

Research Article

Herbicide-Contaminated Soils in Dutsin-Ma Katsina State Nigeria: A Hotbed of Multidrug-Resistant Bacteria

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ABSTRACT

Agricultural intensification in Nigeria has led to increased herbicide usage, raising concerns over environmental contamination and its potential ecological effects. This study investigated the microbiological and physicochemical properties of herbicide-contaminated soils in Dutsin-Ma, Katsina State, Nigeria, and assessed the antibiotic resistance profiles of the indigenous bacterial isolates. Soil samples were collected from agricultural sites with a documented history of glyphosate or Agri-Force herbicide application. Standard microbiological techniques were used to isolate and identify bacterial species. The isolates were characterized morphologically and biochemically, and their antimicrobial susceptibility to tetracycline, ciprofloxacin, amoxicillin/clavulanic acid (augmentin), and sulfamethoxazole was determined using agar disk diffusion technique. The soils samples exhibited a near-neutral to slightly alkaline pH (6.5–8.3). Bacterial growth was prolific (91.7% of the samples), with counts ranging from 1.60×10^5 to 3.81×10^5 CFU/g. Five genera were identified: *Bacillus*, *Pseudomonas*, *Actinomyces*, *Streptomyces*, and *Escherichia coli*. A high prevalence of multidrug-resistant (MDR) isolates was observed, with 95.65% and 75% of the isolates from the two sample sources being resistant to all four antibiotic classes tested. A uniform TET-CIP-AUG-SUL resistance pattern was observed among the MDR isolates. Herbicide-contaminated soils in Dutsin-Ma host robust bacterial communities adapted to herbicide stress. The exceptionally high level of multidrug resistance among these environmental isolates suggests a strong link between herbicide pollution and the co-selection of antibiotic resistance genes. This finding underscores a significant public health and ecological concern, highlighting the urgent need for prudent agrochemical management and further research on the genetic mechanisms underlying this resistance.

Keywords: Antibiotic resistance; Antibiotic-resistant bacteria; Environmental pollution; Herbicide contamination; Soil contamination

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INTRODUCTION

Agricultural intensification in Nigeria has dramatically transformed farming practices, particularly in northern regions like Katsina State, where chemical herbicides have become integral to crop management strategies. According to Adebayo *et al.* (2022), herbicide usage in Nigerian agricultural system has increased by approximately 67% over the past decade, reflecting a significant shift in agricultural technological interventions. The environmental implications of extensive herbicide application represent a critical

research concern. A study by Umar *et al.* (2021) comprehensively documented the long-term ecological consequences of persistent agricultural chemicals. These herbicides, including glyphosate, atrazine, and 2,4-dichlorophenoxyacetic acid (2,4-D), demonstrate remarkable persistence in soil and water ecosystems, potentially disrupting complex ecological interactions. Microbiological interventions, particularly bacterial bioremediation, have emerged as a promising sustainable approach to managing agricultural chemical

contamination. Adekunle *et al.* (2021) emphasized the exceptional metabolic capabilities of specific bacterial genera in degrading complex chemical compounds. Several genera, including *Pseudomonas*, *Bacillus*, and *Arthrobacter*, have been identified for their herbicide-degrading capacities. These bacteria employ enzymatic systems capable of breaking down complex xenobiotics into less toxic or harmless products, thus facilitating natural attenuation processes in contaminated environments (Putra *et al.*, 2024; Zameer *et al.*, 2023). In a study by Jayaraj *et al.* (2023), *Pseudomonas nitroreducens* was isolated from agricultural soil and demonstrated high tolerance and degradation capability against multiple pesticides, suggesting its potential application in large-scale bioremediation efforts (Jayaraj *et al.*, 2023).

