

World News of Natural Sciences

An International Scientific Journal

WNOFNS 64 (2026) 275-289

EISSN 2543-5426

A preliminary comparative study of root – associated microbial communities in plantain (*Musa paradisiaca* L.) grown in natural and herbicide polluted soils in Toru Orua, Bayelsa State

**Philips Ibuomotimi Asaigbe^{1,2,*}, Alice Ebimoboere Manda^{1,3},
and Funlayefa Atoukudu¹**

¹ Department of Biological Sciences, University of Africa, Toru-Orua, Bayelsa, Nigeria

² Department of Biotechnology, University of Africa, Toru-Orua, Bayelsa, Nigeria

³ Bio-Sciences Laboratory, University of Africa, Toru-Orua, Bayelsa, Nigeria

*E-mail address: philips.asaigbe@uat.edu.ng

ABSTRACT

This preliminary comparative study was conducted to assess the impact of herbicides on the microbial communities of plantain (*Musa paradisiaca* L.). Rhizospheric samples were collected from natural and herbicide polluted soils within the campus of the University of Africa, Toru-Orua, Bayelsa State, Nigeria. Samples were serially diluted where 10^{-6} and 10^{-7} were adopted. Microbial populations in both herbicide polluted soil (HP) and Normal Soil (NS) were carefully compared on PDA, MAC and NA media and morphological characteristics differences were carefully analysed. The results revealed clear differences between HP soil and NS. The HP soil sample had a significantly higher overall microbial load and species richness compared to the NS sample. Total culturable bacteria in HP soil made up 43.75% of the isolates while NS soil had 25%. In HP soil, fungi constituted 25% of the isolates while only 6.25% fungi were found in NS soil. Key organisms isolated include *Cryptococcus* spp. and *Fusarium* spp. (pathogenic) were more in occurrence than other commensal fungi such as *Rhizopus* spp., *Basidiomycetes*, *Aspergillus* spp. where *Basidiomycetes* which are known decomposers. Bacterial isolates are *Micrococcus luteus* (mostly abundant) *Enterobacter* spp, *Enterobacter aerogenes*, *Bacillus* spp., *Proteus* spp. and *Pseudomonas* spp. While studies have reported a decline in overall microbial presence and diversity after herbicide use due to general toxicity, the increased abundance and richness in the HP soil reflects an ecological trend of selective adaptation as previously reported by a few authors.

The study concludes that the use of herbicides as effective weed control, compromises the microbial balance of the plantain's rhizosphere. Thus, herbicide application does not directly destroy the plant microbiome but creates an imbalance that lead to the elimination of organisms which are not tolerant and the subsequent selective promotion of herbicide degrading microorganisms.

Keywords: Plantain, Rhizosphere, Herbicide pollution, Plant microbiome, Food Security

1. INTRODUCTION

Soil microbes are an integral part of sustainable plant health, influencing nutrient cycling, disease resistance, growth regulation, and ecological balance. The rhizosphere, which is the narrow region of soil occupied by plant roots and other associated microorganisms, serve as a dynamic interface between plants and the microbial community. This microbial community differs vastly from plant to plant, influenced by location, soil type and other adjourning factors. Wild and cultivated plant species may also have differences in their microbiota due to factors such as domestication, soil management practices, and plant genotype.

In plantain (*Musa paradiasica* L.), like other perennial crops, root-associated microbial communities play vital roles in nutrient cycling, plant growth, nitrogen fixation, and suppression of soil-borne pathogens (Van der Heijden et al., 2008; Teke & Akowei., 2023a). These microorganisms include bacteria, fungi, actinomycetes, and other microflora forming symbiotic relationships with plant roots. However, anthropogenic activities such as the indiscriminate use of herbicides have raised concerns regarding the long-term effects on soil microbial diversity and functions (Cycon, et al., 2013). Herbicides, although beneficial for weed control, can persist in soil, disrupt microbial balance and reduce biodiversity, ultimately affecting plant health and productivity (Ahemad & Kibret, 2014). In contrast, natural soils that are less chemically disturbed typically support more robust microbial ecosystems.

Plantain (*M. paradiasica.*) is not just a staple food crop; it's also a vital source of income for countless smallholder farmers across sub-Saharan Africa (Kaushal, et al., 2022). In Nigeria, plantain is an important staple and economic crop which depends heavily on soil microbial interactions for optimal growth due to its high nutrient demands. Its cultivation is popular in both home gardens and commercial plantations due to its tolerance to varying soil types and climatic conditions. However, plantain is also nutrient-demanding and highly responsive to soil microbial interactions.

Despite this, there is limited research on how herbicide pollution alters the root-associated microbial community in plantains. More so, with the increasing use of agrochemicals such as herbicides in modern farming, there is a growing concern about their possible negative impacts on soil microbial ecosystems that are vital to plantain productivity. It is also worthy of note that many local farmers who have adopted these herbicides as a faster alternative for weed control, lack knowledge of their proper use. Herbicides are chemical agents applied to control or eliminate unwanted vegetation (weeds).

Research has shown that herbicide residues in soil can alter the composition, diversity, and activity of microbial communities, sometimes leading to the dominance of resistant strains and suppression of beneficial microbes (Nwankwo, et al., 2025), thus, understanding these differences is crucial for sustainable soil management, especially in areas where herbicide use is prevalent.

