

Pesticide residue gradients drives culturable bacterial diversity and restructure soil communities in tropical agricultural soils

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ABSTRACT

Soil pollution from intensive pesticide use poses a noteworthy universal risk to agrarian sustainability and ecological wellbeing. This study investigated influence of pesticides usage on soil bacterial abundance, diversity and residual pesticides. Soil samples were collected from pesticide-contaminated Farms, analyzed for physicochemical parameters, pesticide residues, and bacterial populations following standard protocols. Results showed that Aldrin (0.53-1.53 ppb), Endrin ketone (0.01-1.90 ppb), Heptachlor (0.07- 2.8ppb) and P, P¹-DDT (0.25- 2.25 ppb) were the organochlorine pesticide (OCP) residues with the highest frequency of detection while Gamma-BHC was completely absent across Farms. The least OCP concentrations were found in control soil. The highest organochlorine residues were 2.25 ppb (P, P¹-DDT), 2.42 ppb (Alpha- Chlordane) 2.80 ppb (Heptachlor) and 0.61 ppb (P, P¹-DDD) while organophosphate residues were 4.24 ppb (Azinphos-ethyl), 3.24 ppb (Azinphos-ethyl). 3.53ppb (EPN) and 1.70 ppb (Chloropyriphos-methyl) for control, Farms 1, 2 and 3, respectively. Farm 2 recorded the highest organophosphate pesticide residues with Azimophos- ethyl and Pyraclofos having the highest frequency of detection among soils.

Total heterotrophic bacteria were $64.0 \times 10^4 \pm 5.01$ CFU/g, $97.3 \times 10^4 \pm 4.16$ CFU/g, $82.1 \times 10^4 \pm 4.01$ CFU/g, $74.5 \times 10^4 \pm 3.04$ CFU/g for control, Farm I, Farm II and Farm III, respectively. Actinomycete counts were highest in control and least in Farm I. Bacterial isolates identified tentatively included species of *Staphylococcus*, *Arthrobacter*, *Alkaligenes*, *Micrococcus*, *Chryseobacterium*, *Klebsiella*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Proteus* and *Streptomyces*. *Bacillus subtilis* made up 23.08% of bacteria in control; pesticide soil was dominated by *Bacillus subtilis* (28.57%) and *Paenibacillus polymyxa* (17.14%). These findings highlight potential ecological risks, persistence of banned pesticides, and need for improved pesticide management to safeguard soil health and food safety. Understanding these interactions is crucial, as microorganisms play key roles in overall soil fertility; emphasizing the need for careful pesticide management to balance pest control with the preservation of soil biological and chemical integrity.

Keywords: Analytical Profile Index, Bacteria, Pesticide residues, Soil contamination.

1. INTRODUCTION

Agricultural soils are dynamic ecosystems where bacteria drive nutrient cycling, organic matter turnover, and plant health. Key taxa include *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Rhizobium*. *Bacillus* species are notable for their resilience and biocontrol activity, producing enzymes and antimicrobial compounds that suppress pathogens [1,2]. *Pseudomonas* spp. enhance crop performance through siderophore production and disease suppression, making them central to plant growth-promoting rhizobacteria [3,4]. *Streptomyces* contribute to soil fertility by decomposing complex organic matter and producing antibiotics that regulate microbial competition [5,6]. Symbiotic nitrogen fixers such as *Rhizobium* remain vital for legume productivity, while free-living nitrogen fixers like *Azotobacter* enrich soil nitrogen pools [7]. Farming practices strongly shape these bacterial communities [8].

Organic amendments (compost, manure) increase populations of *Bacillus* and *Streptomyces*, improving soil fertility and disease resistance [9] Crop rotation fosters microbial diversity, reducing pathogen buildup and enhancing nutrient cycling [10,11]. On the other hand, intensive chemical fertilization often reduces microbial diversity, favouring copiotrophic taxa at the expense of beneficial symbionts [12,13]. Reduced tillage preserves soil structure and microbial habitats, supporting stable communities dominated by *Actinobacteria* and *Firmicutes* [14,15]. High-throughput sequencing consistently identifies *Proteobacteria*, *Actinobacteria*, and *Firmicutes* as dominant phyla in managed soils, with community shifts reflecting specific agronomic inputs [16]. Associating bacterial assemblages with functional outcomes remains a frontier for microbiome-informed strategies that reduce reliance on synthetic inputs and promote sustainable agriculture [17,18,19,20].

Across tropical Farmlands, pesticides have become an almost inseparable part of modern agriculture. Farmers rely on them to protect crops from relentless pest pressures, yet the very chemicals that secure harvests often linger in the soil long after their intended use. These residues, especially organochlorines and organophosphates, are notorious for their persistence and capacity to disrupt ecological balance. Their presence raises pressing questions about how much soil ecosystems can endure before their natural functions begin to falter [21].

Soil is more than just a medium for plant growth; it is a living system teeming with microorganisms that recycle nutrients, stabilize organic matter, and sustain fertility [16]. When pesticides accumulate, they do not simply vanish into the background; they reshape microbial communities, suppressing sensitive species while favouring those that can withstand chemical stress [22]. Over time, this restructuring alters the diversity and resilience of soil life, leaving behind communities that may function differently and less effectively than before.