Bioremediation can be enhanced through several approaches, such as bioaugmentation (introduction of selected degraders), biostimulation (amendment with nutrients), and phytoremediation-assisted microbial remediation. The combined use of plant-associated microbes and crop species has shown efficacy in glyphosate-contaminated environments, improving both degradation rates and soil health (Masotti *et al.*, 2023). Moreover, microbial communities, rather than individual strains, are now recognized as more effective in degrading herbicides due to synergistic interactions and synergistic metabolic pathways within microbial consortia (Pileggi *et al.*, 2020).

Herbicide application does not only reduce bacterial population and diversity, but also shifts microbial community composition in soils. For instance, herbicides such as atrazine and paraquat had been shown to suppress sensitive bacterial species while enriching tolerant genera including *Bacillus* and *Pseudomonas* (Stanley *et al.*, 2013). These observations support the use of microbial indicators in assessing soil health and selecting candidates for soil bioremediation. Field-scale and experimental studies have shown success in deploying bacterial strains or communities for herbicide degradation. For example, Carles *et al.* (2021) reported accelerated degradation of 2,4-D herbicide when co-applied with the degrading strain *Cupriavidus necator*, reducing herbicide persistence by nearly threefold in agricultural soils (Carles *et al.*, 2021). Similarly, engineering multi-degrading bacterial consortia had been shown to significantly improve soil detoxification efficiency under real agricultural conditions (Thieffry *et al.*, 2024).

Global environmental challenges demand innovative, context-specific scientific interventions. The United Nations Environment Programme (WHO, 2022) has consistently emphasized the critical need for localized

research addressing agricultural sustainability. Katsina State's semi-arid agricultural landscape presents a unique research context for investigating herbicide-induced environmental transformations. Odoh *et al.* (2019) highlighted the region's vulnerability to chemical contamination. Their comprehensive analysis revealed that agricultural herbicides could persist in soil systems for extended periods, with potential half-lives ranging from 30 to 240 days, depending on specific environmental conditions. By focusing on Katsina State's specific ecological context, this research aims to generate meaningful insights into sustainable environmental management practices. The intersection of agricultural productivity, environmental conservation, and technological innovation demands continuous scientific exploration. This research represents a critical step towards developing sustainable, scientifically validated solutions for managing agricultural chemical interventions in sensitive ecological systems.

MATERIALS AND METHODS

Study Area

The study sites were selected based on agricultural intensification and documented herbicide usage. Twenty sampling locations were identified across these zones, ensuring representation of different soil types and farming practices. Each selected site had a minimum five-year history of consistent herbicide application, particularly focusing on commonly used herbicides such as glyphosate, atrazine, and 2,4-D (Figure 1).

Inclusion Criteria

The study included agricultural farmlands that met specific criteria to ensure data quality and consistency. Selected sites had documented herbicide application histories spanning at least five years, with detailed records of application frequencies and concentrations. The fields maintained consistent crop rotation patterns and soil management practices. Only farms that used common commercial herbicides (glyphosate and Agri-Force) were included. The selected sites had no history of bioremediation treatments within two years prior to this study to avoid interference with natural bacterial populations.

Exclusion Criteria

Fields with recent chemical treatments (within six months) other than herbicides were excluded from the study. Areas that showed evidence of industrial contamination, waterlogging, or flooding patterns were not considered. Sites that underwent biological treatments within the year prior to the study were excluded. Additionally, fields with extreme soil

conditions (pH < 4 or > 9) or those lacking proper documentation of herbicide usage were not included in the study.

