



Assessment of Residual Antibiotics, Biocides and their impact on Soil Microbial Communities in Irrigated Farms in Kano State, Nigeria

Suleiman, M^{1.}, Mohammed N^{2.}, Aliyu A^{2.}, Shamsuddeen Umar³ Sani Yahaya^{3.}, and Habibu Usman Abdu³

¹Department of Biology, School of Science, Federal College of Education Technical Bichi Kano State, Nigeria

²Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria

³Department of Microbiology, Bayero University, Kano State Nigeria

Corresponding Author's Email: maisunice03@gmail.com (+234 806 020 6058)

Received on: January, 2025

Revised and Accepted on: February, 2025

Published on: March, 2025

ABSTRACT

This study investigates the presence and impact of residual antibiotics and biocides on soil microbial communities in irrigated farms in Kano State, Nigeria. The growing use of antibiotics and biocides in agriculture, particularly in Kano's extensive irrigated farming systems, has raised concerns about their environmental effects. This research aims to understand how these substances affect soil health by analyzing soil samples from three farms and a control site. Soil samples were collected biweekly over six months from three farms and a control area using a 6-inch sampling probe. Bacterial counts were determined using pour-plating techniques, and bacterial isolates were identified through biochemical tests. Residual antibiotics and biocides in soil were quantified using an Agilent Intuvo 9000 GC system coupled with a detector system 5977B MSD. The total bacterial counts in the farms' soil samples were significantly higher than in the control site, indicating contamination. Ceftriaxone, doxycycline, and amoxicillin-clavulanic acid were the most frequently detected antibiotics, with varying concentrations across different farms. Cypermethrin was the only biocide consistently found in soil samples, with higher concentrations in the irrigated farm soils than in the control. Several bacterial isolates exhibited resistance to multiple antibiotics, particularly amoxicillin-clavulanic acid and doxycycline. This resistance was more pronounced in soil from the farms than the control site. The presence of residual antibiotics and biocides in Kano's irrigated farms significantly affects soil microbial communities, leading to increased bacterial counts and antibiotic resistance. This study underscores the need for sustainable agricultural practices to mitigate the environmental impact of these substances.

Keywords: Antibiotics, Biocides, Cypermethrin, Ceftriaxone, Doxycycline

1.0 INTRODUCTION

Significant environmental concerns, especially to soil microbial populations, are associated with the growing use of antibiotics and biocides in agriculture. In agricultural production and animal husbandry, antibiotics and biocides are widely used to prevent and control illnesses. Nevertheless, their residues may linger in the environment, especially in soils, where they may negatively impact microbial populations that are essential to the fertility and health of the soil (Kotzerke *et al.*, 2008).

The breakdown of organic matter, the cycling of nutrients, and the general health of the soil are all influenced by the microbial populations in the soil. These communities may be disturbed by the presence of leftover antibiotics and biocides, which can kill or selectively inhibit sensitive microbial populations, reducing microbial diversity and changing the structure of the community. The stability of the ecosystem and plant growth may be impacted by this disturbance, which can compromise vital soil processes (Keen & Patrick, 2013). Microbial communities in the soil are vital to the decomposition of organic matter, the cycling of nutrients, and the general health of the soil. Because they selectively

suppress or kill sensitive microbial populations, leftover antibiotics and biocides can upset these communities, reducing microbial diversity and changing the structure of the community. This disturbance may hinder vital soil processes, impacting plant development and the stability of the ecosystem processes (He *et al.*, 2010).

Recent research has demonstrated the substantial effects of biocides and residual antibiotics on soil microbial communities. For instance, processes of Akimenko (2021) and Kotzerke *et al.* (2008) discovered that antibiotics can differentially inhibit the growth of soil microorganisms, resulting in changes in the ecological functionality and composition of the community. Similarly, He *et al.* (2010) and Akimenko (2021) identified factors like exposure levels, degradation rates, and soil texture that affect the distribution and effects of antibiotics in soils.

The improper disposal of pharmaceutical waste and extensive use of agrochemicals has resulted in the persistence of residual antibiotics and biocides in soil environments. The physicochemical properties of these environments influence the persistence and mobility of these contaminants, yet there is limited

comprehensive data on these parameters within the context of irrigated farms in Kano State. Soil is essential for agricultural activities and human survival, and the economy of any nation depends largely on the availability of its land (Hubeny *et al.*, 2019).

One of the main economic sectors in Kano State, Nigeria, is Agriculture. In this region, biocides and antibiotics are frequently used, particularly in irrigated crops. Kano State offers a unique setting for researching how these compounds affect soil microbial populations because of its climate, soil types, and agricultural methods. The ongoing use of antibiotics and biocides can result in notable alterations in soil microbial diversity and function, which may have an impact on crop productivity and soil health, according to earlier research conducted in comparable environments.

Kano's agriculture thrives due to advantageous climatic and soil conditions, facilitating the cultivation of crops such as cotton, groundnuts, and assorted vegetables, in addition to substantial cattle and dairy production. However, the extensive use of pesticides and fertilizers is prevalent, with about 77.3% of farmers using chemicals to enhance production (Oyewole & Ojeleye 2014).

Antibiotics and biocides have been shown to change the microbial populations in the soil in irrigated farming systems in areas with comparable agricultural practices. These changes have the potential to lower soil fertility and agricultural production by reducing microbial diversity and compromising the soil's natural processes (Abakpa & Umoh, 2013). Developing sustainable farming methods that preserve soil health and guarantee long-term output requires an understanding of these effects

Therefore, this widespread application of agrochemicals, including antibiotics and biocides, has raised concerns about their impact on the environment and public health.

2.0 MATERIALS AND METHODS

2.1 Collection of Samples

Using the protocol outlined by Shawn *et al.* (2015), a total of 156 soil sample (comprises of 144 from Fagge (LGA) and 12 from CDA (BUK) as a control) were randomly collected every two (2) weeks for six (6) months. Samples of soil were taken from three farms. Using a 6-inch sampling probe, four soil samples were randomly taken from each farm. To acquire sub-samples, this process was repeated ten to fifteen (10-15) times. The sub-samples were then combined, and a small amount of the soil was moved to a small soil sample container. Similarly, utilizing the same process as previously illustrated, a random sample of

soil from CDA study area was taken and used as a control. After being delivered to the lab, each sample was stored in an ice box held at 40C (American Public Health Association, 2012).

