2.5	3.0
	Aspergillus tamari	3.0	-
	Rhizopus sp.	1.5	2.0
	Fusarium sp.	-	2.0
	Actinomycetes	2.0	1.5

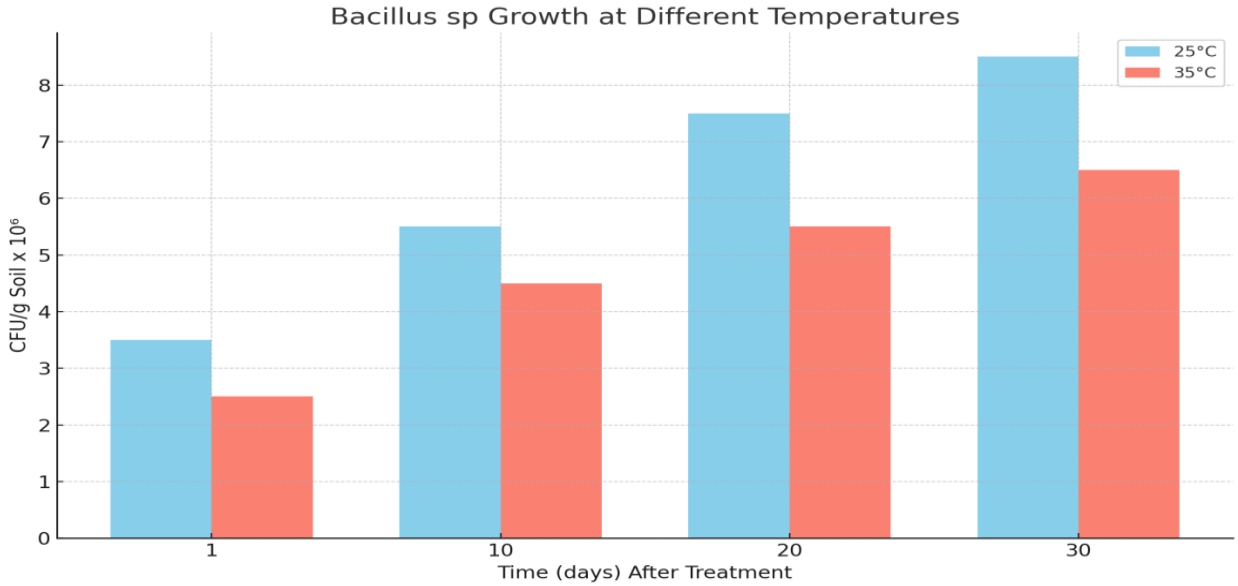

Fig 4: Effects of temperature on bacterial biodegradation of Dimepax in dry loamy sand soil

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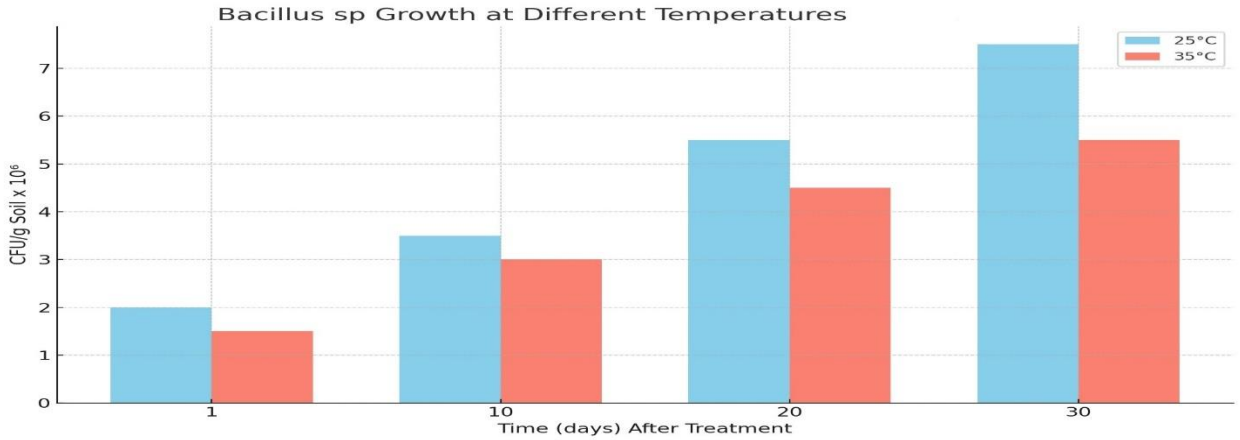