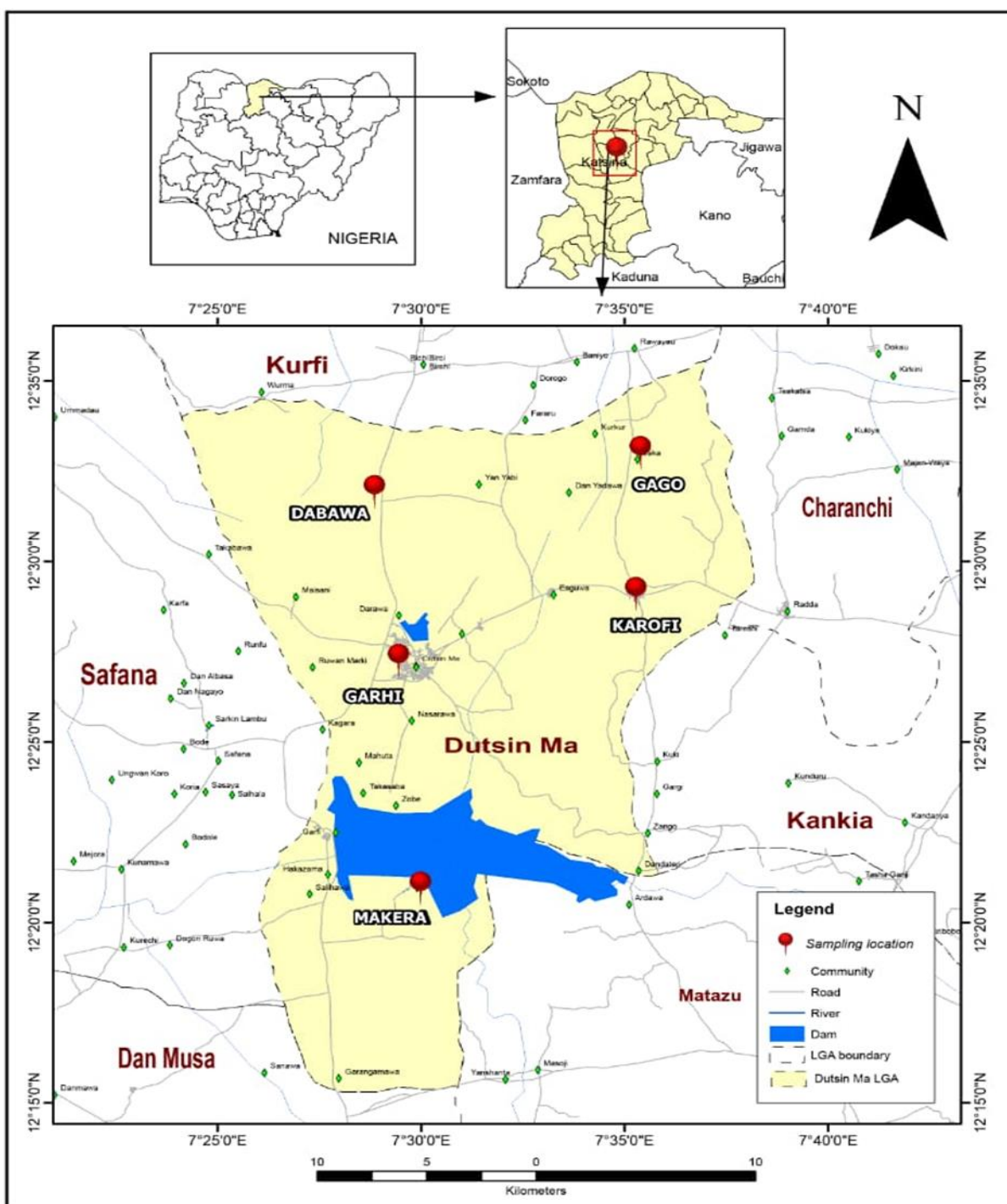


Figure 1. Map of Dutsin-Ma showing sampling sites

Ethical Approval

Prior to commencing the research, ethical approval was obtained from the relevant authorities. This included

clearance from the University Research Ethics Committee, permission from the Katsina State Agricultural and Rural Development Authority. Verbal

consent was secured from landowners and farmers whose fields were selected for sampling.