2.2 Enumeration of Aerobic Mesophilic Bacteria from Soil Samples

This was carried out using the pour-plating technique. A vortexer was used to homogenize the soil sample with peptone water, after which 10 ml of the homogenate was transferred into a 90-ml conical flask containing sterile buffered peptone water and labelled 10^{-1} . After agitation, about 1 ml of 10^{-1} was transferred into another test tube containing 9 ml of buffered peptone water and labeled 10^{-2} . This was repeated to obtain 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} respectively. One (1) millimeter of diluent from 10^{-8} and 10^{-9} was transferred into two sterile petri dishes and labeled accordingly. Cooled molten nutrient agar was poured into each petri dish, swirled clockwise and anti-clockwise, and allowed to solidify. Finally, the plates were incubated at 37°C for 24 hours (International Organization for Standardization, 2017).

2.3 Isolation of Bacteria

Discrete colonies from the pour-plating technique were picked up and subcultured on MacConkey and Salmonella Shigella Agar (SSA) plates and incubated for 24–48 hrs at 37°C (APHA 2012; IOS 2017). Pure isolates were obtained by repeated streaking on MacConkey agar plates and stored in a cryovial (skim milk solution, 150 ml glycerol, and 850 ml distilled water), autoclaved at -80°C for biochemical testing.

2.3.1 Biochemical Test

The colonial features of each isolate, including sizes, forms, colours, margins, elevations, and opacity, were noted. Furthermore, morphological traits such as cell shape, organizational structure, and Gram reaction were ascertained using accepted techniques. The API 20E Kit (Biomerieux) further confirmed the isolates' identities.

2.4 Concentration of Antibiotics and Biocides in Soil

This was carried out, as demonstrated by Lixiang *et al.* (2019); Yun-Feng *et al.* (2022). In detail, extraction was first carried out by weighing 1 gram of the soil sample into a centrifuge tube. About 20 ml of the mixture of methanol, acetonitrile, acetone, and ethyl acetate (2:1:1:1) was added to the soil sample. This was then shaken in a vortex shaker for 30 minutes at 80 rpm. The mixture was then centrifuged at 30 rpm for 10 minutes. The supernatant was decanted and filtered. This was then evaporated to dryness and then reconstituted with 5 ml of methanol. Analysis was performed using an Agilent Intuvo 9000 GC system coupled with the detector system 5977B MSD with split and splitless injectors. A DB-5MS (5% phenyl

dimethylsiloxane) fused silica capillary column (30 m, 320 µm i.d., 0.25 µm of film thickness) was used with helium gas (99.999% purity) as carrier gas at flow rates of 1.2 ml min⁻¹. Inlet temperature was set at 300°C, MS Source at 230°C, and MS Quad at 150°C. The oven temperature was programmed: 50°C for 5 minutes, increased to 300°C at 20°C min⁻¹. Data were acquired by GCMSD/Enhanced MassHunter Software and Processed GCMSD Data Analysis Software incorporated with the 2017 version of the NIST Library. About 1 µL of the extracts was injected in splitless mode into the GC system using the Agilent Automated Liquid Sampler (ALS) G4513A. The analysis was performed in selected ion monitoring mode (SIM).

2.5 Bacterial-Antibiotic Resistance Test

This was carried out by the disc diffusion technique (Kirby-Bauer) using commercially available discs (Clinical and Laboratory Standard Institute, 2010). The bacterial isolates were grown for 24 hours on Nutrient agar. They were suspended in 2 ml sterile normal saline and turbidity was adjusted to match McFarland 0.5 opacity standard. Bacterial suspensions of 0.1 ml were dispensed on the surface of the Mueller-Hinton agar plate and spread evenly using sterile swabs. The inoculum was allowed to dry for 5 minutes, and antibiotic discs were dispensed on the surface of the media and incubated aerobically at 37°C for 24 hours (Clinical and Laboratory Standards

Institute, 2017). A standard strain of *E. coli*, ATCC 25922, was used as a control in this analysis. After 24 hours of incubation, the diameter of the inhibition zones was measured, and the isolates were classified as resistant (R), intermediate (I), and susceptible (S) following the standard antibiotic disc chart. The following antibiotic discs were tested: Amoxicillin Clavulanic acid (AMX, 25 µg), Ceftriaxone (CEF, 30 µg), and Doxycycline (DOX, 30µg) based on availability and use.

3.0 RESULT

3.1 Total Bacterial Counts of Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas

The total bacterial counts (CFU/ml) in soil samples from farms F1, F2, F3, and the control farm (CF) are shown in Table 1. A significant difference was observed between the waste and control soils, as evidenced by the substantial association between F1, F2, F3, and CF (p<0.05), where the waste soil has significantly higher bacterial counts. On the other hand, at a 95% confidence level, no statistically significant variation in the number of bacteria was seen among the waste soil samples (p > 0.05). At 215.06±4.62 CFU/ml x 10⁸, Farm 2 (F2) had a considerably higher bacterial count than Farm 3 (211.63±1.18 CFU/ml x 10⁸), while F1 came last with the least count of (205.60±15.05 CFU/ml x 10⁸).

Table 1: Total Bacterial Counts of Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas

S. No.	Sample code	Bacterial Count (x10 ⁸ CFU/ml)	Standards		
			WHO	NSDWG	FAO
1	F1	205.60±15.05			
2	F2	215.06±4.62			
3	F3	211.63±1.18			
4	CF	61.75±0.00			

Key: Mean ± SD, F1 = farm 1, F2 = farm 2, F3 = farm 3, and CF = control farm; WHO = World Health Organization, FAO = Food and Agriculture Organization, and NSDWQ = National Standards for Drinking Water Quality

3.2 Qualitative Analysis of Antibiotics in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas

A bar chart titled Qualitative Analysis of Residual Antibiotics in soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) is shown in Figure 4.1 below. The picture shows the distribution of four antibiotics along the horizontal axis at several farm locations (F1, F2, F3, and Cf): ceftriaxone (CRO), doxycycline (DOX), ciprofloxacin (CIP), and amoxicillin-clavulanic acid (AMC). Every antibiotic has a unique colour: amoxicillin clavulanic acid is green, ceftriaxone is blue, ciprofloxacin is purple, and

doxycycline is red. With each centimeter on the scale denoting two units, the vertical axis displays the frequency of incidence of antibiotics. According to the findings, doxycycline had the highest frequency, appearing 19 times in F1, 16 times in F2, 14 times in F3, and not at all in CF. Following ceftriaxone were 15 in F1, 13 in F2, 12 in F3, and 2 in CF. Ciprofloxacin was not present in any of the locations, although amoxicillin clavulanic acid was found in F2 (3 instances) and F3 (1 instance).