Among the most concerning pollutants in tropical agricultural soils are pesticide residues, particularly organochlorine pesticides (OCPs) and organophosphate pesticides (OPPs). Organochlorine pesticides such as aldrin, heptachlor, and DDT are characterized by their chemical stability and persistence, often detected decades after their use has been restricted or banned. Their ubiquity and refractoriness contribute to soil degradation, fertility decline, and long-term ecological imbalance [23]. In contrast, OPPs including chlorpyrifos, azinphos-ethyl, and parathion are less persistent but exert acute toxicity, with degradation products that can disrupt nutrient cycles and microbial activity [24,25]. The coexistence of these two classes of residues in tropical soils creates a dual burden: OCPs drive chronic contamination and microbial restructuring, while OPPs cause short-term but intense ecological disturbances. Recent assessments in West African soils have revealed residue gradients of both OCPs and OPPs, directly influencing bacterial diversity and favouring resistant genera such as *Bacillus* and *Klebsiella* while suppressing more sensitive taxa [26]. These findings highlight the urgent need for integrated pesticide management strategies that address both legacy pollutants and ongoing chemical inputs to protect soil health and ensure sustainable food production.

In tropical regions, the challenge is compounded by climate conditions that accelerate both pesticide breakdown and leaching. Ironically, this means residues can be both more mobile and more persistent, creating uneven gradients of contamination across Farms. Studies comparing contaminated and uncontaminated soils have demonstrated significant differences in microbial community composition, with pesticide residues driving reductions in culturable bacterial diversity and altering the abundance of genera such as *Bacillus*, *Klebsiella*, and *Staphylococcus* [27]. These shifts are not trivial; they signal deeper ecological risks, including reduced soil fertility and compromised food safety.

Despite growing awareness of pesticide pollution, little attention has been paid to how residue gradients specifically drive changes in culturable bacterial diversity in tropical agricultural soils. This gap matters because microbial diversity is not just a measure of ecological health; it is the foundation of sustainable agriculture. By investigating how pesticide residues restructure soil communities, this study aims to shed light on the hidden costs of chemical dependence and underscore the urgent need for better pesticide governance.

Protecting soil health is not only about preserving biodiversity; it is about safeguarding the very systems that sustain food production for future generations.

2. MATERIAL AND METHODS

2.1 Study area

The study was conducted in the environs of Abraka, Delta State, Nigeria, focusing on pesticide contaminated agriculture Farms soil. Soil samples were collected from different locations in Abraka, Delta State, with coordinates of 5°47'57.15816 N latitude and 6°7'21.77148"E longitude (Farm I), 5°48'13.6548" N latitude and 6°7'24.15648" E longitude (Farm II), 5°48'14.00184" N latitude and 6°7'29.78904"E longitude (Farm III). This geographical spread ensured a comprehensive analysis of the bacteria and quality of agricultural Farming soils within the study area.

2.2 Sample collection

Soil samples were collected from agricultural Farms with pesticides usage in Abraka, Delta State. Debris were removed from the topsoil, and an auger was inserted to a depth of 15cm. The collected samples were then placed in a sterile bag and tightly sealed to preserve them for transport to the laboratory in a cooler containing icepacks at 4°C.

2.3 Soil physicochemical analysis

The physicochemical properties of the soil samples were examined in line with standard protocol. The pH of the soil was determined using a calibrated pH meter after mixing 10 g of soil with 25 mL of deionized water, while temperature was recorded with a digital thermometer once the reading stabilized [28]. In addition, sulphate concentration was measured through the turbidimetric method, where spectrophotometric readings were compared against a calibration curve, and nitrate levels were quantified by UV-Vis spectrophotometry at 540 nm following cadmium reduction [29]. Furthermore, electrical conductivity (EC) was assessed using a calibrated probe placed in a soil-water suspension prepared with 10 g of soil and 50 mL of deionized water [28]. Moisture content was determined gravimetrically by oven-drying fresh samples at 105°C for 24 hours and calculating the percentage loss in weight [28]. The cation exchange capacity (CEC) was established by saturating the soil with 1M ammonium acetate at pH 7, displacing ammonium ions with sodium chloride, and quantifying the displaced ions spectrophotometrically [29]. Finally, total organic carbon (TOC) was measured using the Walkley-Black method [30], which involved digestion with potassium dichromate and sulfuric acid, followed by titration with ferrous ammonium sulfate to obtain values relative to a standard curve.

2.3 Residues

The United States Environmental Protection Agency method 3540C [31] was adopted for the extraction and analysis of organochlorine and organophosphate pesticides (OCPs and OPPs) residues from the samples. A mass of 10.0 g of the soil was homogenized with an equal mass of anhydrous sodium sulfate (Na_2SO_4) and spiked with 20 ng of TCmX and PCB-209 as surrogate standards before extraction. A Soxhlet extraction was carried out on the homogenate with a 100 mL aliquot of 1:1 (v/v) DCM/hexane for 15 hrs. The extract was concentrated to 2 mL by vacuum rotary evaporation and subsequently, purified on an alumina/ silica gel packed column.