Collection of Samples

Soil samples were collected using a systematic grid sampling approach. At each selected site, samples were taken from two depth profiles: 0–15 cm and 15–30 cm. Sampling was conducted using sterile tools and containers to prevent cross-contamination. Each sampling point was geo referenced using GPS coordinates. Composite samples were collected from five points within each sampling grid, following a W-pattern. Each selected sampling site had been assigned a unique sample code for proper identification and traceability throughout the study. This coding system was used consistently in data logs, sample containers, and laboratory analysis records to ensure accuracy and traceability. The samples were immediately stored in sterile polyethylene bags and transported to the laboratory in ice boxes maintained at 4°C. Basic soil physicochemical parameters (pH and temperature) were measured on site.

Sample Processing

The collected soil samples were air-dried at room temperature (approximately 25°C) for 48 hours under sterile conditions to prevent microbial contamination. After drying, the samples were sterile sieved through a 2 mm mesh to remove stones, plant debris, and other large particles, ensuring a uniform and homogenous sample texture suitable for microbiological analysis. From each processed soil sample, Ten-fold serial dilutions were prepared using sterile physiological saline solution (0.85% NaCl). This involved adding 1 gram of the sieved soil into 9 mL of saline to create the initial 10^{-1} dilution, followed by successive ten-fold dilutions up to 10^{-6} . Each dilution step was carried out using sterile test tubes and pipettes to maintain aseptic conditions throughout the procedures as previously described by Hojdová *et al.*, (2015).

Primary Isolation

Aliquots (0.1 mL) from appropriate serial dilutions were spread-plated on Mineral Salt Medium (MSM), which contained (g/L): K_2HPO_4 (1.5), KH_2PO_4 (0.5), NaCl (0.5), $(NH_4)_2SO_4$ (0.5), $MgSO_4 \cdot 7H_2O$ (0.2), and herbicide (50–100 mg/L), depending on the compound under investigation. The herbicides used included glyphosate and Agri-Force added individually to assess microbial tolerance and potential degradation. The plates were incubated at $28 \pm 2^\circ C$ for 48 to 72 hours under aerobic conditions. Following incubation, colonies that exhibited distinct morphological characteristics (e.g., colour, shape, elevation, and edge) were counted and documented. Colony counts were used as a preliminary indication of herbicide-tolerant microbial populations.

Pure Culture Isolation

Morphologically distinct colonies were selected from the herbicide-supplemented plates and purified using the streak plate method on Nutrient agar. This process was repeated multiple times to ensure that pure cultures were obtained, eliminating any potential mixed growth. The purified isolates were maintained on double strength Nutrient agar slants and stored at 4°C for routine laboratory use. For long-term preservation, the isolates were suspended in sterile 20% phosphate buffered glycerol solution and stored at $-80^\circ C$, ensuring viability and genetic stability over extended periods.

Bacterial Identification

Morphological Characterization

Each isolated bacterial strain was characterized based on its cultural morphology, as observed on Nutrient agar plates. In addition, the isolates were Gram stained, aiding in preliminary classification. Microscopic examination of the stained cells was carried out using a compound light microscope to observe basic cell features and staining response. For more detailed visualization of surface structures.

Biochemical Characterization

The isolates were subjected to various biochemical tests to characterize their enzymatic and metabolic activities:

Catalase Test: The catalase test was used to determine the ability of the bacterial isolates to produce the catalase enzyme, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. A small amount of each bacterial culture was placed on a clean glass slide, and a drop of 3% hydrogen peroxide solution was added. The immediate formation of gas bubbles indicated a positive catalase reaction, confirming the production of the catalase enzyme (Cheesbrough, 2006).

Oxidase test

The oxidase test was performed to detect the presence of cytochrome c oxidase, an enzyme involved in the electron transport chain of aerobic respiration. A small portion of each bacterial culture was transferred onto an oxidase reagent strip impregnated with tetramethyl-p-phenylenediamine. A blue or purple color change within 10–30 seconds indicated a positive oxidase reaction, confirming the presence of cytochrome c oxidase in the isolate (Cheesbrough, 2006).