2. LITERATURE REVIEW

As most local studies focus on herbicide residue analysis or their phytotoxicity on crops, leaving a knowledge gap on biological soil quality indicators, particularly microbial diversity and activity (Ogunmwonyi et al., 2008), this research seeks to bridge this gap by assessing the comparative effects of herbicide on root-associated microbial populations in plantain, thereby providing insights into best agronomic practices for soil health preservation. The plantain rhizosphere is a hub of biological activities influenced by root exudates that attract and support specific microbial populations, hence a diverse and robust microbial community in the rhizosphere is essential for plantain health.

Here, Nigerian and other external studies have extensively documented the beneficial microbes associated with plantain roots in natural, undisturbed settings. First, research carried out in Nigeria has identified a rich assortment of bacteria and fungi in the rhizosphere of healthy plantain plants (Solanke & Falade, 2010). These microorganisms according to the research, perform vital functions such as nitrogen fixation, phosphorus solubilization, and the production of plant growth-promoting substances like indole-3-acetic acid (Adegboye, 2018). Key microbial genera identified include *Pseudomonas*, *Bacillus*, and *Acinetobacter*, which are known for their plant-beneficial properties (Adegboye, 2018). More so, a study conducted in Bayelsa, Nigeria, underscored the connection between a high population of these microorganisms and improved plant nutrition, highlighting the importance of organic matter accumulation and zero-tillage practices (Teke & Akowei., 2023a).

Similarly, on a broader note, other African studies have characterized the plantain rhizosphere across diverse agroecologies. A study spanning Nigeria, Cameroon, and Gabon revealed significant variations in microbial profiles depending on the agroecological zone, but also identified a core microbiome common to plantain (Birt et al., 2022a). This core microbiome, consisting of key bacterial and fungal species, is thought to be essential for the plant's health and resilience, particularly in resource-poor conditions prevalent in smallholder farming systems (Birt et al., 2022a). Studies in Kenya further confirmed the presence of beneficial bacterial phyla like Proteobacteria, Actinobacteria, and Cyanobacteria in banana and plantain rhizospheres, linking their presence to improved soil health and crop yield (Zhang et al., 2021).

It is true that bacterial species are the most prevalent group of microorganisms in cultivated soil and these organisms partake in intricated rhizospheric activities (Ahemad & Kibret, 2014). Reports suggests that these organisms have between 106 and 108 cell per soil cubic centimetre. This simply imply that in a gram of soil, there occur between a few million and a few billion bacteria cells. That is, the topmost layers of soil, up to 30cm of arable soil lies the highest concentration of bacteria (Teke & Akowei., 2023b). In natural soil, the microbial community is characterized by high diversity and stability. These microbes form complex networks and synergistic relationships that benefit the plant (Iqbal et al., 2025). This includes a high abundance of plant growth-promoting rhizobacteria (PGPRs) and arbuscular mycorrhizal fungi (AMF), which enhance nutrient uptake and provide protection against disease (Adegboye, 2018; Birt et al., 2022b). The natural soil environment is a balanced, self-regulating system that supports long-term plant health.

In contrast, herbicide-polluted soil presents a different picture. The microbial community here is often less diverse and functionally compromised. The application of herbicides acts as a filter, eliminating sensitive species and leaving behind those that are either resistant or can

metabolize the chemical (Tyagi et al., 2025). This leads to a shift in community composition from a balanced, cooperative system to one dominated by a few opportunistic or stress-tolerant species. The reduction in beneficial microbes can impair nutrient cycling, weaken the plant's immune system, and ultimately affect crop productivity (Onwudiegwu et al., 2025).

3. MATERIAL AND METHODS

The study location is selected plantain plantations within the campus of the University of Africa Toru-Orua, Bayelsa State, Nigeria, which cover few areas with a history of herbicide use, allowing for a comparison with a nearby area with natural, unpolluted soil. Samples of soil from plantain root rhizosphere were collected in sterile sample bags, transported to the Lab and stored under optimum conditions before serial dilution. Adopting a completely randomised design, all samples were replicated in triplicates for the serial dilution. Using dilutions 10^{-6} and 10^{-7} , samples were cultured on three separate media (PDA, MAC and NA). The PDA was used to select colonial fungi while NA and MAC were used for selecting colonial bacteria of plantain root rhizosphere. The bacterial isolates were subcultured and subjected to biochemical tests such as Catalase Test, Indole Test, Simmons Citrate Test and Triple Sugar iron Agar (TSIA) Test to further identify specific bacteria genera/species. Antibiotic resistance test was also conducted to ascertain which antibiotic will be effective enough to manage these organisms as the case arises.

4. RESULTS AND DISCUSSION

The results presented show the researcher's observations of the four samples based on the condition, colonies morphology, Sub-culturing, biochemical test (catalase and indole test, Triple sugar iron Agar test (TSIA test), Simmon Citrate Test, sensitivity test and Gram staining of microorganisms isolated both from herbicide polluted soil (HP) and normal soil (NS) of Plantain root systems. First, it was generally observed that herbicide polluted samples had the highest microbial loads counts both for fungi at 25% and bacteria at 43.75%. Samples from the normal non-polluted soils had low microbial loads with 6.25% of fungi and 25% of bacterial isolates as shown in Table 1. It was also observed that *Cryptococcus* spp and *Fusarium* spp. which are pathogenic, were more in occurrence than other commensal fungi such as *Rhizopus* spp., *Basidiomycetes*, *Aspergillus* spp. and *Basidiomycetes* where are known decomposers. Many studies over the years have reported a decline in overall microbial biomass and diversity after herbicide use due to general toxicity (Onwudiegwu et al., 2025; Solanke & Falade, 2010). However, the increased abundance and richness in the HP sample reflect the ecological trend of selective enrichment or adaptation (Devi et al., 2018). Herbicides serve as a strong selective pressure, killing sensitive, non-target microbes (Cycoń et al., 2012). This drop in generalist competition lets resistant or herbicide-degrading specialists use the xenobiotic compound as a new carbon source. They can then multiply quickly, increasing their viable count and relative occurrence. This adaptation accounts for the larger number of colonies in the HP sample. Thus, the unique makeup of the HP community, particularly the presence of specific genera of fungi and their high relative abundance, supports the idea that the exposed soil has favoured herbicide-degrading organisms.