The OCPs and OPPs were eluted individually from the column with 30 mL of 3:1 (v/v) DCM/hexane. The eluate was reduced to 1 mL with a gentle stream of nitrogen gas. The identities and concentrations of OCPs and OPPs in the samples were determined with an Agilent 7980A gas chromatography coupled to an Agilent 5975C mass selective detector. The separation was effected on a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) with 99.9 % purity helium as the carrier gas at a constant flow rate of 1 mL/min. Sample injection was by a pulsed splitless mode with an injection volume of 1 μL. The oven temperature was initially set at 70°C for 3 min, then raised at 15°C min⁻¹ to 180°C, and further raised at 30°C min⁻¹ to 290°C.

2.4 Microorganisms

The total colony-forming units (CFU) counts of total heterotrophic bacteria were quantified by plating serial decimal dilutions (10⁻² and 10⁻⁵) of soil samples diluted in sterile distilled water (1% (w/v)) on plate count agar and MacConkey agar, then incubated for 24 hours at 30°C, as previously described by Ataikiru and Okorhi-Damisa [32]. The isolation of total heterotrophic bacteria (THB) was done by the spread plate method on nutrient agar. Discrete colony forming units were picked and sub-cultured onto nutrient agar (NA) for further purification. Purification was done by streaking twice and transferring onto agar slant for storage, and identified with the biochemical tests described in Bergey's Manual for Determinative Bacteriology [33]. Furthermore, isolates were identified using analytical profile index (API) test kits (BioMérieux).

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of the different Farms

The soil analyses across Farm I, Farm II, Farm III, and the Control (C) revealed distinct differences in texture, chemical composition, and fertility status, with implications for soil productivity and crop management. Farms I, II, and III were all classified as sandy clay, while the Control was a Sandy clay loam (Table 1). The control soil has a higher sand proportion (61.3%) and lower clay content (26.1%) than the Farms, giving it a coarser texture and likely better drainage. Farm I had the highest clay proportion (40.4%), followed by Farm II (37.7%) and Farm III (35.6%), which may influence water retention and nutrient-holding capacity. Other soil physicochemical properties are shown in Fig.1. Farm II showed the highest pH (6.24), close to neutral, while Farm I was slightly acidic (5.99). Farm III was distinctly more acidic (4.55), which could limit nutrient availability and microbial activity. The control was moderately acidic (5.48). These variations suggest that Farm III may face stronger acidity-related constraints compared to the other soils.

Soil salinity was generally low across all sites, with EC values declining from Farm I (193 μS/cm) and Farm II (171 μS/cm) to Farm III (110 μS/cm) and the Control (102 μS/cm). This pattern indicated that soluble salts were more concentrated in Farm I and II soils, though still within non-problematic ranges for crop growth. Moisture content decreased gradually from Farm I (23.7%) to Farm III (20.5%) and the Control (18.6%). This trend aligned with textural differences, where higher clay content contributes to higher water retention in Farm I relative to the sandier control.

All soils showed low organic matter levels, with Farm I having the highest TOC (1.21%) and TOM (1.36%), followed closely by Farm II (1.19% and 1.32%).

Farm III (1.12% and 1.21%) and the Control (1.11% and 1.19%) were the lowest. The pattern suggested progressive depletion of soil organic matter across the Farms and control, with potential implications for soil fertility, structure, and microbial activity. Chloride levels were highest in Farm I (60.37 mg/kg) followed by Farm II (53.49 mg/kg), dropping substantially in Farm III (34.41 mg/kg) and Control (31.91 mg/kg). Available phosphate followed a similar declining pattern, from Farm I (5.55 mg/kg) to Control (2.87 mg/kg). Nitrate and sulphate concentrations also decreased consistently from Farm I through the Control, with Farm I showing 1.17 mg/kg (nitrate) and 53.04 mg/kg (sulphate), compared to 0.82 mg/kg (nitrates) and 28.14 mg/kg (sulphate) in the Control. This downward gradient indicated nutrient depletion from Farm I to Control, with the Control soil being poorest in available macronutrients.

The CEC values were low overall but decline markedly following the trend > Farm I (2.15) > Farm II (1.97) > Farm III (1.21) > Control (0.61). The higher values in Farms I and II reflected their greater clay and organic matter content, which enhances nutrient retention. The Control soil, with the lowest CEC, had limited nutrient-holding capacity, making it less fertile.

Table 1: Soil textural characteristics

	Farm I	Farm II	Farm III	Control
% Sand	49.9	52.1	57.3	61.3
% Silt	9.7	10.2	7.1	12.6
% Clay	40.4	37.7	35.6	26.1
Textural Class	Sandy Clay	Sandy Clay	Sandy Clay	Sandy Clay Loam