IMViC tests

The IMViC series of tests—Indole, Methyl Red, Voges-Proskauer, and Citrate utilization were carried out to further evaluate the metabolic characteristics of the isolates:

Indole test

The isolates were inoculated into tryptone broth and incubated at the appropriate temperature. After

incubation, Kovac's reagent was added to each tube. A red to cherry-red layer in the reagent indicated a positive indole test, signifying the ability of the organism to degrade tryptophan into indole (Cheesbrough, 2006).

Methyl red test

The isolates were inoculated into glucose phosphate broth and incubated. After incubation, methyl red indicator was added. A stable red coloration indicated a positive methyl red test, suggesting the production of stable acid end-products during glucose fermentation (Cheesbrough, 2006).

Voges-Proskauer test

The isolates were inoculated into the same glucose phosphate broth and incubated. Following incubation, α -naphthol and potassium hydroxide (KOH) were added. The development of a pink to red color indicated a positive Voges-Proskauer test, demonstrating the production of acetoin as a fermentation product (Cheesbrough, 2006).

Citrate utilization test

The isolates were streaked onto Simmons Citrate agar and incubated. Growth on the medium and a colour change from green to blue indicated a positive citrate utilization test, confirming the organism's ability to utilize citrate as the sole carbon and energy source (Cheesbrough, 2006).

Sugar Fermentation Patterns

The bacterial isolates were tested for their ability to ferment various carbohydrates, including glucose, lactose, sucrose, and mannitol, as part of their biochemical characterization. Each isolate was inoculated into specific carbohydrate fermentation broths, each containing a single carbohydrate, phenol red as a pH indicator, and an inverted Durham tube to capture any gas produced during fermentation. The inoculated media were incubated under standard conditions, and observations were made for both acid production (indicated by a color change from red to yellow) and gas production (evidenced by bubbles in the Durham tube). These changes were recorded after 24–48 hours of incubation. The resulting carbohydrate fermentation profiles based on the presence or absence of acid and gas for each sugar were used as additional biochemical markers to aid in the identification and

differentiation of the bacterial species (Cheesbrough, 2006).

Antimicrobial Susceptibility Testing

Isolates were screened for phenotypic resistance and susceptibility to Gram-positive and Gram-negative antibiotic disks. The procedure includes inoculation of stock cultures stored at 4°C on nutrient agar slants into 10ml of nutrient broth, which was then incubated overnight at 37 °C. Thereafter, the bacterial suspension was standardized to an OD600 of 0.5 in M üeller-Hinton broth, and 0.1 mL was spread onto Mueller-Hinton agar plates. Antibiotic disks were then applied, and the plates were incubated at 37°C for 24 hours. Following incubation, the inhibition zones were measured according to CLSI guidelines. (CLSI, 2021). Multidrug-resistant isolates were selected based on their resistance to ≥ 3 classes of antibiotics.

RESULTS

The physicochemical parameters of the various soil samples show that the soil samples across different locations had near-neutral to slightly alkaline pH (6.5 - 8.3) and temperatures typical of the region (27°C - 33°C) (Table 1).

The bacterial distribution across the various sampling areas showed that 91.7% (55 out of 60) of the samples were positive for bacterial growth. Agri-Force-contaminated sites (ACS) showed 100% bacterial growth. In contrast, glyphosate-contaminated soil had lower bacterial growth. Fisher's exact test indicated a statistically significant association between sampling location and bacterial prevalence ($p = 0.008$) (Table 2). Total bacterial counts were high, ranging from $\sim 1.60 \times 10^5$ to 3.81×10^5 CFU/g of soil. The Agri-Force (ACS)-contaminated soil samples from Makera and Gago had the highest bacterial counts. A statistically significant difference in mean bacterial counts (CFU/g) was observed across the sample locations in herbicide-contaminated soils ($p = 0.021$) (Table 3).