Table 1. Percentage Occurrence of Microorganisms across samples.

Sample	Test	Microbial isolate	No per Organism	No of Fungi Per Sample Type	% Occurrence
FUNGAL ISOLATES					
HP	PDA	<i>Rhizopus</i> spp. <i>Cryptococcus</i> spp <i>Fusarium</i> spp. <i>Basidiomycetes</i> <i>Aspergillus</i> spp.	1 3 2 1 1	8	25
NS	PDA	<i>Rhizopus</i> spp.	1	2	6.25
		<i>Basidiomycetes</i>	1		
Total Fungi Species			10	10	
BACTERIA ISOLATES					
HP	MAC & NA	<i>Enterobacter</i> spp <i>Enterobacter aerogenes</i> <i>Micrococcus luteus</i> <i>Bacillus</i> spp <i>Proteus</i> spp.	2 1 6 4 1	14	43.75
NS	MAC & NA	<i>Enterobacter aerogenes</i> <i>Pseudomonas</i> spp. <i>Bacillus</i> spp <i>Micrococcus luteus</i> .	1 1 1 5	8	25
Total Bacteria Species			22		100
Total Rhyzospheric Microbes			32		

HP = Herbicide Polluted Soil Sample; NS = Normal Soil Sample

Source: Lab report

Table 2 show the results of fungi isolates from both sample types. Here, the HP sample included four genera of fungi that were absent in the NS sample: *Cryptococcus* spp., *Fusarium* spp., *Basidiomycetes* (undifferentiated), and *Aspergillus* spp. The detection of *Aspergillus* spp. and *Fusarium* spp. is key for xenobiotic remediation. Filamentous fungi are known for their ability to break down complex pollutants using non-specific extracellular enzymes (Makut Bello, 2018).

Aspergillus species are well-documented for their effectiveness in degrading various herbicides, including glyphosate and other pesticides (Dinakarkumar et al., 2024; Matúš et al., 2023). Their presence only in HP soil indicates they are relying on the herbicide or its breakdown products for growth (Plate 1).

Table 2. Colony Morphology on Potato Dextrose Agar (PDA) for fungal isolation.

PDA Plate	Fungi count	Colony	Size	Shape	Elevation	Surface	Colour	Margin	Opacity	Most probable organism
HP P1 10 ⁻⁶	11	Colony 1	2 cm	Irregular	Raised, dry	-	White	Irregular	Transparent	<i>Rhizopus</i> spp.
		Colony 2	1 mm	Circular	Raised	Moist	White	Entire	Opaque	<i>Cryptococcus</i> spp.
		Colony 3	1 mm	Circular	Flat	Wet	Fluffy white	Entire	Opaque	<i>Fusarium</i> spp.
HP P1 10 ⁻⁷	1	Colony 1	4 cm	Circular	Raised	Dry	White	Irregular	Opaque	<i>Basidiomycetes</i>
HP P2 10 ⁻⁶	1	Colony 1	1 mm	Circular	Raised	Moist	White	Entire	Opaque	<i>Cryptococcus</i> spp.
HP P2 10 ⁻⁷	15	Colony 1	5 cm	Circular	Raised	Dry	White	Irregular	Opaque	<i>Fusarium</i> spp.
		Colony 2	1 cm	Circular	Convex	Dry	Green	Entire	Opaque	<i>Aspergillus</i> spp.
		Colony 3	1 cm	Irregular	Flat	Moist	Milky	Lobate	Opaque	<i>Candida</i> spp.
NS P1 10 ⁻⁶	1	Colony 1	5 cm	Circular	Flat	Dry	Fluffy white	Irregular	Transparent	<i>Rhizopus</i> spp.
NS P1 10 ⁻⁷	NG	—	—	—	—	—	—	—	—	—
NS P2 10 ⁻⁶	NG	—	—	—	—	—	—	—	—	—
NS P2 10 ⁻⁷	4	Colony 1	3 cm	Circular	Raised	Dry	White	Irregular	Opaque	<i>Basidiomycetes</i>

HP P1 = Herbicide Polluted Sample 1; HP P2 = Herbicide Polluted Sample 2;
 NS P1 = Normal Soil Sample 1; NS P2 = Normal Soil Sample 2; NG = No Growth
 Source: Lab report.

Result of bacterial isolates are reported on MacConkey Agar – MAC and Nutrient Agar – NA (Table 3 & 4). The bacterial community in both soils (as shown in Plates 2 & 3) was dominated by common soil-dwelling and facultative pathogenic genera. *Micrococcus* spp. and *Bacillus* spp. were prominent in both HP and NS, where *Micrococcus* had the highest count in HP soil (6 isolates) vs NS (5 Isolate). Both genera (Gram-positive) are often recognized as degraders of various pesticides, including oxyfluorfen and other complex organic compounds (Pal et al., 2006, Moneke et al., 2010).

Their thick peptidoglycan cellwall often help them withstand chemical stress, enabling them to thrive in the contaminated environment. *Enterobacter* spp. and *Proteus* spp. were also

recorded, whose presence in HP soil points to a generalist response, as these Gram-negative bacteria are common in soil and water. Enterobacter has further been linked to the breakdown of some organic pollutants (Maikalu et al., 2023).