Fig 5: Effects of temperature on bacterial biodegradation of Primextra in dry loamy sand soil.

Table 6: Effect of temperature on fungal biodegradation of Dimepax in wet loamy sand soil

Time (days) After treatment	**Fungal Isolates**	**Propagules per gram soil x 10^6**	
		Temperature (°C)	
1	*Aspergillus flavus*	25	35
	Fusarium sp.	0.5	-
	Rhizopus sp.	0.5	1.0
10	*Aspergillus flavus*	1.5	2.0
	Fusarium sp.	1.0	0.5
	Rhizopus sp.	1.0	1.5
	Penicillium notatum	0.5	0.5
20	*Aspergillus flavus*	2.5	2.5
	Fusarium sp.	2.0	1.5
	Rhizopus sp.	1.5	2.0
	Penicillium notatum	1.5	1.5
	-	-	1.0
	Actinomycetes	0.5	1.0
30	*Aspergillus flavus*	3.0	3.5
	Fusarium sp.	2.5	2.5
	Rhizopus sp.	2.0	2.5
	Penicillium notatum	2.5	2.0
	Actinomycetes	2.0	2.5

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Table 7: Effect of temperature on fungal biodegradation of Primextra in wet loamy sand soil

Time (days) After treatment	**Fungal Isolates**	**Propagules per gram soil x 10^6**	
		Temperature (°C)	
		25	25
1	*Aspergillus flavus*	0.5	0.5
	Furasium sp.	0.5	1.0
	Rhizopus sp.	0.5	0.5
10	*Aspergillus flavus*	1.5	1.5
	Fusarium sp.	1.0	0.5
	Rhizopus sp.	1.0	1.5
	Aspergillus niger	1.0	0.5
20	*Aspergillus flavus*	2.5	2.5
	Fusarium sp.	2.0	2.0
	Rhizopus sp.	1.5	2.0
	Aspergillus niger	1.5	1.0
	Actinomycetes	0.5	1.5
30	*Aspergillus flavus*	2.5	2.5
	Fusarium sp.	2.5	2.0
	Rhizopus sp.	2.0	2.0
	Aspergillus niger	2.0	1.5
	Actinomycetes	2.0	2.5