Bacillus species was the most common genus isolated (44 out of 107 isolates), followed by *Actinomyces* species and *Pseudomonas* species (19 each). Conversely, *Streptomyces* species and *E. coli* were the lowest species (Table 4).

Table 1. Physicochemical Parameters of herbicide-contaminated soil of Dutsin-Ma, Katsina State

Sample locations	Herbicide types used in the sample locations	pH	Temperature (°C)
Gago	GCS	7.0	27
Dabawa	ACS	8.3	30
Garhi	GCS	6.5	30
Karofi	GCS	7.0	33
Makera	GCS	7.6	28
Gago	ACS	7.4	30

Keys: GCS = lyphosate contaminated soil; CS =Agri-Force contaminated soil

Table 2. Percentage bacterial growth per sample from herbicide-contaminated soil of Dutsin-Ma, Katsina State, Nigeria

Sample location	Sample types	Number of samples tested	Number of samples positive for bacterial growth	Percentage bacterial growth per sample (%)
Gago	GCS	10	7	70
Dabawa	ACS	10	10	100
Garhi	GCS	10	8	80
Karofi	GCS	10	10	100
Makera	GCS	10	10	100
Gago	ACS	10	10	100
Total		60	55	91.7

Keys:GCS = glyphosate contaminated soil; CS =Agri-Force contaminated soil

Table 3. Mean Bacterial counts of herbicide-contaminated Soil of Dutsin-Ma, Katsina State, Nigeria

Sample locations	Sample types	Mean Bacterial Counts (CFU/g)
Gago	GCS	1.74 X 10 ⁵
Dabawa	ACS	2.61 X 10 ⁵
Garhi	GCS	1.60 X 10 ⁵
Karofi	GCS	1.1 X 10 ⁵
Makera	ACS	3.81 X 10 ⁵
Gago	ACS	2.91 X 10 ⁵

Keys: GCS = glyphosate contaminated soil; CS =Agri-force contaminated soil.

Table 4. Distribution of bacteria from Herbicide-Contaminated Soil

Bacteria genera	Number of bacterial isolates	Percentage occurrence (%)
<i>Actinomyces</i>	19	17.76
<i>Bacillus</i>	44	41.12
<i>E. coli</i>	12	11.21
<i>Pseudomonas</i>	19	17.76
<i>Streptomyces</i>	13	12.15
Total	107	100

Antibiotic susceptibility patterns among the five groups of bacterial species recovered from herbicide-contaminated soil revealed that *Actinomycetes*, *Bacillus*, *Pseudomonas*, and *Streptomyces* species exhibited high resistance profiles. In contrast, *Escherichia coli* showed complete susceptibility to all the tested antibiotics (TET, CIP, AUG, and SUL). Pearson's Chi-Square and Fisher's exact tests revealed a statistically significant association between the bacterial isolate groups and their antibiotic resistance patterns ($p = 0.000$, $p < 0.001$), (Table 5).

All recovered MDR isolates show similar resistance patterns, with resistance to tetracycline, ciprofloxacin, augmentin, and sulphamethoxazole being the common resistance phenotypes (Table 6).

The percentage of resistance in the bacterial isolates varied across the sampling sites. Isolates from source A showed complete (100%) resistance to tetracycline and sulfamethoxazole and approximately 95.65% resistance

to ciprofloxacin and augmentin. Isolates from source B exhibited slightly lower resistance patterns than those from source A, with 86.36% resistance to tetracycline and 77.27% resistance to ciprofloxacin. In contrast, isolates recovered from source E were completely susceptible to all antibiotics tested (Table 7). Post-hoc pairwise comparisons (Bonferroni-corrected $\alpha = 0.0167$) revealed significant differences between Groups A and E and between Groups B and E ($p < 0.001$ for both Chi-square and Fisher's Exact Tests). No significant difference was found between Groups A and B (chi-square $p = 0.094$; Fisher's exact $p = 0.033$), a result that was consistent across tests when considering the corrected alpha.