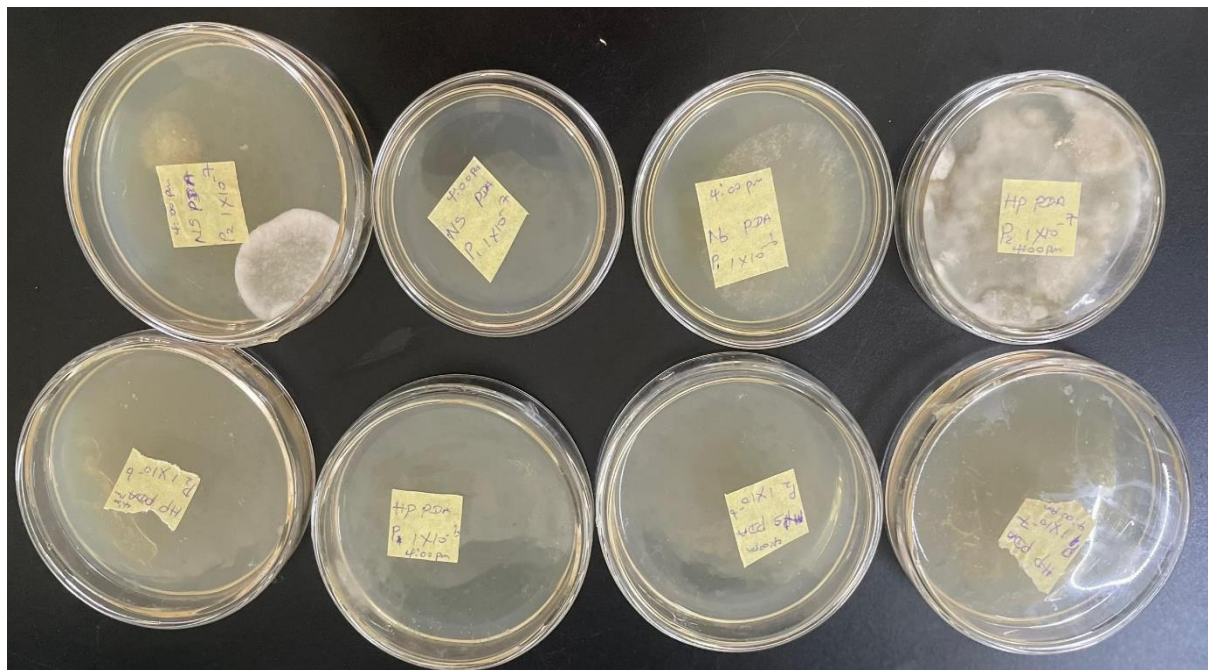


Plate 1. Potato dexteros agar (PDA) cultures plates.

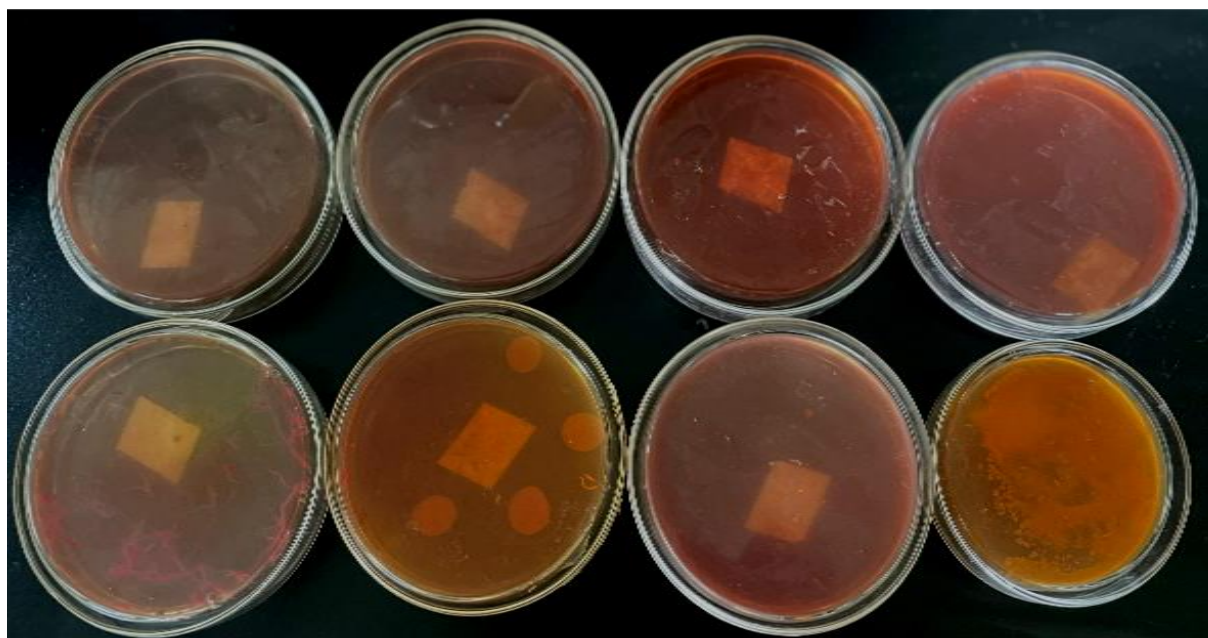


Plate 2. MacConkey Agar cultures plates.

Literatures strongly support the role of these organisms identified in this study particularly *Bacillus*, *Pseudomonas*, and various fungi as active agents in remediating herbicide contaminated soils. More so, it is believed that bacteria are the dominant microbial group, constituting the majority (a total of 32 isolates).