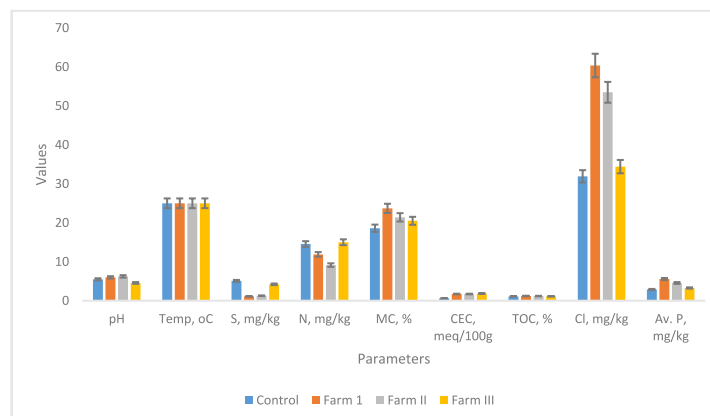


Fig.1: Physicochemical properties of soil used in the study

*Values are in means

3.2 Identification of pesticides residues in the soil

The residual organochlorine pesticides are shown in Table 1 from the Farm soils. There was a reduction in concentrations of DDT in Farm control (1.05 ppb) and Farm II (0.25 ppb), although, Farms I and Farm III had the same concentration (2.25ppb) as seen in Table 1. The same trend was observed for Beta-BHC with values of 0.25 ppb in all soils except Farm II. Heptachlor had varying concentrations during the study; 0.07 ppb (Farm II), 2.80 ppb (Farm III) and 0.10 ppb (control and Farm I). Alpha-BHC increased in Farm II (2.37 ppb) while significantly reducing in control (0.37ppb), however, there was no detectable value in Farm III. Endrin ketone values were low in Farm I (0.40 ppb) and control (0.01 ppb) and high in Farm III (1.90 ppb). Heptachlor epoxide (1.56 ppb) in the pesticide-contaminated soil (Farm II) was above the Maximum Residue Limits (MRL) of 0.6ppb.

The concentrations of the organophosphorus pesticide residues are shown in Table 2. Azinphos-ethyl consistently showed the highest concentration among all OPPs with Farm I showing the highest concentration of (4.24 ppb), Farm II (3.24 ppb), and Farm III (1.24 ppb). Quinalphos was notably high and reached the same concentration in both Farm I and Farm III (2.33 ppb) respectively, moderately present in Farm II (1.33 ppb). O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate (EPN) concentration in Farm III was (3.53 ppb) and control soil (0.53ppb) but remained undetected across other soil samples. Diazinone and chlorpyrifos-methyl were absent across all soil samples except the control soil, while Isazophos concentration was at 0.14 ppb across all samples and not detected in the control. Triphenyl phosphate was detected only in Farm II (1.63 ppb) while pyrazophos concentration was 1.96 ppb in Farm III as it remained undetected across other soil sample.

Table 1: Organochlorine pesticides (OCPs) residues concentration (ppb)

S/N	Target Compounds	Control	Farm 1	Farm 2	Farm 3	Maximum Residue Limit (ppb)	Reference
1.	Alpha-BHC	0.37	1.17	2.37	N.D	>7.4	[34]
2.	Beta-BHC	0.25	0.25	N.D	0.25	30	[35]
3.	Gamma-BHC	N.D	N.D	N.D	N.D	9	[31]
4.	Heptachlor	0.10	1.10	0.07	2.80	20	[36]
5.	Delta-BHC	N.D	N.D	N.D	0.11	400	[34]
6.	Aldrin	0.53	1.53	0.53	1.53	5	[35]
7.	Heptachlor epoxide	N.D	N.D	1.56	N.D	0.6	[35]
8.	Gamma-chlordane	0.02	2.02	N.D	0.82	>4	[35]
9.	Alpha-chlordane	0.42	0.42	2.42	N.D	>4	[35]
10.	Endosulfan I	0.59	N.D	N.D	0.57	4	[31]
11.	P, P'-DDE	N.D	1.07	N.D	N.D	2000	[34]
12.	Dieldrin	0.24	N.D	1.03	1.59	20	[31]
13.	Endrin	N.D	0.12	0.19	2.79	10	[31]
14.	P, P'-DDD	0.61	1.61	2.01	N.D	100	[36]
15.	Endosulfan II	N.D	N.D	N.D	0.66	4	[31]
16.	P, P'-DDT	0.25	2.25	1.05	2.25	10	[31]
17.	Endrin aldehyde	0.15	N.D	N.D	N.D	10	[35]
18.	Endosulfan sulphate	N.D	0.07	0.07	1.07	4	[35]
19.	Methoxychlor	0.35	N.D	N.D	N.D	20000	[37]
20.	Endrin ketone	0.01	0.40	0.10	1.90	NA	NA

*ASTDR: Agency for Toxic Substances and Disease Registry, EU: European Union and Regulatory Central Baltic, FAO: Food and Agricultural Organization of the United Nations, N.D: Not detected, WHO: World Health Organization, US EPA: United States Environmental Protection Agency, NA: Not available, ppb: parts per billion

Table 2: Organophosphate pesticides (OPPs) concentration (ppb)