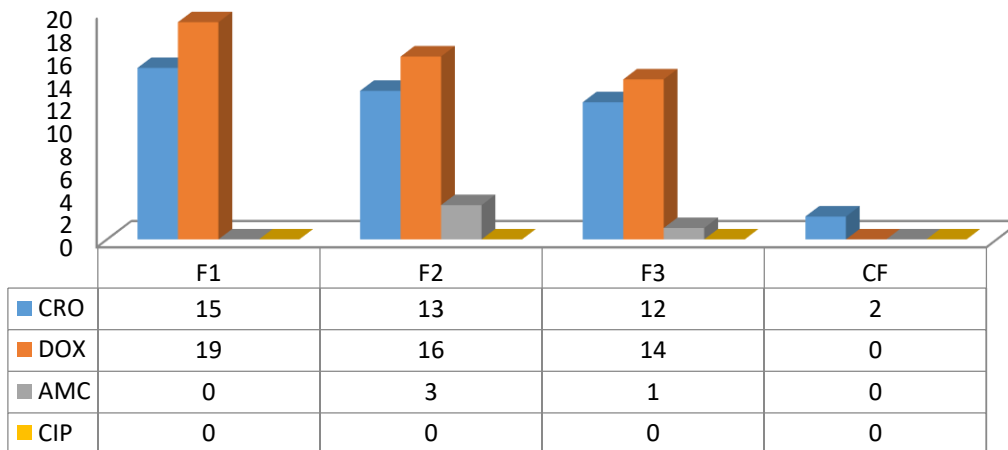


Figure 1: Qualitative Residual Analysis of Antibiotics in Soil

Key: CRO = Ceftriaxone, Dox = Doxycycline, AMX = Amoxicillin Clavulanic Acid, and CIP = Ciprofloxacin. F1 = farm 1, F2 = farm 2, F3 = farm 3, and CF = control farm

3.3 Quantitative Analysis of Antibiotics in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas

A bar chart titled Quantitative Analysis of Residual Antibiotics in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) is shown in Figure 4.6 below. The horizontal axis of the picture displays the presence of four antibiotics: ciprofloxacin (CIP), doxycycline (DOX), amoxicillin-clavulanic acid (AMC), and ceftriaxone (CRO) at several farm locations (F1, F2, F3, and CF). The colours blue (ceftriaxone), red (doxycycline), green (amoxicillin clavulanic acid), and purple (ciprofloxacin) correspond to each drug. The vertical axis displays the antibiotic concentration in $\mu\text{g}/\text{kg}$, with one centimeter

on the scale equal to two units. Ceftriaxone was shown to have greater concentrations in F1 and F2, measuring $13.63 \mu\text{g}/\text{kg}$ and $12.87 \mu\text{g}/\text{kg}$, respectively. However, in F3 and CF, the concentrations of the drug gradually dropped to $9.13 \mu\text{g}/\text{kg}$ and $1.34 \mu\text{g}/\text{kg}$, respectively. The amounts of doxycycline (DOX) were $8.69 \mu\text{g}/\text{kg}$ in F1, $7.17 \mu\text{g}/\text{kg}$ in F2, $1.34 \mu\text{g}/\text{kg}$ in F3, and $0 \mu\text{g}/\text{kg}$ in CF. Amoxicillin clavulanic acid was found in F1, F2, F3, and CF at $0 \mu\text{g}/\text{kg}$, $0.56 \mu\text{g}/\text{kg}$, and $0.22 \mu\text{g}/\text{kg}$, respectively. No samples or locations had any detectable levels of ciprofloxacin ($0 \mu\text{g}/\text{kg}$).

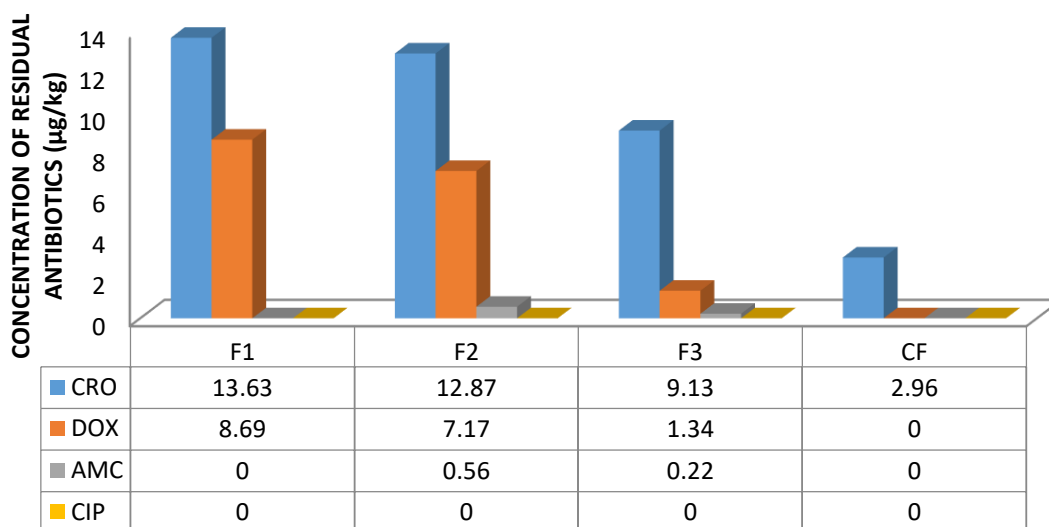


Figure 2: Quantitative Analysis of Antibiotics in Soil

KEY: CRO = ceftriaxone, DOX = doxycycline, AMC = amoxicillin-clavulanic acid, and CIP = ciprofloxacin. F1 = farm 1, F2 = farm 2, F3 = farm 3, and CF = control farm