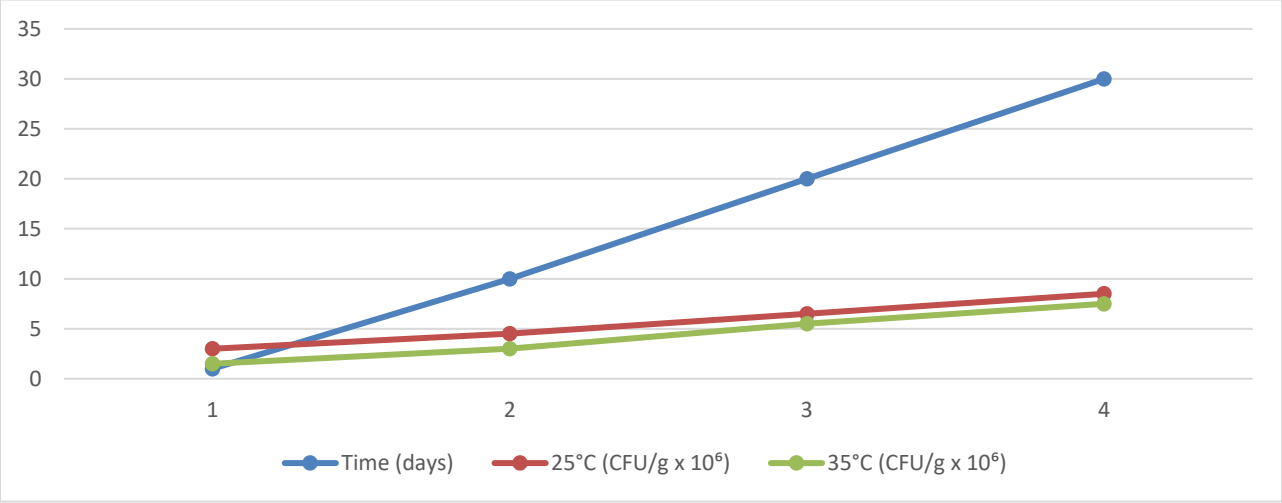

Fig 6: Effect of temperature on bacterial biodegradation of Dimepax in wet loamy sand soil

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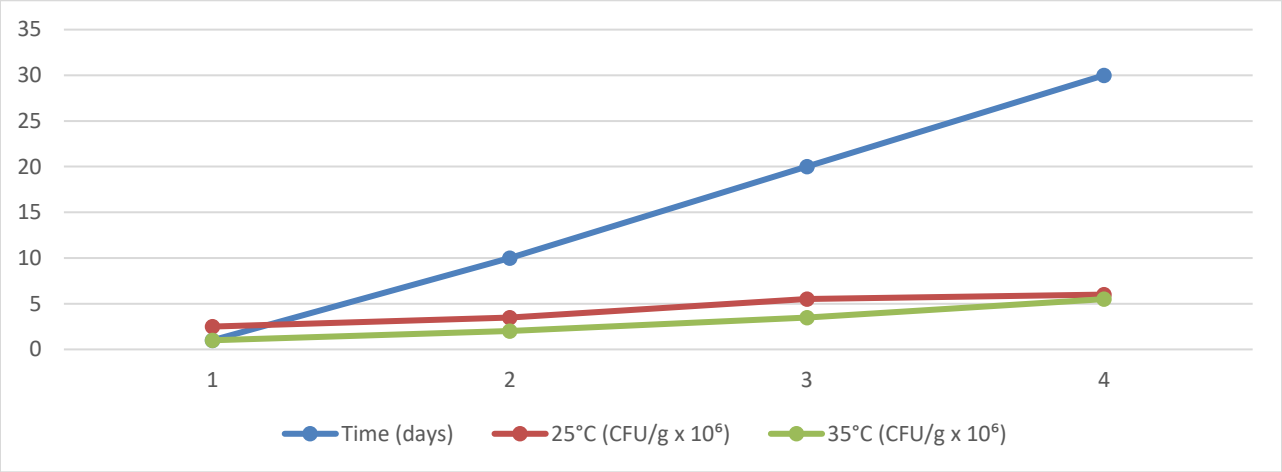

Fig 7: Effect of temperature on bacterial biodegradation of Primextra in wet loamy sand soil

The initial assessment of the microbial communities in the three soil samples, varying in pH (6.4, 6.8, and 7.7), revealed a diverse range of fungal and bacterial isolates (Table 1). Among fungal isolates, *Aspergillus tamari*, *A. niger*, *A. flavus*, *Rhizopus* sp., *Penicillium notatum*, and *Fusarium* sp. were consistently detected across all pH conditions. *Cladosporium* sp. was absent at pH 6.4 but present at higher pH levels. Fungal propagule counts were generally highest at pH 7.7, with *Fusarium* sp. exhibiting the greatest abundance (10.5×10^6 CFU/g soil), followed by *P. notatum* (9.5×10^6 CFU/g soil). In contrast, lower propagule counts were observed at pH 6.4 for most isolates. Bacterial isolates also exhibited broad distribution across all pH levels, with *Bacillus* sp. displaying the highest abundance at pH 6.4 (10.5×10^6 CFU/g soil), which decreased with increasing pH. Other bacterial isolates included *Enterobacter helicola* and *Xanthomonas* sp., both showing moderate propagule counts that tended to increase at higher pH levels (Table 1).