S/N	Target Compounds	Control	Farm 1	Farm 2	Farm 3	Maximum Residue Limit (ppb)	Reference
1.	1,3-dimethyl-2-nitrobenzene	N.D.	1.79	N.D.	2.79	NA	NA
2.	Diazinone	0.18	N.D.	N.D.	N.D.	10	[35]
3.	Isazophos	N.D.	0.14	0.14	0.14	NA	NA
4.	Pirimiphos-methyl	N.D.	0.3	1.3	N.D.	30	[31]
5.	Chlorpyrifos-methyl	1.7	N.D.	N.D.	N.D.	NA	NA
6.	Fenitrothion	0.22	N.D.	N.D.	0.22	NA	NA
7.	Pirimiphos-ethyl	0.46	N.D.	0.46	N.D.	30	[31]
8.	Quinalphos	N.D.	2.33	1.33	2.33	NA	NA
9.	Chlorpyrifos	N.D.	0.88	N.D.	0.88	30	[31]
10.	Triphenyl phosphate	N.D.	N.D.	1.63	N.D.	NA	NA
11.	EPN	0.53	N.D.	N.D.	3.53	NA	NA
12.	Phosalone	N.D.	1.21	0.21	N.D.	NA	NA
13.	Pyrazophos	N.D.	N.D.	N.D.	1.96	NA	NA
14.	Azinphos-ethyl	0.24	4.24	3.24	1.24	NA	NA
15.	Pyraclufos	0.14	1.14	0.14	0.14	10	[35]

*US EPA: United States Environmental Protection Agency, NA: Not available, N.D: Not detected, ppb: parts per billion; EU: European Union and Regulatory Central Baltic

3.3 Enumeration of microorganisms in the soil

Table 3 shows the microbial counts in colony-forming units per gram soil across the various Farm soils while Table 4 shows the bacteria isolated from the different soil samples. Total heterotrophic bacteria were $64.0 \times 10^4 \pm 5.01$ CFU/g, $97.3 \times 10^4 \pm 4.16$ CFU/g, $82.1 \times 10^4 \pm 4.01$ CFU/g, $74.5 \times 10^4 \pm 3.04$ CFU/g for control, Farm I, Farm II and Farm III, respectively. Actinomycete counts were $42.6 \times 10^3 \pm 2.11$ CFU/g, $32.5 \times 10^3 \pm 1.15$ CFU/g, $36.3 \times 10^3 \pm 1.35$ CFU/g, $35.2 \times 10^3 \pm 2.02$ CFU/g for control, Farm I, Farm II and Farm III, respectively. Phosphate solubilizers were $34.2 \times 10^3 \pm 1.12$ CFU/g - $43.7 \times 10^3 \pm 0.00$ CFU/g while nitrogen fixers were between $31.2 \times 10^3 \pm 2.31$ CFU/g - $41.2 \times 10^3 \pm 5.03$ CFU/g across the various Farms.

Table 3: Microbial populations from Farm soils (Mean \pm Standard deviation)

Sample code	TCHB ($\times 10^4$ CFU/g)	Actinomycetes ($\times 10^3$ CFU/g)	Phosphate solubilizers ($\times 10^3$ CFU/g)	Nitrifying bacteria ($\times 10^3$ CFU/g)
Control	64.0 \pm 5.01	42.6 \pm 2.11	43.3 \pm 0.24	41.2 \pm 5.03
Farm I	97.3 \pm 4.16	32.5 \pm 1.15	34.2 \pm 1.12	32.6 \pm 0.92
Farm II	82.1 \pm 4.01	36.3 \pm 1.35	43.7 \pm 0.00	32.8 \pm 1.91
Farm III	74.5 \pm 3.04	35.2 \pm 2.02	37.6 \pm 0.05	31.2 \pm 2.31

Note: TCHB: Total culturable heterotrophic bacteria, CFU/g: colony forming units per gram

3.4 Gram Reaction and Biochemical Characteristics of the Bacterial Isolates

The bacterial isolates were identified based on colonial and cellular morphologies, including their reactions to biochemical tests. Table 4 shows the Gram's reaction and biochemical characteristics of the bacterial isolates. The bacterial isolates identified were *Alkaligenes* spp., *Arthrobacter* spp., *Streptomyces* spp., *Staphylococcus* spp., *Chryseobacterium* spp., *Micrococcus* spp., *Paenibacillus* spp., *Bacillus* spp., *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp.

Table 4: Gram reaction and biochemical characteristics of bacterial isolates

S/N	Isolate' s code	Gram' reaction	Shape	Urease	Citrate	Motility	H ₂ S	Gas	Catalase	MR	VP	Indole	Probable identity of isolates
1	B1	+	Rods	-	+	+	-	-	+	-	-	-	<i>Arthrobacter</i> spp
2	B2	+	Rods	+	-	-	+	-	+	-	-	-	<i>Paenibacillus</i> spp
3	B3	+	Rods	-	+	+	-	-	+	-	+	-	<i>Bacillus</i> spp
4	B4	-	Rods	-	+	-	-	-	+	-	-	-	<i>Chryseobacterium</i> spp
5	B5	-	Rods	+	+	-	-	+	+	-	+	+	<i>Klebsiella</i> spp
6	B6	-	Rods	-	+	+	-	-	+	-	-	-	<i>Alkaligenes</i> spp
7	B7	-	Rods	+	+	-	-	+	+	-	+	-	<i>Klebsiella</i> spp
8	B8	+	Filamentous	-	-	-	-	-	+	-	+	-	<i>Streptomyces</i> spp
9	B9	+	Cocci	+	-	-	-	-	+	-	+	-	<i>Staphylococcus</i> spp
10	B10	+	Cocci	+	-	-	-	-	+	-	+	-	<i>Staphylococcus</i> spp
11	B11	+	Cocci	+	-	-	-	-	+	-	-	-	<i>Micrococcus</i> spp
12	B12	-	Rods	-	+	-	-	-	+	-	-	-	<i>Pseudomonas</i> spp
13	B13	-	Rods	+	+	+	+	+	+	+	-	+	<i>Proteus</i> spp
14	B14	+	Rods	-	+	+	-	-	+	-	+	-	<i>Bacillus</i> spp