3.4 Qualitative Analysis of Biocides in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas

A bar chart titled "Qualitative Analysis of Biocides in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano)" can be found in Figure 4.4 below. The horizontal axis of the picture shows the distribution of two biocides, Triclosan and cypermethrin, across several farm locations (F1, F2, F3, and CF). Each biocide is represented by a distinct colour: red for triclosan and blue for cypermethrin. The frequency of biocides is displayed on the vertical axis, where one unit is represented by each centimeter on the scale. The findings demonstrated that the insecticide cypermethrin, which is frequently used in agricultural contexts, was consistently found in soil samples from all three farms (F1, F2, and F3) with six occurrences each. Cypermethrin was likewise detected at the control farm, however it did so less frequently (3 instances). Contrarily, triclosan—which is frequently present in consumer goods like detergents and soaps—was not present in any of the soil samples that were examined (F1, F2, F3, and CF).

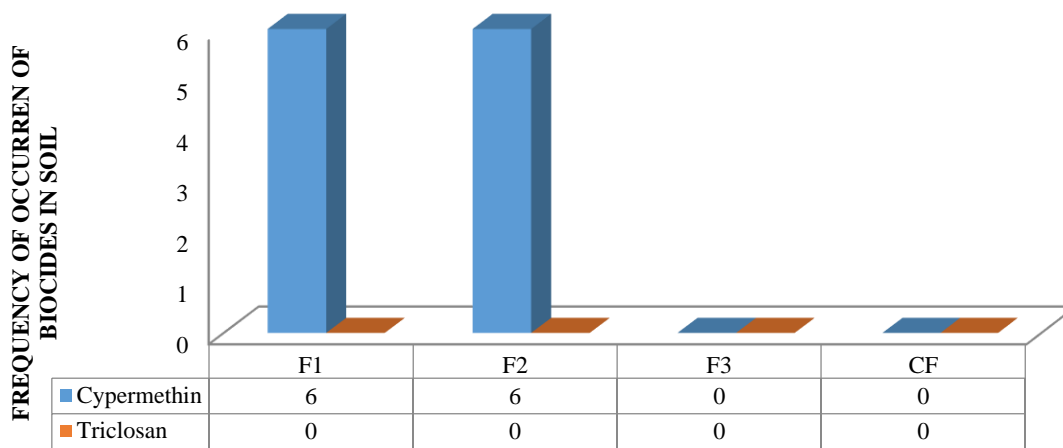


Figure 3: Qualitative Analysis of Biocides in Soil
Key: F1 = farm 1, F2 = Farm 2, F3 = farm 3, CF= control farm

3.5 Quantitative Analysis of Biocides in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas

A bar chart titled Quantitative Analysis of Biocides in Soil from Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) is shown in Figure 4.8 below. The horizontal axis of the picture shows the presence of two biocides: triclosan and cypermethrin, at several farm (soil) locations (F1, F2, F3, and CF). Two distinct colours are used to indicate each biocide: red (triclosan) and blue (cypermethrin). The concentration of biocides is displayed on the vertical axis as $\mu\text{g}/\text{kg}$, with one centimetre on the scale denoting 0.05 units. Cypermethrin was found in farm 1 (F1) and farm 2 (F2), with amounts of 0.014 $\mu\text{g}/\text{kg}$ and 0.485 $\mu\text{g}/\text{kg}$, respectively, according to the data. However, neither farm 3 (F3) nor the control farm (CF) had any cypermethrin identified. Conversely, no trace of triclosan ($\mu\text{g}/\text{kg}$) was found in any of the examined areas (F1, F2, F3, and CF).

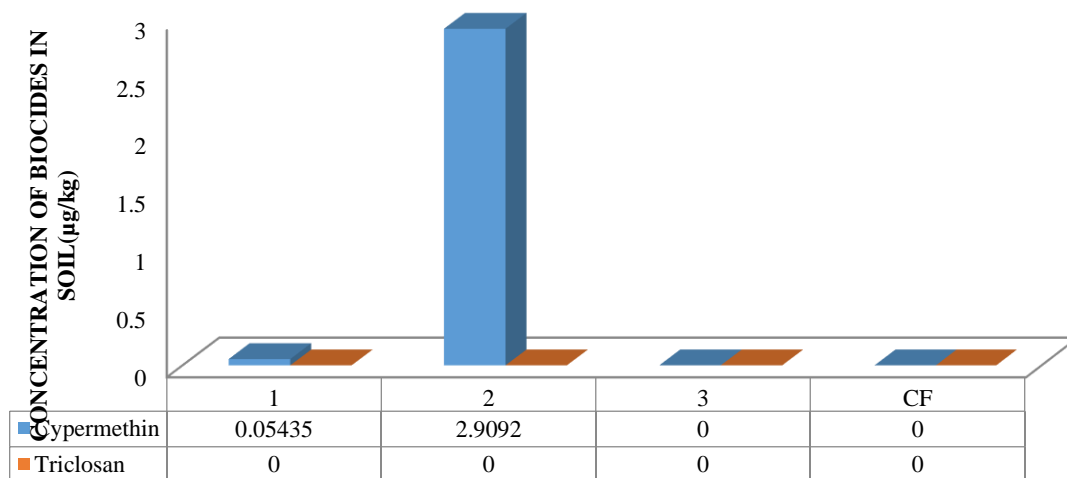


Figure 4: Quantitative Analysis of Biocide in Soil
KEY: F1= farm 1, F2 = f 2, F3 =farm 3, and CF = control farm

3.6 Antibiotic Susceptibility Profile of Bacterial Isolates in Soil from Kwakwaci (Fagge LGA) Study Area

The antibiotic susceptibility profiles of several isolates of Gram-negative bacteria in soil (F1) from the Fagge (LGA) Study Area are shown in Table 2. The isolates were tested against three antibiotics: Doxycycline (DOX), Ceftriaxone (CRO), and Amoxicillin-Clavulanic acid (AMC). The proportion of isolates in each group is indicated by numbers and percentages, and the categories are susceptible (S), intermediate (I), and resistant (R). According to the findings, at least 50% of the isolates of *Klebsiella oxytoca*, *Proteus mirabilis*, *E. coli*, and *Bordetella* sp. displayed some degree of susceptibility. In comparison to AMC, no clinical or laboratory standard for *Stenotrophomonas maltophilia* was found. AMC resistance was 100% displayed by *Citrobacter freundii*, *Providencia rettgeri*, and *Pseudomonas fluorescens*.