Importantly, the Herbicide-Polluted (HP) soil sample exhibited both a higher total microbial count (22 isolates) and greater species diversity for both bacteria and fungi when compared to the Normal Soil (NS) sample (10 isolates). The most frequently isolated bacterium was *Micrococcus luteus* (6 isolates in HP), suggesting it is either highly tolerant to the herbicide or possesses the metabolic capacity to utilize or degrade the compound, a key characteristic for potential bioremediation applications.

Table 3. Morphological characteristics of bacterial isolates on MacConkey agar (MAC).

Sample	Colony count	Size	Shape	Elevation	Surface	Colour	Margin	Opacity	Most probable organism
HP P1 10^{-6}	4	3 cm	Circular	Flat	Moist	Light pink	Entire	Transparent	<i>Enterobacter</i> spp.
	2	2.5 cm	Circular	Raised	Moist	Rose pink	Entire	Opaque	<i>Enterobacter aerogenes</i>
HP P1 10^{-7}	1	3 mm	Circular	Raised	Moist	Light pink	Entire	Opaque	<i>Enterobacter</i> spp.
HP P2 10^{-6}	NG	—	—	—	—	—	—	—	—
HP P2 10^{-7}	252	—	Irregular, round, filamentous	—	—	Rose pink/light pink	—	—	<i>Proteus</i> spp.
NS P1 10^{-6}	5	1 mm	Circular	Raised	Moist	Light pink	Entire	Opaque	<i>Enterobacter aerogenes</i>
	3	1.5 mm	Irregular	Raised	Moist	Rose pink	Undulate	Opaque	<i>Pseudomonas</i> spp.
NS P1 10^{-7}	NG	—	—	—	—	—	—	—	—
NS P2 10^{-6}	258	—	Irregular, round, filamentous, fluffy	—	—	White and light pink	—	—	<i>Proteus</i> spp.
NS P2 10^{-7}	NG	—	—	—	—	—	—	—	—

HP P1 = Herbicide Polluted Sample 1; HP P2 = Herbicide Polluted Sample 2;
 NS P1 = Normal Soil Sample 1; NS P2 = Normal Soil Sample 2; NG = No Growth
 Source: Lab report

Table 4. Colony morphology of bacteria for nutrient agar (NA).

Sample	Colony count	Size	Shape	Elevation	Surface	Colour	Margin	Opacity	Most probable organism
(HP P1 10 ⁻⁶)	6	2.5 cm	Circular	Raised	Dry	Fluffy white	Entire	Opaque	<i>Micrococcus</i> spp.
	6	1 mm	Circular	Flat	Moist	Light yellow	Entire	Transparent	<i>Micrococcus</i> spp.
	2	3 cm	Irregular	Raised	Moist	Fluffy white	Undulate	Opaque	<i>Bacillus cereus</i>
	4	1.5 cm	Circular	Flat	Dry	Fluffy white	Entire	Transparent	<i>Micrococcus</i> spp.
(HP P1 10 ⁻⁷)	4	1.5 mm	Circular	Raised	Moist	Fluffy white	Entire	Opaque	<i>Bacillus</i> spp.
	2	1mm	Wrinkled	Flat	Dry	Fluffy white	Undulate	Transparent	<i>Bacillus cereus</i>
HP P2 10 ⁻⁶	6	2 mm	Circular	Raised	Moist	Light yellow	Entire	Opaque	<i>Micrococcus</i> spp.
	5	1.5 mm	Wrinkled	Raised	Moist	Fluffy white	Irregular	Opaque	<i>Bacillus</i> spp.
	1	1 mm	Circular	Flat	Dry	Fluffy white	Entire	Transparent	<i>Micrococcus</i> spp.
HP P2 10 ⁻⁷	2	2 cm	Circular	Raised	Moist	Light yellow	Entire	Opaque	<i>Micrococcus luteus</i>
NS P1 10 ⁻⁶	3	1 mm	Circular	Flat	Moist	Milky white	Entire	Transparent	<i>Bacillus subtilis</i>
	1	3 mm	Wrinkled	Raised	Dry	Fluffy white	Undulate	Opaque	<i>Micrococcus</i> spp.
NS P1 10 ⁻⁷	3	3 mm	Circular	Flat	Dry	Fluffy white	Entire	Transparent	<i>Micrococcus</i> spp.
	2	1 mm	Circular	Flat	Dry	Milky white	Entire	Opaque	<i>Micrococcus</i> spp.
	1	1.5 cm	Irregular	Flat	Dry	Fluffy white	Irregular	Transparent	<i>Micrococcus</i> spp.
	2	2 mm	Concentric	Flat	Dry	Fluffy white	Undulate	Transparent	<i>Micrococcus</i> spp.
NS P2 10 ⁻⁶	1	1.5 cm	Circular	Flat	Dry	Fluffy white	Entire	Opaque	<i>Micrococcus</i> spp.
	2	2 mm	Circular	Raised	Dry	Milky white	Entire	Transparent	<i>Micrococcus</i> spp.
NS P2 10 ⁻⁷	1	1 mm	Circular	Flat	Moist	Fluffy white	Entire	Transparent	<i>Bacillus</i> spp.