The dynamics of fungal populations during Dimepax biodegradation were strongly influenced by soil pH and incubation time (Table 2). At 1 day post-treatment, fungal activity was relatively low across all pH levels. *A. tamari* and *A. flavus* exhibited higher propagule counts at pH 6.4 and 6.8, while *Fusarium* sp. was notably active at pH 7.7. By day 10, there was a marked increase in fungal activity, particularly for *A. flavus* (2.0×10^6 at pH 6.8 and 1.5×10^6 at pH 7.7), *A. niger* (1.5×10^6 at pH 6.4), and *P. notatum* (1.5×10^6 at both pH 6.8 and 7.7). At 20 days, fungal populations continued to expand, with *A. tamari* reaching up to 2.0×10^6 at pH 6.4 and 2.0×10^6 at pH 7.7. The appearance of *Actinomycetes* at pH 6.4 and 6.8 ($0.5–1.0 \times 10^6$) indicated a possible complementary role in biodegradation. By day 30, maximum fungal propagule counts were recorded, particularly for *A. niger* ($2.5–3.0 \times 10^6$), *A. tamari* (2.5×10^6 across all pH levels), and *P. notatum* (up to 3.0×10^6 at pH 7.7). *Actinomycetes* populations also increased, suggesting progressive community involvement in herbicide degradation.

Fungal biodegradation of Primextra followed a similar pH- and time-dependent pattern (Table 3). On day 1, *A. niger* and *A. tamari* demonstrated initial activity at pH 6.4 and 6.8, while *A. flavus* and *Rhizopus* sp. were active across all pH levels. By day 10, fungal activity significantly increased, with *A. niger* (2.0×10^6 at pH 6.4), *A. flavus* (1.5×10^6 at both pH 6.8 and 7.7), and *A. tamari* (1.5×10^6 at both pH 6.4 and 7.7) dominating. Notably, *Fusarium* sp. began to appear at pH 7.7. At 20 days, fungal populations were sustained or further elevated, with *A. niger* reaching 2.5×10^6 at pH 6.4, *A. tamari* peaking at 2.5×10^6 at pH 6.8, and *Fusarium* sp. rising to 2.5×10^6 at pH 7.7. The detection of *Actinomycetes* at all pH levels indicated an emerging role in the degradation process. By day 30, peak fungal populations were recorded: *A. niger* (3.0×10^6 at pH 6.4), *A. tamari* (up to 3.0×10^6 at pH 6.8), and *Fusarium* sp. (3.0×10^6 at pH 7.7). *Actinomycetes* populations also increased, especially at pH 6.4 (2.5×10^6). These results underscore the influence of soil pH and time on fungal community dynamics during Primextra biodegradation.

Bacterial response to Dimepax and Primextra treatment is shown in Figures 2 and 3. In general, bacterial counts increased over time, with *Bacillus* sp. showing particularly robust growth across all pH levels under both herbicide treatments. The results indicate that bacteria, alongside fungi, actively participate in the biodegradation process, with soil pH influencing the rate and extent of their response.