Except for *Bordetella* sp. and *Bulkholderia cepacia*, every isolate exhibited 100% ceftriaxone sensitivity. *Bulkholderia cepacia* and *Bordetella* sp. exhibited varied susceptibility to ceftriaxone; 33% of the isolates were intermediately sensitive and 67% were sensitive.

Additionally, the results demonstrated that *Aeromonas hydrophilia*, *E. coli*, *Serratia liquefaciens*, *Stenotrophomonas maltophilia*, *Proteus mirabilis*, and *Klebsiella pneumoniae* were all 100% susceptible to doxycycline.

Nevertheless, *Bordetella* sp., *Providencia rettgeri*, and *Klebsiella oxytoca* exhibited varying degrees of susceptibility and resistance to doxycycline.

3.7 Antibiotic Susceptibility Profile of Gram-negative Bacteria in Soil (F2)

The antibiotic susceptibility profile of several isolates of Gram-negative bacteria in a soil (F2) from the Fagge (LGA) Study Area is shown in Table 3. The isolates were tested against three antibiotics: doxycycline (DOX), ceftriaxone (CRO), and amoxicillin-clavulanic acid (AMC). Numbers and percentages represent the percentage of isolates in each of the three categories: susceptible (S), intermediate (I), and resistant (R).

The findings revealed that *Stenotrophomonas maltophilia* exhibited complete susceptibility to doxycycline and ceftriaxone. *Pseudomonas fluorescens* responded to both AMC and doxycycline with mixed resistance (57%) and intermediate resistance (43%) and with 100% sensitivity to ceftriaxone. *Pantoea* sp. exhibited complete sensitivity to ceftriaxone (100%) but exhibited strong resistance to AMC (60%) and doxycycline (60%). *Vibrio fluvialis* exhibited 100% sensitivity to CRO but was fully resistant to AMC and DOX. *Aeromonas hydrophilia* showed 100% sensitivity to dox and CRO and mixed susceptibility to AMC with 50% intermediate resistance. *Acinetobacter baumannii*, *Citrobacter freundii*, and *Serratia ficaria* were all 100% responsive to every antibiotic that was tested. Conversely, *Pseudomonas oryzae* exhibited mixed susceptibility to AMC (20% sensitive and resistant) and DOX (60% sensitive and 20% resistant), while demonstrating significant sensitivity to CRO (100%) in contrast. *Bulkholderia cepacia* exhibited variable responses to DOX (33% for sensitive, intermediate, and resistant), and mixed resistance to AMC (67% resistant and 33% sensitive). *Bordetella* sp. was 100% sensitive to CRO and resistant to AMC and DOX (100% and 50%), respectively. *Klebsiella*



oxytoca exhibited variable susceptibility to CRO and DOX and was 100% resistant to AMC. With 50% sensitivity and resistance to AMC and DOX, *E. coli* showed mixed susceptibility, but 100% sensitivity to CRO. *Proteus mirabilis* exhibited 100% and 50% resistance to AMC and dox, respectively, and 100% sensitivity to CRO. *Serratia rubidaea* and *Enterobacter cloacae* exhibited 100% resistance to AMC and 100% sensitivity to CRO, respectively. *Providencia rettgeri* exhibited complete sensitivity to CRO but exhibited great resistance to AMC and DOX (100%).

3.8 Antibiotic Susceptibility Profile of Gram-negative Bacteria in Soil (F3)

The antibiotic susceptibility profiles of three antibiotics—amoxicillin-clavulanic acid (AMC), ceftriaxone (CRO), and doxycycline (DOX)—for different Gram-negative bacterial isolates in soil (F3) from the Fagge (LGA) Study Area are shown in Table 4. The proportion of isolates in each group is indicated by numbers and percentages, and the categories are susceptible (S), intermediate (I), and resistant (R).

According to the findings, *Pantoea* sp., *Proteus mirabilis*, *E. coli*, and *Hafnia alvei* were 100% sensitive to CRO and DOX and 100% intermediate to AMC. Mixed susceptibility was exhibited by *Klebsiella pneumoniae*, with 100% resistance to DOX, 100% sensitivity to CRO, and 100% intermediate to AMC. AMC and DOX demonstrated intermediate resistances of 29 and 71%, respectively, while CRO exhibited intermediate resistances of 14 and 86% in *Pseudomonas fluorescens*. *Roaultella terrigena* exhibited 100% sensitivity to DOX and 100% resistance to AMC and CRO. *Providencia rettgeri* and *Aeromonas hydrophilia* exhibited mixed susceptibility, meaning they were 100% susceptible to CRO and DOX and 100% resistant to AMC. All three medicines showed 100% sensitivity to *Acinetobacter baumannii*. *Citrobacter freundii* exhibited 50% intermediate sensitivity to AMC, 50% resistance to DOX, and 100% sensitivity to CRO. *Pseudomonas oryzihabitans* was 33% resistant, 17% sensitive, and 50% intermediate to AMC, however, it exhibited 100% sensitivity to CRO. Its susceptibility to DOX, however, varied (33% sensitive, 33% intermediate, and 33% resistant). *Bordetella* sp. showed 67% sensitivity to CRO and DOX, 33% resistance to DOX, and 100% resistance to AMC.