HP P1 = Herbicide Polluted Sample 1; HP P2 = Herbicide Polluted Sample 2;

NS P1 = Normal Soil Sample 1; NS P2 = Normal Soil Sample 2

Source: Lab report

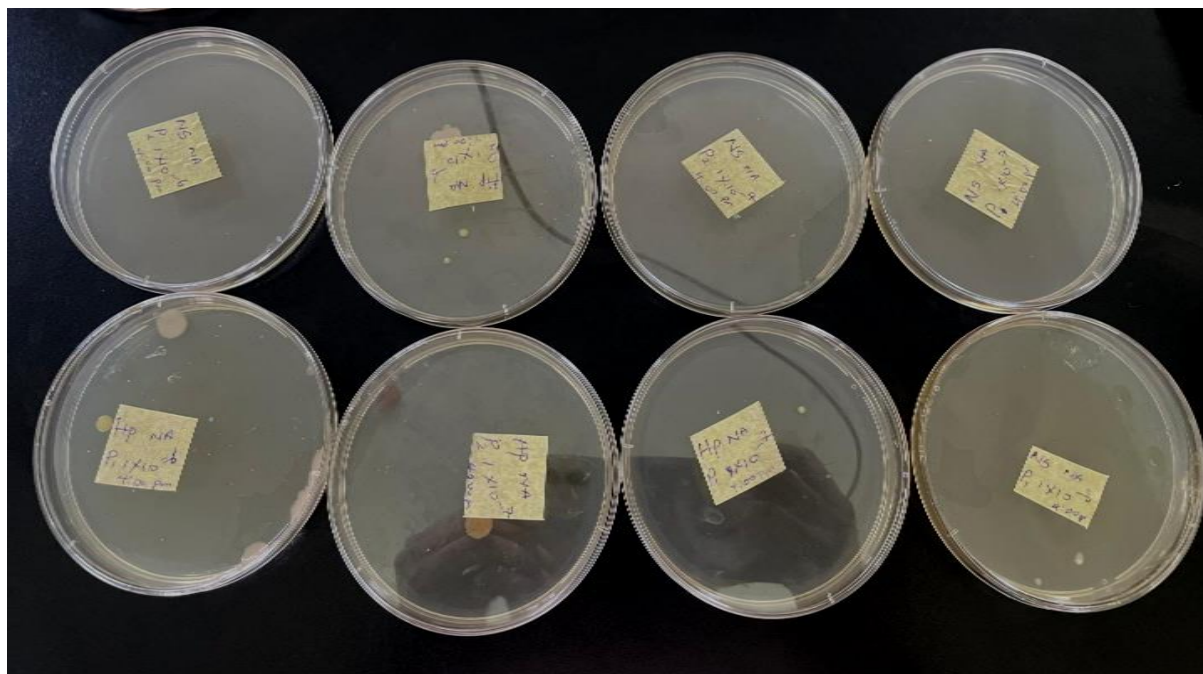


Plate 3. Nutrient agar cultures plates.

Table 5 presents the results of three key biochemical tests; Catalase, Indole, and Simons Citrate Test. These are basically employed for a preliminary presumptive identification of various bacterial isolates, including members of the Enterobacteriaceae family and common Gram-positive organisms. These tests are crucial as they exploit metabolic differences to rapidly differentiate between bacterial genera and species.

Catalase Test: All of the successful isolates listed (*Enterobacter aerogenes*, *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus*, *Lactobacillus* spp., *Bacillus* spp.) were found to be Catalase-positive (+ve). This result is consistent with their identification as most obligate aerobes and facultative anaerobes possess the catalase enzyme. For instance, *Staphylococcus aureus* (HP P1 10⁻⁶ NA) is routinely distinguished from catalase-negative *Streptococcus* species using this test. Similarly, the positivity of *Bacillus* spp. (HP P2 10⁻⁶ NA) is characteristic of this genus.

Indole and Citrate Tests (IMViC): The Indole and Simmons Citrate tests are often used together as part of the IMViC battery to differentiate Gram-negative enteric bacteria. The IMViC test comprises a set of four distinct biochemical tests utilized for the identification and differentiation of bacteria, particularly those belonging to the Enterobacteriaceae family. While it can be employed (and is indeed used) for identifying various types of bacteria, its primary application is in the identification of Gram-negative bacteria.

The majority of the isolates, including *Enterobacter aerogenes*, *Proteus* spp., and *Bacillus* spp., were Indole-negative. Indole production relies on the presence of the enzyme tryptophanase, which cleaves tryptophan into indole, pyruvic acid, and ammonia (Roy et al., 2023). A negative result indicates the organism lacks this enzyme. A notable exception in the table is *Staphylococcus aureus* (HP P1 10⁻⁶ NA), which was recorded as Indole-positive.

Table 5. Results of Biochemical Tests on Bacterial Isolate.