3.5 API Identification of bacterial Isolates

The bacteria were identified tentatively using API test kits. Tables 5, 6 and 7 showed the API results of the Gram-negative, Gram-positive bacterial and actinomycetes isolates, respectively. Bacterial Isolates from the pesticide contaminated soil and control soil were mostly Gram-positive bacteria from the Genus *Bacillus*. The bacterial isolates identified were *Alkaligenes faecalis*, *Arthrobacter globiformis*, *Micrococcus luteus*, *Streptomyces coelicolor*, *Staphylococcus aureus*, *Staphylococcus* spp., *Chryseobacterium indologenes*, *Klebsiella pneumoniae*, *Paenibacillus polymyxa*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella* spp.

Table 5: Identification of bacterial isolates using Analytical Profile Index (API 20E) test

Isolate ID	ONPG	ADH	LDC	ODC	CIT	H ₂ S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO ₂	N ₂	MOB	OF-0	OF-F	% API similarity identity	Identity of Organisms
B4	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	-	+	+	98	<i>Chryseobacterium indologenes</i>
B5	+	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	82.5	<i>Klebsiella</i> species
B6	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	95	<i>Alkaligenes faecalis</i>
B7	+	-	+	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	99.1	<i>Klebsiella pneumoniae</i>
B12	-	+	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	+	+	-	+	+	+	+	+	+	97.8	<i>Pseudomonas aeruginosa</i>
B13	-	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	+	-	-	-	-	+	-	+	-	+	93	<i>Proteus vulgaris</i>

Table 6: Identification of bacterial isolates using Analytical Profile Index (API 50CH) test

Isolate ID	GLY	DARA	LARA	GAL	GLU	FRU	SOR	RHA	MNE	DUL	CEL	ESC	MAN	INO	LAC	TRE	SAC	MEL	MAL	MDG	NAG	MDX	SBE	RIB	DXYL	LXYL	ADO	% API similarity identity	Tentative Identity of Organisms
B1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90.0	<i>Arthrobacter globiformis</i>
B2	+	+	+	+	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	95.0	<i>Paenibacillus polymyxa</i>
B3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	99.0	<i>Bacillus subtilis</i>
B9	+	-	-	+	+	+	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	93.5	<i>Staphylococcus</i> spp.
B10	+	-	-	+	+	+	-	-	+	-	+	+	+	-	-	+	+	-	+	+	+	+	-	+	+	+	-	95.1	<i>Staphylococcus aureus</i>
B11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	89.5	<i>Micrococcus luteus</i>
B14	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	-	+	95.0	<i>Bacillus cereus</i>

Table 7: Identification of actinomycetes using Analytical Profile Index (API 20A) test

ID	CAT	URE	GLU	GEL	IND	MAN	LAC	SAC	MAL	SAL	XYL	ARA	ESC	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE	API % Similarity	Tentative Identification
B8	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	-	-	-	+	98.0	<i>Streptomyces coelicolor</i>

3.6 Comparative bacterial diversity across the pesticide contaminated soils and control

The comparative bacterial diversity of both soils are displayed in Figs. 2 and 3. The control soil was dominated by *Bacillus subtilis* (23.08%) as seen in Fig. 2 while pesticide-contaminated Farm soils were dominated by members of the phylum Firmicutes [*Bacillus subtilis* (28.57%) and *Paenibacillus polymyxa* (17.14%)] (Fig. 3),