Table 2: Antibiotic Susceptibility Profile of Gram-Negative

Isolates	Antibiotics								
	AMC			CRO			DOX		
	S	I	R	S	I	R	S	I	R
<i>Pantoea</i> sp.	0	3(60)	2(40)	5(100)	0	0	4(80)	0	1(20)
<i>Klebsiella oxytoca</i>	1(50)	2	1(50)	2(100)	0	0	1(50)	0	1(50)
<i>Serratia liquefaciens</i>	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Pseudomonas fluorescens</i>	0	0	6(100)	5(83)	0	1(17)	0	2(33)	4(67)
<i>Stenotrophomonas maltophilia</i>	NC	NC	NC	2(100)	0	0	2(100)	0	0
<i>Proteus mirabilis</i>	1(100)	0	0	1(100)	0	0	1(100)	0	0
<i>Klebsiella pneumonia</i>	0	3(100)	0	2(67)	1(33)	0	3(100)	0	0
<i>Citrobacter freundii</i>	0	0	1(100)	1(100)	0	0	0	0	1(100)
<i>Escherichia coli</i>	1(100)	0	0	1(100)	0	0	1(100)	0	0
<i>Providencia alcalifaciens</i>	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Aeromonas hydrophilia</i>	0	1(50)	1(50)	2(100)	0	0	2(100)	0	0
<i>Bordetella</i> sp.	1(33)	1(33)	1(33)	2(67)	1(33)	0	2(67)	0	1(33)
<i>Bulkholderia cepacia</i>	0	1(33)	2(67)	2(67)	1(33)	0	0	1(33)	2(67)
<i>Providencia rettgeri</i>	0	0	2(100)	2(100)	0	0	1(50)	0	1(50)

Bacterial Isolates in Soil (F1)

Key: AMC = amoxicillin-clavulanic acid, CRO = ceftriaxone, DOX= doxycycline, F1= farm 1, NC = no clinical and laboratory standard for AMC (for *Stenotrophomonas. maltophilia*), S = susceptibility, I = intermediate, R = resistance.



Isolates	Antibiotics								
	AMC			CRO			DOX		
	S	I	R	S	I	R	S	I	R
<i>Stenotrophomonas maltophilia</i>	NC	NC	NC	1(100)	0	0	1(100)	0	0
<i>Pseudomonas fluorescens</i>	0	3(43)	4(57)	7(100)	0	0	0	3(43)	4(57)
<i>Pantoea</i> sp.	1(20)	1(20)	3(60)	5(100)	0	0	2(40)	0	3(60)
<i>Vibrio fluvialis</i>	0	0	1(100)	1(100)	0	0	0	0	1(100)
<i>Aeromonas hydrophilia</i>	0	1(50)	1(50)	2(100)	0	0	2(100)	0	0
<i>Serratia ficaria</i>	1(100)	0	0	1(100)	0	0	1(100)	0	0
<i>Pseudomonas oryzihabitans</i>	1(20)	3(60)	1(20)	5(100)	0	0	3(60)	0	2(40)
<i>Bulkholderia cepacia</i>	1(33)	0	2(67)	3(100)	0	0	1(33)	1(33)	1(33)
<i>Bordetella</i> sp.	0	0	2(100)	2(100)	0	0	0	1(50)	1(50)
<i>Klebsiella oxytoca</i>	0	0	2(100)	1(50)	0	1(50)	1(100)	0	0
<i>Acinetobacter baumannii</i>	1(100)	0	0	1(100)	0	0	1(100)	0	0
<i>Citrobacter freundii</i>	1(100)	0	0	1(100)	0	0	1(100)	0	0
<i>Escherichia coli</i>	1(50)	0	1(50)	2(100)	0	0	1(50)	0	1(50)
<i>Proteus mirabilis</i>	0	0	2(100)	2(100)	0	0	1(50)	0	1(50)
<i>Enterobacter cloacae</i>	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Serratia rubidaea</i>	0	0	1(100)	1(100)	0	0	1(100)	0	0
<i>Providencia rettgeri</i>	0	0	2(100)	2(100)	0	0	0	0	2(100)

Table 3:
Antibiotic

Susceptibility Profile of Gram-Negative Bacterial Isolates in Soil (F2)



laboratory standard for AMC (for *Stenotrophomonas. maltophilia*), S = susceptibility, I = intermediate, R = resistance.

Table 4: Antibiotic Susceptibility Profile of Gram-Negative Bacterial Isolates in Soil (F3)

Isolates	Antibiotics								
	AMC			CRO			DOX		
	S	I	R	S	I	R	S	I	R
<i>Pantoea</i> sp.	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Klebsiella pneumonia</i>	0	1(100)	0	1(100)	0	0	0	0	1(100)
<i>Pseudomonas fluorescens</i>	0	2(29)	5(71)	6(86)	0	1(14)	0	2(29)	5(71)
<i>Citrobacter freundii</i>	0	1(50)	1(50)	2(100)	0	0	1(50)	0	1(50)
<i>Proteus mirabilis</i>	0	3(100)	0	3(100)	0	0	3(100)	0	0
<i>Pseudomonas oryzihabitans</i>	1(17)	3(50)	2(33)	6(100)	0	0	2(33)	2(33)	2(33)
<i>Bordetella</i> sp.	0	0	2(100)	2(67)	1(33)	0	2(67)	0	1(33)
<i>Roaultella terrigena</i>	0	0	1(100)	0	0	1(100)	1(100)	0	0
<i>Escherichia coli</i>	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Acinetobacter baumannii</i>	1(100)	0	0	1(100)	0	0	1(100)	0	0
<i>Hafnia alvei</i>	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Aeromonas hydrophilia</i>	0	0	1(100)	1(100)	0	0	1(100)	0	0
<i>Bulkholderia cepacia</i>	0	0	6(100)	6(100)	0	0	0	3(50)	3(50)
<i>Providencia rettgeri</i>	0	0	1(100)	1(100)	0	0	1(100)	0	0

Key: AMC = Amoxicillin-clavulanic acid, CRO = ceftriaxone, DOX = doxycycline, F3 = farm 3, S = susceptibility, I = intermediate, R = resistance.

Table 5: Antibiotic Susceptibility Profile of Gram-Negative Bacterial Isolates in Soil (CF)

	AMC			CRO			DOX		
	S	I	R	S	I	R	S	I	R
<i>Aeromonas hydrophilia</i>	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Pantoea</i> sp.	0	1(100)	0	1(100)	0	0	1(100)	0	0
<i>Pseudomonas fluorescens</i>	0	0	1(100)	1(100)	0	0	0	0	1(100)

Key: AMC = amoxicillin- clavulanic acid, CRO = ceftriaxone, DOX= doxycycline, CF= control farm, S = susceptibility, I = intermediate, R = resistance.