Sample	Test	Catalase	Indole	Simons Citrate	Most Probable organism
HP P1 10 ⁻⁶ MAC	Rose pink, moist, Transparent	+ve	-ve	+ve	<i>Enterobacter aerogenes</i>
HP P1 10 ⁻⁷ MAC	light pink, moist, opaque	+ve	-ve	-ve	<i>Enterobacter</i> spp
HP P2 10 ⁻⁶ MAC	NG	-	-	-	-
HP P2 10 ⁻⁷ MAC	Rose pink, round, filamentous	+ve	-ve	+ve	<i>Proteus</i> spp
NS P1 10 ⁻⁶ MAC	Rose pink, moist opaque	+ve	-ve	+ve	<i>Pseudomonas</i> spp.
NS P1 10 ⁻⁷ MAC	NG	-	-	-	
NS P2 10 ⁻⁶ MAC	Round, filamentous, light pink	+ve	-ve	+ve	<i>Proteus</i> spp.
NS P2 10 ⁻⁷ MAC	NG	-	-	-	-
HP P1 10 ⁻⁶ NA	Moist, flat, light yellow Transparent	+ve	+ve	+ve	<i>Staphylococcus aureus</i>
HP P1 10 ⁻⁷ NA	Dry, fluffy-white, undulate, transparent	+ve	-ve	+ve	<i>Lactobacillus</i> spp.
HP P2 10 ⁻⁶ NA	Moist, irregular, fluffy-white, opaque	+ve	-ve	+ve	<i>Bacillus</i> spp.
HP P2 10 ⁻⁷ NA	Moist, light yellow, circular opaque	+ve	-ve	+ve	<i>Micrococcus luteus</i>
NS P1 10 ⁻⁶ NA	Moist, flat, milky-white, transparent	+ve	-ve	+ve	<i>Bacillus subtilis</i>
NS P1 10 ⁻⁷ NA	Milky-white, dry, flat, opaque	+ve	-ve	-ve	<i>Staphylococcus aureus</i>
NS P2 10 ⁻⁶ NA	Circular, dry, milky-white, opaque	+ve	-ve	-ve	<i>Micrococcus</i> spp.
NS P2 10 ⁻⁷ NA	Flat, moist, fluffy-white, transparent	+ve	-ve	+ve	<i>Lactobacillus</i> spp..

HP P1 = Herbicide Polluted Sample 1; HP P2 = Herbicide Polluted Sample 2;

NS P1 = Normal Soil Sample 1; NS P2 = Normal Soil Sample 2

Source: Lab report

The results of Simons Citrate Test further revealed that *Enterobacter aerogenes*, *Proteus* spp., and *Pseudomonas* spp. were all Citrate-positive. A positive result in Simmons Citrate medium indicates that the bacterium can utilize citrate as its sole carbon source. For this to occur, the organism must possess the enzyme citrate permease to transport citrate into the cell. As the utilization of citrate produces alkaline byproducts, the bromothymol blue indicator changes the medium from green to blue. The positive citrate result for *Enterobacter aerogenes*, paired with its negative indole result, is the classic biochemical signature used to differentiate it from *Escherichia coli*, which is typically Citrate-negative and Indole-positive.

Thus, the isolate identified as *Enterobacter aerogenes* (HP P1 10-6 MAC) showed an Indole-negative (-ve) and Citrate-positive (+ve) reaction. This metabolic profile (negative/positive) is a classic characteristic used to distinguish *E. aerogenes* from other enteric bacteria. Consequently, the isolates identified as *Proteus* spp. (HP P2 10-7 MAC, NS P2 10-6 MAC) and *Pseudomonas* spp. (NS P1 10-6 MAC) were all Citrate-positive (+ve), which is common for these organisms.

Triple Sugar Iron Agar (TSIA) was also conducted to assess an isolate's ability to ferment glucose, lactose, and sucrose, and to produce hydrogen sulfide (H₂S) which show that all six bacterial isolates exhibited a non-fermenters, a finding common among environmental and soil-associated microorganisms, such as those found in a plant root. The overall non-fermentative pattern aligns with the documented characteristics of the genera the isolated (Microbe Online, 2013), where both are key genera of Plant Growth-Promoting Rhizobacteria (PGPR) commonly isolated from soil and the rhizosphere which are generally non-fermentative on TSIA (Shah et al., 2024; Papade et al., 2024). For instance, a standard *Pseudomonas aeruginosa* control strain is documented to produce a Red/Red, Gas-negative, H₂S negative reaction (Microbe Notes, 2023).

Table 6. Isolate results for Triple Sugar Iron Agar (TSIA) Test.

ISOLATES	SLANT	BUTT	GAS	(H ₂ S)	Most Probable Organism
Milky White irregular	Red	Red	negative	positive	<i>Bacillus</i> spp.
Milky Transparent, Moist	Red	Red	positive	negative	<i>Bacillus</i> spp.
Dark pink	Red	Red	positive	negative	<i>Pseudomonas</i> spp.
Light pink	Red	Red	positive	positive	<i>Pseudomonas</i> spp.
Round light	Red	Red	positive	negative	<i>Pseudomonas</i> spp.
Milky Round dry	Red	Red	positive	positive	<i>Pseudomonas</i> spp.

Source: Lab report

5. CONCLUSION

Although studies over the years have reported an apparent decline in overall microbial presence and diversity after herbicide use due to perceived general toxicity, this study however, observed an increased abundance and richness of microbial activity in the HP sample reflecting an ecological trend of selective adaptation as previously reported by a few authors. This study thus report that the use of herbicides as effective weed control, actually compromises the microbial balance of the plantain's rhizosphere. Thus, herbicide application does not totally destroy the plant microbiome but creates an imbalance that lead to the elimination of non-

tolerant microorganisms and the subsequent selective promotion of herbicide degrading microorganisms.