A total of 95.65% of the isolates from Source A and 75% of the isolates from Source B were classified as MDR isolates. On the other hand, none of the isolates from source E exhibited MDR phenotype (Table 8).

Table 5. Antibiotic susceptibility profiles of the bacteria isolated from herbicide-contaminated Soil of Dutsin-Ma, Katsina State, Nigeria

Bacterial Isolates	MDR Phenotypes	Intermediate Phenotypes	Susceptible Phenotypes	Total Isolates recovered
<i>Actinomycetes</i> species	22	1	0	23
<i>Bacillus</i> species	28	7	5	40
<i>Escherichia coli</i>	0	0	12	12
<i>Pseudomonas</i> species	19	0	0	19
<i>Streptomyces</i> Species	9	3	1	13
Total	78	11	18	107

Table 6. Resistant patterns of MDR isolates from herbicide-contaminated soil of Dutsin-Ma, Katsina State, Nigeria

Isolates	Number of antibiotics tested	Resistant phenotype	Frequency of occurrence
<i>Actinomycetes</i> species	4	TET-CIP-AUG-SUL	22
<i>Bacillus</i> species	4	TET-CIP-AUG-SUL	28
<i>Pseudomonas</i> species	4	TET-CIP-AUG-SUL	19
<i>Streptomyces</i> species	4	TET-CIP-AUG-SUL	9
Total			78

Table 7. Resistant profiles of the isolates from herbicide-contaminated soil

Source	Class of antibiotics	Antibiotics	Disc potency. (µg)	Resistant number (%)	Susceptible number (%)	Intermediate number (%)
A	Tetracycline	Tetracycline	30	100.00	0.00	0.00
	Fluoroquinolone	Ciprofloxacin	10	95.65	4.35	0.00
	β-lactam	Augmentin		95.65	4.35	0.00
	sulfonamide	Sulphamethoxazole	25	100.00	0.00	0.00
B	tetracycline	tetracycline	30	86.36	13.64	0.00
	fluoroquinolone	ciprofloxacin	10	77.27	22.73	0.00
	β-lactam	augmentin		88.64	11.36	0.00
	sulfonamide	sulphamethoxazole	25	81.82	18.18	0.00
E	tetracycline	tetracycline	30	0.00	100	0.00
	fluoroquinolone	ciprofloxacin	10	0.00	100	0.00
	β-lactam	augmentin		0.00	100	0.00
	sulfonamide	sulphamethoxazole	25	0.00	100	0.00

Table 8. Percentage occurrence of MDR within the sample sources

Sources	% of MDR
A	95.65
B	75.00
E	0.00

DISCUSSION

Herbicide contamination remains a problem in low-resource settings, with negative consequences for ecological niches. This study provides baseline data on the microbiological and physicochemical properties of herbicide-contaminated soils in the Dutsin-Ma Local Government Area of Katsina State, Nigeria.

Physicochemical analysis revealed that the soil pH ranged from slightly acidic (6.5) to slightly alkaline (8.3), while the temperatures were consistently warm (27–33°C). These conditions are generally conducive to microbial activity and proliferation. The near-neutral to alkaline pH observed, particularly in Agri-Force contaminated sites (ACS), is consistent with other studies that have reported that herbicide application

can alter soil pH over time, influencing microbial community structure and function (Araujo *et al.*, 2019). A high percentage of bacterial growth was recovered from the herbicide-contaminated soil samples (91.7%), suggesting that the native bacterial communities have developed tolerance or degradative capabilities against the applied herbicides. This was corroborated by the mean bacterial counts, which were substantial, ranging from 1.60×10^5 to 3.81×10^5 CFU/g. Notably, ACS-contaminated soil samples from Dabawa and Makera showed higher bacterial counts than some glyphosate-contaminated soil (GCS) samples, indicating a significant association between sampling location and bacterial prevalence ($p = 0.008$). This aligns with the findings of Liu *et al.* (2021), who discovered that soils with a history of herbicide exposure often harbor enriched populations of degradative microbes, a phenomenon known as microbial adaptation. The specific formulation of "Agri-Force" may provide a carbon/nitrogen source for a broader consortium of bacteria compared to glyphosate alone, which is primarily degraded through specific pathways, such as the C-P lyase enzyme (Singh and Walker 2016).