4.0 DISCUSSION

All the bacteria isolated were gram negative, this confirms by many scientific literatures that widely reported the gram-negative bacteria as highly prevalent at contaminated sites (Khafilzadeh *et al.*, 2013). This is because these bacteria have an outer membrane and a negatively charged lipopolysaccharide surface, which allow them to withstand high concentrations of environmental contaminants and toxic substances like antibiotics and heavy metals (Yakoubi *et al.*, 2013).

The total count of bacteria in soil from the Kwakwaci (Fagge LGA) and CDA (Bayero University Kano) Study Areas. The irrigated fields with wastewater had mean values ranging from 211.63±1.18 to 205.60±15.05 cfu/ml, which was higher than the control farm's 61.75±0.00 cfu/ml. The remarkably high bacterial load in soil that was irrigated with wastewater may result from a variety of factors, including runoff from fresh manure applied to amend the soil, fertilizers applied to farms, open defecation near water bodies, and industrial effluents (Cobbina *et al.*, 2013).

An additional contributing factor could be the presence of nutrients in the effluents from polluted soil that microorganisms can consume (Rabah *et al.*, 2010). There were fewer bacteria at the control site. This might result from reduced human activity and controlled farming methods used in the control location. This study supports the findings of (Ja'afaru *et al.*, 2020), who found that in the Runde-Doubeli irrigated field in Yola, Nigeria, soil irrigated with wastewater had higher bacterial counts than control soil irrigated with borehole water.

In the current study, ciprofloxacin was absent from wastewater-irrigated soils (F1, F2, and F3), and control farm (CF). This could be because of the drug's strong sorption, poor extractability, uptake by plants, runoff, and transport into the ground (Carter *et al.*, 2014; Martínez-Hernández *et al.*, 2016, Pan *et al.*, 2017). This study refutes the findings of Hussain *et al.* (2016) who

discovered that high quantities of ciprofloxacin ranged from 2.03–3.51 µg/kg in soil and 3.0-5.25 mg/ml in wastewater of Lahore, Pakistan. Furthermore, the study defies the findings of Mahmood *et al.* (2018) who reported that in a wastewater treatment plant in Baghdad, Iraq, ciprofloxacin was the most common antibiotic, appearing 11 times out of 36 water samples and having a concentration of 1.270 mg/ml.

With a concentration of amoxicillin-clavulanic acid found four times in soil (3 in F2 and 1 in F3), with a detection rate of 3% in soil, out of 145 wastewater-irrigated soil samples with values ranging from 0.56 to 0.22µg/kg. The low levels of amoxicillin-clavulanic acid in soil could be attributed to hydrolytic degradation, which occurs because amoxicillin has chemically unstable rings that easily undergo hydrolysis, making the antibiotic difficult to detect in soil. The current investigation of amoxicillin-clavulanic acid content was lower than that of Shipingana *et al.* (2022) who found 8.76µg/kg in the hospital effluents of Ghana's Sunyani municipality.

Among the four antibiotics, ceftriaxone had occurrence and concentration of 15–13.63 µg/l in F1, 13–12.47 µg/kg in F2, 12-9.13 µg/kg in F3, and 2-2.96 µg/kg in CF, it was the second most frequently identified residual antibiotic in soil. Its detectable rate was 28% in wastewater-irrigated soil and 17% in control soil. The high consumption of ceftriaxone, its presence in home sewage, its discharge from hospitals or the antibiotic production industry, livestock farms, and aquaculture into water bodies used for irrigation could all be contributing factors to the high occurrence of ceftriaxone in soil in the current study (Zeng *et al.*, 2022). The release of ceftriaxone into the environment by humans and animals, the use of animal faeces as fertilizer and manure, and irrigation with ceftriaxone-containing water are further reasons why it can be found in soil (Boy-Roura *et al.*, 2018).

With a detectable rate of 34% in wastewater-irrigated soil, ceftriaxone was the most often detected antibiotic

in soil samples, with occurrence and concentrations of 19–8.69 µg/kg in F1, 16–7.17 µg/kg in F2, 14–1.34 µg/kg in F3, and none of it was detected in the control site. In the soil samples used for this investigation, doxycycline was more prevalent. This may be attributed to its high application in veterinary medicine and feed additives, use of poultry and livestock manure which contain doxycycline, and landfill disposal (Zeng *et al.*, 2022).

This study's findings suggest that triclosan may not have been present because of microbial degradation, adsorption to sludge and soil particles, chemical oxidation and transformation, photodegradation, and improved treatment techniques from wastewater treatment plants (Chen *et al.*, 2018; Zhang *et al.*, 2021). The result of this study contradicts the findings of (Bakare and Adeyinka, 2022) who studied the fate mechanisms of parabens, triclocarban, and triclosan during wastewater treatment assessment using field measurements and model simulations, discovered high quantities of triclosan to range from 1.732 to 6.980 µg/L in wastewater. A well-known antiseptic broad-spectrum biocide with numerous uses in medicine and the home, triclosan is a member of the bisphenol family. Due to its great prevalence and capacity for accumulation, triclosan finds its way into the ecosystem through soil and water bodies (Jablonska-Trypuc, 2023).

According to studies conducted by (Bragança *et al.*, 2019; Farina *et al.*, 2018) cypermethrin is a pyrethroid that is thought to be less hazardous than other insecticides used in soil habitats, where frequent and extensive use can make it hazardous to non-target organisms. Because of the organic matter and its extremely hydrophobic qualities, this enables it to penetrate groundwater through soil pores and a variety of crops (Environmental Agency, 2019). The concentration of Cypermethrin detected in the current study in wastewater-irrigated soil was found to be 6–0.014 µg/kg in F1, 6–0.485 µg/kg in F2, and nd not detected in F3 and CF. There was also a detectable rate of 8% in wastewater-irrigated soil. The concentration of cypermethrin in wastewater-irrigated soil may be caused by a variety of factors, including industrial processes like wool processing, domestic sources from home and garden use, loss from hard standings on farms after treating sheep and cattle, and surface runoff after its application to (Deng *et al.*, 2020). The current study's soil cypermethrin concentration was lower than that of soil from the Yangtze River Delta, China, as reported by (Boattini *et al.*, 2016).