Temperature significantly influenced fungal biodegradation efficiency in dry loamy sand soil. Under Dimepax treatment (Table 4), fungal propagule counts increased progressively with time at both 25°C

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and 35°C, with generally higher counts recorded at 35°C by day 30. *A. flavus*, *A. niger*, *Rhizopus* sp., and *Fusarium* sp. were the dominant fungi, with *A. flavus* reaching 4.0 × 10^6 CFU/g at 35°C on day 30. For Primextra (Table 5), similar trends were observed. *A. flavus*, *A. niger*, *A. tamari*, *Rhizopus* sp., and *Fusarium* sp. demonstrated increased propagule counts over time, with temperature-dependent differences. At 35°C, *A. flavus* and *A. niger* exhibited the highest counts, suggesting that elevated temperature may enhance fungal enzymatic activity involved in Primextra degradation. Temperature significantly influenced fungal biodegradation efficiency in dry loamy sand soil. Under Dimepax treatment (Table 4), fungal propagule counts increased progressively with time at both 25°C and 35°C, with generally higher counts recorded at 35°C by day 30. *A. flavus*, *A. niger*, *Rhizopus* sp., and *Fusarium* sp. were the dominant fungi, with *A. flavus* reaching 4.0 × 10^6 CFU/g at 35°C on day 30.

Figures 4 and 5 depict bacterial biodegradation dynamics under Dimepax and Primextra treatment at different temperatures. Increased bacterial activity was observed at 35°C compared to 25°C, particularly among *Bacillus* sp. and other dominant isolates, indicating temperature-enhanced bacterial degradation capacity. In wet loamy sand soil, fungal community dynamics during Dimepax degradation (Table 6) showed similar trends to those observed in dry soil. *A. flavus*, *Fusarium* sp., *Rhizopus* sp., and *P. notatum* exhibited progressive increases in propagule counts at both temperatures, with slightly enhanced counts at 35°C. *Actinomycetes* also emerged during the later stages (days 20–30), suggesting synergistic fungal-bacterial interactions.

Under Primextra treatment in wet soil (Table 7), *A. flavus*, *Fusarium* sp., *Rhizopus* sp., and *A. niger* exhibited increasing propagule counts over time. Notably, *Actinomycetes* also showed marked increases by day 30, particularly at 35°C, further supporting the role of microbial synergy in ineffective herbicide degradation. Figures 6 and 7 illustrate bacterial dynamics during Dimepax and Primextra degradation in wet soil. As in dry soil, bacterial propagule counts increased progressively, with higher values observed at 35°C. These results indicate that bacterial activity, like that of fungi, is enhanced by elevated temperature, contributing to the overall biodegradation process.

Discussion and Conclusion

This study demonstrates that soil pH and temperature are key environmental factors shaping the dynamics of fungal and bacterial communities involved in the biodegradation of Dimepax and Primextra herbicides in loamy sand soil systems. Our findings align with and extend previous reports on the critical role of abiotic factors in modulating microbial activity and degradation efficiency of xenobiotic compounds in soil ecosystems (Zhang *et al*., 2023; Singh *et al*., 2021). The initial assessment of soil microbial communities showed that both fungal and bacterial taxa were broadly distributed across the three tested pH levels, but their abundance and activity patterns varied with pH. Fungal isolates such as *Aspergillus tamari*, *A. niger*, *A. flavus*, *Rhizopus* sp., *Penicillium notatum*, and *Fusarium* sp. dominated across all pH conditions, consistent with their known resilience in a range of soil environments and their established role in herbicide biodegradation (Zhao *et al*., 2020). Higher fungal propagule counts at pH 7.7 suggest that slightly alkaline conditions may support optimal fungal growth and enzymatic activity, facilitating effective herbicide degradation. Similar pH preferences have been reported for *Fusarium* and *Penicillium* species during the biodegradation of organophosphate and chlorinated herbicides (Ghosh *et al*., 2021).