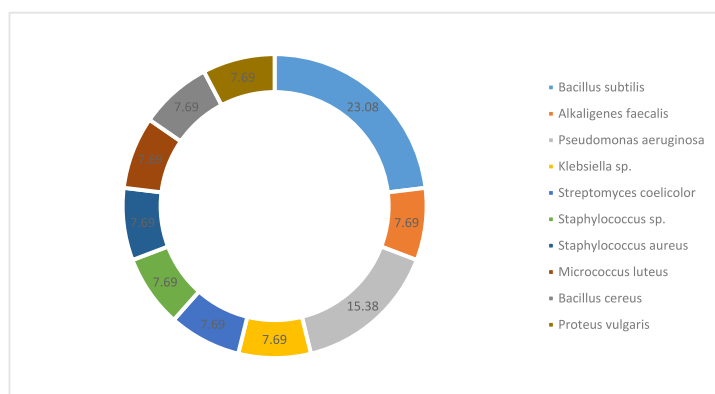


Fig. 2. Bacterial diversity in control soil

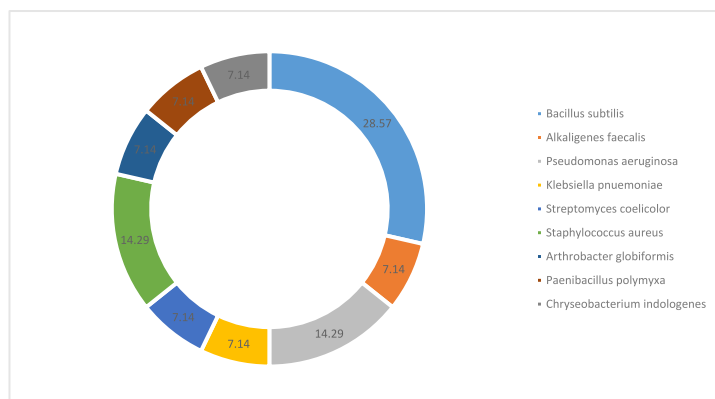


Fig. 3. Bacterial diversity across agrochemical Farms

4.0 Discussion

Soil pH values were within the acidic range 4.55 - 6.24, while temperature stayed constant at 25.0°C across all samples. The pH values were within the same range for all soil samples, this indicated that the pesticides had no effects on the tested soils relative to the control and this may be due to the nature of the soil in the study area. It has been reported that acidic, sandy soils tend to prolong organochlorine half-life and modulate organophosphate degradation [24]. This corroborates research outcomes that persistent pesticides may possibly modify soil pH, organic matter, and nutrient cycling [38]. Different researchers have reported pH range values of 5.5 to 8.0 for most Farm soils but may increase or reduce under different agricultural practices. The solubility of soil macronutrients, micronutrients or essential trace elements is influenced by soil pH [39,40].

Nitrate contents were 9.14 mg/kg (Farm II), 11.88 mg/kg (Farm I), 15.0 mg/kg (Farm III) and 14.55 mg/kg in the control soil.

The present-day study has shown that pesticides had an effect on the nitrates content of the soil relative to the control probably, as a result of their sustained use on the soil [41]. Available phosphate content levels were also higher in the pesticide contaminated soil (5.55 mg/kg, 4.55 mg/kg and 3.26 mg/kg for Farm I, Farm II, Farm III, respectively) compared to the control (2.87 mg/kg), which could indicate nutrient enhancement resulting from microbial degradation of these pesticides [39,42].

Cation exchange capacity (CEC) showed slight increases in Farm I (1.71 meq/100g), Farm II (1.71 meq/100g), and Farm III (1.84 meq/100g). This pattern suggests that herbicide use did not significantly alter CEC compared to the control, likely because the soils had already been exposed to pesticides for a long time. The higher values may also reflect the influence of organic matter at the soil surface, which naturally boosts cation exchange capacity. Similar observations have been reported elsewhere [43]. For total organic carbon, Farm II recorded a rise to 0.19%, Farm III showed a slight decline to 0.12% compared to the control (0.11%). Previous studies highlight that herbicide concentrations in soil often depend on carbon content. Bradbury et al. [44] noted that soils with higher carbon and nitrate levels tend to retain fewer herbicides, most likely due to more active microbial communities. In essence, soil organic matter plays a decisive role in herbicide fate, helping to break them down and speed up their disappearance.

Different OCP residues were identified from the Farm soils. Aldrin values were 2.25 ppb in Farms I and III, although there was no noticeable change between control and Farm II. The same values (0.25 ppb) were observed for Beta-BHC in relation to the control with no detectable value in Farm II. Dichlorodiphenyltrichloroethane (DDT) reaching up to 2.25 ppb and heptachlor to 2.80 ppb indicated potential environmental implication. This report is in corroboration with the findings of Xu et al. [45].

Methoxychlor and DDT were detected across all samples (0.35 ppb and 0.25–2.25 ppb, respectively), suggesting potential illegal application or use in agriculture and vector control. Dichlorodiphenyldichloroethylene (DDE) was not detected in most Farms except Farm I.

Dichlorodiphenyltrichloroethane and metabolites (p,p'-DDE, DDD) with values of (0.61–2.01 ppb) suggest ongoing environmental presence and may lead to potential ingestion through food chains. The presence of compounds like endosulfan sulfate, DDT, dieldrin, and endrin in the environment pose moderate to high ecological risk. Endosulfan and DDT have been shown to present moderate environmental risk in coastal sediments, with likelihood of exceeding predicted no-effect concentrations [46].

Also, OPPs were characterized from the various Farm soils. Azinphos-ethyl exhibited the highest concentrations across the samples, reaching 4.24 ppb in some Farms. This finding aligns with recent research that highlights the persistence of Azinphos-ethyl in agricultural soils [47].

Quinalphos was found at significant concentrations in three of the Farms. Quinalphos remains one of the more frequently detected OPPs in soils from regions with intensive pesticide application; the findings are in agreement with the reports of Garg et al. [48]. Its presence here may be attributed to its widespread use as an insecticide in crop protection, especially in tropical and subtropical regions. Also, 1,3-dimethyl-2-nitrobenzene was detected only in Farms I (1.79 ppb) and III (2.79 ppb). While not a classic OPP, derivatives of nitrobenzene compounds have been reported as degradation products or impurities associated with pesticide formulations [49]. The presence of EPN in Farm III (3.53 ppb) but its absence in other samples (Farms I and II) may indicate localized application or point-source contamination. These findings align with the results of Zhang et al. [50].