References

- [1] Adegboye, O. C. The role of beneficial microbes in sustainable plantain production in Nigeria. *Nigerian Journal of Microbiology*, 32(1), (2018) 45-56
- [2] Ahemad, M. & Kibret, M. Mechanisms and Applications of Plant Growth Promoting Rhizobacteria: Current Perspective. *Journal of King Saud University - Science*, 26, (2014) 1-20
- [3] Birt, H.W.G., Pattison, A.B., Skarszewski, A., Daniells, J., Raghavendra, A. and Dennis G. P. The core bacterial microbiome of banana (*Musa* spp.). *Environmental Microbiome* 17, (2022 a) 46
- [4] Birt, H. W. G., Tharp C. L., Custer, G. F., Dini-Andreote F. Root phenotypes as modulators of microbial microhabitats. *Frontiers in Plant Science* 13, (2022 b) 1-9
- [5] Cycoń M, Żmijowska A, Wójcik M, Piotrowska-Seget Z. Biodegradation and bioremediation potential of diazinon-degrading *Serratia marcescens* to remove other organophosphorus pesticides from soils. *Journal of Environmental Management* 117, (2013) 7-16
- [6] Dinakarkumar, Y., Ramakrishnan, G., Gujjula, K.R., Vasu, V., Balamurugan, P. & Murali, G. Fungal bioremediation: An overview of the mechanisms, applications and future perspectives. *Environmental Chemistry and Ecotoxicology*, 6, (2024) 293-30
- [7] Iqbal, S., Begum, F., Nguchu, B.A., Claver, P. U. & Shaw, P. The invisible architects: microbial communities and their transformative role in soil health and global climate changes. *Environmental Microbiome* 20(36) (2025) 1-21
- [8] Kaushal, M., Kolombia, Y., Alakonya, A. E., Kuate, A. F., Alejandro Ortega-Beltran, A. Amah, D. & Masso, C. Subterranean Microbiome Affiliations of Plantain (*Musa* spp.) Under Diverse Agroecologies of Western and Central Africa. *Microbial Ecology* 84 (2022) 580-593
- [9] Maikalu, R. B., Igere, B, E. & Odjadjare, E. E.O. Enterobacter species Distribution, emerging virulence and multiple antibiotic resistance dynamics in effluents: A countrified spread-hub and implications of abattior release. *Total Environment Research Themes*, 8 (2023) 1-9
- [10] Makut, M. D. & Bello, A. Assessment of the Biodegradation of Herbicides by Bacteria Isolated from the Soil. *Asian Journal of Biotechnology and Bioresource Technology* 4(1) (2018) 1-6
- [11] Matúš, P., Littera, P., Farkas, B., & Urik, M. Review on Performance of *Aspergillus* and *Penicillium* Species in Biodegradation of Organochlorine and Organophosphorus Pesticides. *Microorganisms*, 11(6) (2023) 1485

- [12] Moneke, A. N., Okpala, G. N., & Anyanwu, C. U. Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields. *African Journal of Biotechnology*, 9(26) (2010) 4067-4074
- [13] Nwankwo, C. C., Arimaha C.O. and Ezeonuegbu, B. A. (2025). Comparative Analysis of the Physiochemical Characteristics and Bacterial Communities of Healthy and Infected Root Soil of Plantain Plant. *Scientia Africana*, 24 (2) (2025) 247-262
- [14] Ogunmwonyi I. N, Igbinosa O. E, Aiyegoro O. A and Odjadjare E. E. Microbial analysis of different top soil samples of selected site in Obafemi Awolowo University, Nigeria. *Scientific Research and Essay* 3(3) (2008) 120-124
- [15] Onwudiegwu, A. K., Okonofua, P. E., & Obe, O. J. Environmental and public health implications of pesticide residues: From soil contamination to policy interventions. *Greener Journal of Medical Sciences*, 15(1) (2025) 1-11
- [16] Pal, R., Chakrabarti, K., Chakraborty, A. & Chowdhury, A. Degradation and Effects of Pesticides on Soil Microbiological Parameters-A Review. *International Journal of Agricultural Research*, 1 (2006) 240-258
- [17] Papade, S. E., Mohapatra, B. & Phale, P. S. Pseudomonas and Acinetobacter spp. capable of metabolizing aromatics displays multifarious plant growth promoting traits: Insights on strategizing consortium-based application to agro-ecosystems. *Environmental Technology & Innovation*, 36 (2024) 103786
- [18] Roy, B., Das, T. & Bhattacharyya, S. Overview on Old and New Biochemical Test for Bacterial Identification. *Journal of Surgical Case Reports and Images* 6(5) (2023) 1-11
- [19] Solanke, T., & Falade, O. A. The microbial diversity of plantain (Musa paradisiaca) root-associated soil in Southwestern Nigeria. *Journal of Agricultural Science and Technology*, 5(2) (2010) 112-119
- [20] Shah, B. A., Malhotra, H., Papade, S. E., Dhamale, T., Ingale, O. P., Kasarlwar, S. T., & Phale, P. S. Microbial degradation of contaminants of emerging concern: metabolic, genetic and omics insights for enhanced bioremediation. *Frontiers in Bioengineering and Biotechnology*, 12 (2024) 1470522
- [21] Teke E. C. & Akowei J. S. Assessment of microorganisms in the rhizosphere region of plantain in Bayelsa, Nigeria. *World News of Natural Sciences*, 50 (2023 a) 178-196
- [22] Teke, E. C. & Akowei, J. S.. Population, Morphological and Biochemical Characterization of Microorganism in Plantain Root across different Farmlands in Toru-Orua Metropolis, Bayelsa State, Nigeria. *Journal of Applied Science and Environmental Management*, 27(8) (2023 b) 1681-1685
- [23] Tyagi, S., Kumar, A., & Yadav, K. K. Effect of different herbicides on soil microbial population dynamics in Rabi maize (Zea mays L.). *International Journal of Current Microbiology and Applied Sciences*, 7(5) (2025) 1-9
- [24] Van der Heijden M. G, Bardgett, R. D, & van Straalen, N. M. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11(3) (2008) 296-310

- [25] Zhang, J., Cook, J., Nearing, J. T., Zhang, J., Raudonis, R. Bernard R. Glick, R. B., Morgan G.I. Langille, G. I. M. and Cheng, Z. Harnessing the plant microbiome to promote the growth of agricultural crops. *Microbiological Research*, 245 (2021) 126690