The progressive increase in fungal propagule counts during Dimepax and Primextra treatment further underscores the time-dependent adaptation and proliferation of fungal degraders under favorable pH conditions. The appearance and rise of *Actinomycetes* populations during the later stages of treatment suggest a successional pattern, wherein bacteria and fungi collaborate to enhance biodegradation efficiency (Chakraborty *et al*., 2023). The complementary roles of these microbial groups are supported by recent findings demonstrating cross-kingdom cooperation during the breakdown of complex agrochemicals in soil (Li *et al*., 2022). Bacterial community responses mirrored those of fungi, with *Bacillus* sp. consistently exhibiting robust growth across all pH levels under both herbicide treatments. *Bacillus* spp. are well-documented for their capacity to degrade diverse herbicide classes via multiple enzymatic pathways (Meena *et al*., 2024), and their increasing abundance in this study indicates their substantial contribution to the overall biodegradation process. The modulation of bacterial activity by pH further highlights the importance of maintaining near-neutral to slightly alkaline soil pH for effective bioremediation interventions (Niu *et al*., 2023).

Temperature had a significant impact on both fungal and bacterial biodegradation dynamics in both dry and wet loamy sand soils. In dry soil, higher propagule counts of *A. flavus*, *A. niger*, *Rhizopus* sp., and *Fusarium* sp. at 35°C compared to 25°C suggest that elevated temperatures enhance fungal metabolic activity and enzymatic efficiency. These findings are consistent with prior work showing that thermophilic fungi exhibit increased ligninolytic and hydrolytic

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enzyme activity under higher temperatures, facilitating more efficient herbicide degradation (Xu *et al*., 2022). Likewise, bacterial communities showed temperature-enhanced activity, with greater propagule counts recorded at 35°C. This is in line with the metabolic plasticity of bacteria such as *Bacillus* sp., which can modulate enzyme production and biofilm formation in response to thermal cues (Yang *et al*., 2021). The consistent patterns observed in both dry and wet soils suggest that temperature-driven metabolic acceleration is a key factor in enhancing the biodegradation capacity of soil microbial communities. Interestingly, the emergence of *Actinomycetes* in both dry and wet soils, particularly at elevated temperatures, highlights their potential role in complementing fungal activity. Recent studies have shown that Actinomycetes can contribute to the final breakdown of herbicide metabolites, thereby reducing the persistence of toxic residues (Rai *et al*., 2024). Their late-stage appearance in this study suggests that they may play a crucial role in the secondary degradation of intermediate metabolites, complementing the primary enzymatic actions of fungi and bacteria.

Collectively, these findings emphasize that optimizing soil pH and temperature can substantially improve the efficiency of microbial degradation of herbicides like Dimepax and Primextra. Maintaining a slightly alkaline pH and promoting warm soil conditions (≥35°C) could accelerate microbial-driven detoxification processes, reducing the risk of herbicide accumulation in agricultural soils. Moreover, the demonstrated involvement of diverse microbial taxa including fungi, bacteria, and Actinomycetes supports the concept of community-level biodegradation, where synergistic interactions among taxa enhance degradation outcomes (Zhang *et al*., 2024). This suggests that future bioremediation strategies should leverage microbial consortia, rather than single strains, to maximize degradation efficiency across varying soil environments.

This study demonstrated that soil pH and incubation time significantly influence fungal community dynamics and herbicide biodegradation in loamy sand soils. Indigenous fungal isolates particularly *A. niger*, *A. tamari*, *A. flavus*, *P. notatum*, *Fusarium* sp., and *Rhizopus* sp. exhibited considerable resilience and biodegradation potential in the presence of Dimepax and Primextra. Neutral to slightly alkaline soil pH (6.8–7.7) generally supported higher fungal propagule counts and more sustained biodegradation activity. The emergence of *Actinomycetes* at later stages suggests a possible synergistic role between fungi and actinobacteria in herbicide degradation.

These findings highlight the adaptability of indigenous fungal communities under herbicide stress and underscore the importance of optimizing soil pH to enhance bioremediation outcomes. The study provides a strong basis for further research into fungal-based or integrated microbial strategies for remediating herbicide-contaminated soils and the focus should be on elucidating the specific enzymatic pathways utilized by dominant microbial taxa under different pH and temperature regimes. Scaling these findings to field-level applications will be essential for developing effective, practical bioremediation interventions for contaminated agricultural soils.

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