During analysis, all the isolates displayed some level of resistance to amoxicillin-clavulanic acid in all locations

(F1, F2, F3 and CF). *Pseudomonas fluorescens* (100%, 57%, 71%, 100%, 50%, 100%, 100% etc), *Citrobacter freundii* (100%, 50%, 50%, 50%, etc), *Aeromonas hydrophilia* (50%, 50%, 100%, etc), *Bulkholderia cepacia* (67%, 67%, etc), *Providencia rettgeri* (100%, 100%, 100%, etc), *Pantoea* sp. (60%, 100%, etc), *Vibrio fluvialis* (100%, etc), *Pseudomonas oryzihabitans* (33%, 100%, etc), *Bordetella* sp. (100%, 100%, etc), *E. coli* (50%, 100%, etc), *Klebsiella oxytoca* (100%, etc), *Proteus mirabilis* (100%, 100% etc), *Serratia rubidaea* (100%, etc), *Roaultella terrigena* (100%, etc), *Klebsiella pneumoniae* (50%, etc), *Providencia stuartii* (100%, etc), *Rahnella aquatilis* (100%, etc) and *Kluyvera* sp. (100%, etc).

Similarly, some isolates such as *Pseudomonas fluorescens* showed resistance to doxycycline in F1, F2, F2, CF : *Citrobacter freundii*, *Aeromonas hydrophilia*, *Bulkholderia cepacia*, *Providencia rettgeri*, *Pantoea* sp., *Vibrio fluvialis*, *Pseudomonas oryzihabitans*, *Bordetella* sp., *E. coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Serratia rubidaea*, *Roaultella terrigena*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Rahnella aquatilis* and *Kluyvera* sp. Likewise, some isolates showed an intermediate susceptibility to Amoxicillin clavulanate and doxycycline from the locations under study (F1, F2, F3 and CF): Excluding *Roaultella terrigena*, every gram-negative isolate in this study displayed susceptibility to ceftriaxone. (Boattini *et al.*, 2016) found that out of 121 clinical isolates of *Roaultella* spp. from France, 16 percent were resistant to amoxicillin-clavulanic acid, 4% to ceftriaxone, 6% to quinolones, and 10% to trimethoprim (TMP/SMX).

In a different Portuguese study, one (1) clinical isolate of *Roaultella* sp. out of fifty-seven (57) was found to be resistant to amoxicillin-clavulanic acid (Appel *et al.*, 2021). There is little research on *Roaultella* species' resistance to antibiotics in environmental settings as several authors have proposed that *Roaultella* species are uncommon and emerging pathogens (Nagulapally, 2007). *Pseudomonas fluorescens* was the most resistant gram-negative bacterium to amoxicillin-clavulanic acid, and doxycycline among all the isolates in the current study. The reason behind this bacterium's high resistance to doxycycline and amoxicillin-clavulanic acid in soil could be attributed to various factors such as its ability to form biofilm at varying rates, environmental exposure to these antibiotics, acquisition of plasmid-mediated genes, and horizontal gene transfer (Nagulapally R.S, 2007, Duyuncu and Ulusoy 2019: Redondo-Salvo *et al.*, 2020).



In contrast to the current investigation, (Seng *et al.*, 2016) found that strains of *Pseudomonas fluorescens* isolated from Nile tilapia fish in Egypt had a high sensitivity to doxycycline (92.3%) and AMC (89%). The present result is contradicted by the findings of (Lamba and Ahammad, 2017) which found that *Pseudomonas* sp. isolated from a salmon processing environment on the coast of mid-Norway had a high level of ceftriaxone resistance. The current investigation was in line with the report by (Avatsingh *et al.*, 2023) from samples obtained from the Sirmaur and Solan districts of Himachal Pradesh, India. The authors found the resistance of gram-negative bacteria, including *E. coli*, *Enterobacter* spp., *Hafnia* spp., *Shigella* spp., *Citrobacter* spp., and *Klebsiella* spp., to Amoxicillin-clavulanic acid. Gram-negative bacteria *E. coli*, *Proteus* spp., and *Klebsiella* spp. were found to be 100% resistant to amoxicillin-clavulanic acid in a different study carried out in Brazzaville, the Republic of Congo, but extremely sensitive to ceftriaxone. This fits with what the present investigation found. Previous studies have reported the presence of antibiotic-resistant strains of gram-negative bacteria from various regions of India, including *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., *Citrobacter* spp., *Hafnia* spp., *Acinetobacter* spp., *Shiga* spp., and *Aeromonas* spp. (Lamba and Ahammad 2017; Avatsingh *et al.*, 2023; Chaturvedi *et al.*, 2020; Chaturvedi *et al.*, 2021). Antibiotics (beta-lactam and certain cephalosporins) might cause strain resistance since they are used without restriction in both human and veterinary treatment (Leiva *et al.*, 2021) It's possible that horizontal gene transfer (HGT) in which mobile genetic components like plasmids or transposons coding for resistance are shared between bacterial species, even those that are not closely related, is the cause of the antibiotic resistance of these isolates from various locales in the present study.

Antibiotics have two possible entry points into the agricultural ecosystem. First, by fertilizing with antibiotic-containing sediments, sewage sludge, and animal biosolids; second, by irrigating with reclaimed water contaminated by groundwater or surface water, as these sources are frequently contaminated with antibiotics (Moyen *et al.*, 2021; Leiva *et al.*, 2021).

5.0 CONCLUSION

The study evaluated soil microbial populations in irrigated farms in Kano State, Nigeria, as well as the amount of biocides and antibiotic residues in the soil. Ceftriaxone and doxycycline, as well as biocides like cypermethrin, were found to have significantly contaminated the soil, according to the findings.

Significant variations in community structure and a decrease in microbial diversity were linked to the presence of these pollutants in soil microbial communities.

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