Other compounds, such as Diazinone and Chloropyrifos-methyl, were not detected across any of the Farm soil samples analyzed, except control. Their absence could reflect either restricted usage in the study area or rapid degradation due to environmental conditions favouring microbial breakdown [51]. Meanwhile, Isazophos was detected consistently at 0.14 ppb in Farm samples excluding the control, suggesting low-level, widespread contamination likely from past applications. Moreover, Triphenyl phosphate was found only in Farm II (1.63 ppb), while Pyrazophos was detected exclusively in Farm III (1.96 ppb). The isolated detection of these compounds is consistent with findings that show their selective application for specific pests and crops [52]. In conclusion, despite the presence of OCPs and OPPs across the Farms, these residues were below International Maximum Residue Limits (MRLs) except heptachlor epoxide.

Bacterial counts were higher in Farm soils with a history of pesticide use in comparison with the control in the study. This can be attributed to the microorganisms' capabilities to utilize the pesticides as a nutrient source. Interestingly, while our results show an increase in bacterial counts in pesticide-treated soils, other studies have reported contrasting outcomes. For instance, Reitstetter et al. [53] reported that the bacterial populations were adversely affected with the subsequent increase in exposure to pesticides. Furthermore, Némethová et al. [54] demonstrated that indaziflam residues can suppress sensitive bacterial taxa while favoring resistant groups, with soil organic matter, soil type and chemical nature of the pesticide formulation playing decisive roles in shaping microbial responses. These findings reinforce the idea that pesticide exposure leaves lasting microbial legacies in agricultural soils.

In this study, several bacterial isolates were identified, including *Staphylococcus aureus*, *Chryseobacterium indologenes*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Bacillus cereus*, *Paenibacillus polymyxa*, *Micrococcus luteus*, *Klebsiella* spp., *Arthrobacter globiformis*, *Staphylococcus* spp., *Streptomyces coelicolor*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Alkaligenes faecalis*. The diversity observed reflects the adaptability of soil microorganisms to environments with a history of pesticide application. Our findings are consistent with earlier reports by Nisha et al. [55], who isolated three distinct *Bacillus* species from carbofuran-enriched soils, underscoring the resilience of this genus under chemical stress. Likewise, Ataikiru et al. [56] documented four different *Bacillus* species in pesticide-amended soils, further supporting the observation that members of this genus are particularly well adapted to metabolize xenobiotic compounds.

The presence of *Klebsiella* spp. and *Pseudomonas aeruginosa* in our samples also aligns with the work of Ajuzieogu et al. [57], who reported these genera as common inhabitants of Farm soils exposed to herbicides. These organisms are often linked to biodegradation processes, suggesting that their persistence may be tied to their ability to use pesticides as alternative nutrient sources [20].

Taken together, these comparisons highlight the dual nature of microbial adaptation in agricultural soils. Genera such as *Bacillus* and *Klebsiella* appear to flourish under pesticide pressure, while others like *Staphylococcus* spp. may be more vulnerable. This pattern underscores the ecological importance of microbial diversity in shaping pesticide fate. The consistency of our findings with those of Nisha et al. [55], Ataikiru et al. [56], and Ajuzieogu et al. [57] reinforces the view that these isolates are not incidental contaminants but represent normal soil flora adapted to agricultural environments.

These isolates are not incidental contaminants but part of the soil flora adapted to agricultural environments. Pesticide contamination substantially reduces microbial richness and alters community composition, favoring stress-tolerant Firmicutes such as *Bacillus subtilis* and *Paenibacillus polymyxa*, whereas control soils sustain broader diversity with taxa like *Pseudomonas* and *Streptomyces*. Comparative profiles (Figs. 2 and 3) show control soils dominated by *B. subtilis* (23.08%), *Pseudomonas aeruginosa* (15.38%), and *Streptomyces coelicolor* (7.67%), while contaminated soils exhibit reduced diversity with *B. subtilis* (28.57%) and *P. polymyxa* (17.14%) prevailing. Recent studies [58,59,60] corroborate that pesticide exposure diminishes microbial richness but enriches Firmicutes due to their resilience and biodegradation potential. Although this dominance may facilitate pesticide breakdown, it signals ecological imbalance with implications for soil fertility and long-term sustainability.

4. CONCLUSION

This study shows that pesticide residues, particularly organochlorines (OCPs) and organophosphates (OPPs), strongly influence soil chemistry and microbial life. Across Farms, soil pH rose slightly, with Farm II reaching 6.24, signaling a shift toward neutrality that may not disrupt nutrient cycling. Moisture content and electrical conductivity also increased, reflecting changes in soil structure and ionic buildup from pesticide breakdown. In contrast, sulfates and nitrates declined, suggesting interference with microbial nutrient transformations. Bacterial diversity shifted, with species such as *Arthrobacter*, *Bacillus*, *Paenibacillus*, *Chryseobacterium*, and *Pseudomonas* identified. Persistent residues like DDT and Endrin ketone remain problematic, while OPPs such as Azinphos-ethyl were prominent. These findings highlight the persistence of pesticide pollutants and emphasize the urgent need for integrated management to protect soil health, fertility, and food security.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author Tega Lee-Ann Ataikiru designed the study, wrote the protocol, and managed the literature searches. Author Busayo Beatrice Adimula managed the analyses of the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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