Phenotypic characterization and distribution of bacteria from herbicide-contaminated soil revealed *Bacillus* spp. (41.12%) as the most dominant genus, followed by *Actinomyces* spp. and *Pseudomonas* spp. (17.76%). The prevalence of *Bacillus* and *Pseudomonas* is a common finding in polluted environments, as these genera are renowned for their metabolic versatility and ability to utilize a wide range of xenobiotic compounds, including herbicides, as energy sources (Ortiz-Hernández *et al.*, 2013; Cycoń and Piotrowska-Seget, 2016). On the other hand, the isolation of *Streptomyces* and *Actinomyces* is also significant, as these actinobacteria are known for their prolific production of extracellular enzymes capable of breaking down complex organic molecules (Elbendary *et al.*, 2016).

Alarming, antibiotic resistance among the recovered isolates was high; 95.65% of the isolates from source A and 75% of those from source B exhibited multidrug-resistant phenotypes. In contrast, all isolates from source E were susceptible to all the antibiotics tested. The resistance profiles for the MDR isolates were uniform, with resistance to all tested antibiotics: tetracycline, ciprofloxacin, augmentin, and sulfamethoxazole. Notably, resistance to tetracycline and sulphamethoxazole was absolute (100%) in isolates recovered from source A.

This high prevalence of MDR in soil bacteria, particularly in strains not typically associated with human infection (*Bacillus* and *Actinomyces*), is a serious public health and environmental concern. Pearson's Chi-square and

Fisher's exact tests revealed a statistically significant association between the bacterial isolates and their resistance phenotypes ($p = 0.000$, $p < 0.001$). These findings strongly suggest that herbicide contamination may co-select for antibiotic resistance, which can occur through several mechanisms: (1) co-resistance, where genes for herbicide degradation and antibiotic resistance are located on the same mobile genetic element (e.g., plasmid); or (2) cross-resistance, where a single mechanism, such as an efflux pump, can expel both the herbicide and unrelated antibiotics from the bacterial cell (Andersson and Hughes 2014; Kurenbach *et al.*, 2015). Studies have increasingly demonstrated that agricultural practices, including the use of herbicides and pesticides, can exert a selective pressure that enhances the reservoir of antibiotic resistance genes (ARGs) in the soil microbiome, a phenomenon known as the "soil resistome" (Nesme and Simonet, 2015; Van Bruggen *et al.*, 2018). Our results are consistent with those of Dai *et al.* (2018), who found a significant correlation between glyphosate exposure and increased antibiotic resistance in soil bacteria.

The uniform susceptibility patterns of the isolates recovered from source E were intriguing. This suggests that the selective pressure from herbicides may not affect all bacterial genera equally. It is also possible that these sites were recently contaminated with fecal matter or manure and had not been exposed long enough to develop or acquire resistance traits under herbicide pressure.

CONCLUSION

This study demonstrates that soils contaminated with glyphosate and Agri-Force herbicides in Dutsin-Ma host a robust community of bacteria, predominantly *Bacillus* and *Actinomyces* species, that have not only adapted to the herbicides but also exhibit disturbingly high levels of multi-drug resistance. This provides compelling evidence of a link between herbicide pollution and the emergence and persistence of antibiotic resistance in soil bacteria. This underscores the urgent need for the prudent use of agrochemicals and further research into the genetic mechanisms linking herbicide tolerance to antibiotic resistance, as this poses a potential pathway for the transfer of resistance genes to clinically important pathogens.

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