

ORIGINAL RESEARCH ARTICLE

Frequency of Residual Biocide-Resistant Gram-Negative Bacteria in Wastewater Samples from Different Irrigated Farms in Kano State

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ABSTRACT

The sustainability of water resources is increasingly compromised by anthropogenic activities, including industrial and agricultural processes that introduce such as biocides into the environment. This study investigates the presence and frequency of residual biocide-resistant Gram-negative bacteria in wastewater samples from irrigated farms in Kano State, Nigeria. Over six months, 36 wastewater samples from streams in Kwakwachi Fagge LGA and 12 control samples from boreholes of the Center for Drylands Agriculture (CDA) Bayero University Kano were collected and analyzed for physicochemical parameters and bacterial load. Physicochemical analysis revealed significant variations in parameters such as pH, Electrical Conductivity, Total Suspended Solids, and Turbidity across different sampling locations. Notably, downstream locations exhibited higher pollutant levels, likely due to accumulated agricultural runoff and domestic effluents. The bacteriological analysis identified various Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescens*, with a notable presence of biocide-resistant strains. The study also detected Cypermethrin in wastewater samples, while Triclosan was absent, indicating possible degradation or effective removal during wastewater treatment processes. The findings underscore the need for improved wastewater management practices to mitigate the contamination of water resources used for irrigation. Additionally, the study highlights the public health risks associated with the use of contaminated water, emphasizing the importance of regular monitoring and stringent regulation of water quality in Agricultural settings.

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INTRODUCTION

Everyone understands the importance of water to human life, yet we have not been able to sustain current consumption. Various human-caused activities, including industrial and agricultural operations, have resulted in this. These operations discharge large quantities of pollutants into nearby areas and aquatic bodies, such as biocides, which are harmful to living organisms (Yazdankhah *et al.*, 2018; Huang *et al.*, 2021). However, these acts lead to a reduction in the quality of water suitable for agricultural and home usage.

Water and soil cannot be separated since they share their contents and are always in contact. Water is necessary for agriculture, human survival, and the economics of many nations (Ajmal *et al.*, 2021). Agriculture uses over 70% of the water extracted, making it one of the industries with the highest water demands. Residential use accounts for

22% of the remaining area, while the industrial sector occupies 8% (Aivazidou *et al.*, 2016). Population increase, economic expansion, wealth, and rising living standards are all contributing to the growing scarcity of water in many nations worldwide (Islam and Karim, 2019).

In many developing countries, including Nigeria, municipal and industrial wastes are dumped directly into streams and rivers, where they are used to fertilize and irrigate crops due to inadequate or nonexistent treatment infrastructure (Ilyas *et al.*, 2019). These practices seriously harm the agriculture industry and, consequently, people and may also result in a shortage of fresh water for farming (Khalid *et al.*, 2018). Most developing and underdeveloped countries, including Nigeria, have farmers who reuse sewage and industrial wastewater for irrigation to offset

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the insufficient supply of freshwater (Vouk *et al.*, 2015; Drechsel and Hanjra, 2018).

Wastewater contains high concentrations of a variety of pathogens, organic contaminants, and other chemical pollutants, including biocides (Imran *et al.*, 2019). The use of artificial fertilizers exacerbates the situation, and a number of soil microbial species are affected by wastewater irrigation (Jiang *et al.*, 2017). A number of hazardous substances, including biocides, pesticides, and other organic pollutants from wastewater and environmental sources, affect the microbial strains that inhabit soil and water bodies (Bharagava *et al.*, 2018).

Wastewater is a selectively active environment for resistant microorganisms because it contains chemicals with antimicrobial potential, such as antibiotics and biocides (Manaia *et al.*, 2018).

Gram staining, a chemical procedure, is used to classify gram-negative bacteria according to the red color they produce (Larry, 2022). Gram-negative bacteria have an outer membrane under their capsule that protects them from certain antibiotics, such as penicillin (Larry, 2022). Endotoxins are dangerous substances that are released when this membrane changes. During a bacterial infection, the endotoxins that Gram-negative bacteria produce affect the severity of symptoms. Among the illnesses brought on by Gram-negative bacteria are meningitis, bloodstream infections, wound or surgical site infections, and pneumonia. The majority of currently available antibiotics and numerous other drugs are ineffective against gram-negative bacteria (CDC, 2019a).

Gram-negative bacteria have developed a variety of resistance mechanisms and can pass on genetic elements that make other bacteria of the same or different species also resistant to drugs (CDC, 2019a). Gram-negative bacteria pose one of the greatest risks to public health worldwide due to their high level of antibiotic resistance. Because they often need patients to stay in the intensive care unit (ICU), which raises the risk of morbidity and mortality, they are clinically significant in hospital settings (Oliveira and Reygaert, 2022).

MATERIALS AND METHODS

Collection of Water Samples

A total of 48 water samples consisting of thirty-six (36) wastewater from streams and twelve (12) control water from boreholes were collected [Figure 1](#). Throughout six (6) months, wastewater samples were taken every two weeks from the upstream, midstream, and downstream of the stream located at Kwakwaci in Nomansland along Zungero Road, Sabon Gari in Fagge Local Government Area of Kano State. It has a latitude of 8.313°E and a longitude of 12.121°N. The sampling location is highly polluted with industrial effluents from the tannery, automobile, groundnut, and plastic industries, abattoir wastes from major slaughtering homes in Kano, domestic and municipal waste from homes and urban activities,

animal feces, and it is a major irrigation site in Kano supplying ready to eat vegetables to major markets in Kano and neighboring States (Dawaki *et al.*, 2016). Similarly, control samples (water) were collected from underground water of the Centre for Dryland Agriculture (CDA) of Bayero University Kano, where controlled farming is practiced. It is far from any industry and less urban activities around the location, and they practice controlled farming where their ready to eat vegetables are grown in screen houses.

The center was established to develop sustainable agricultural technologies that would boost crop and livestock productivity in semi-arid and dry-humid environments in Nigeria and the wider West African Sub-region, and it is located at the latitude of 8.252°E and longitude of 11.585°N (Tukur *et al.*, 2018). All samples collected were kept in a refrigerator before analyses.

Determination of Physicochemical Parameters of Water Samples

pH and Temperature

Following the manufacturer's recommendations, the pH was measured at the sampling location using a calibrated pH meter (ATC pocketed pH meter). A specific volume (10 ml) of the sample was put into a beaker, and readings were obtained straight from the pH meter after it had been submerged in the beaker for a minute. A mercury-in-glass thermometer was also used on the spot to measure the temperature (APHA, 2012).

Electrical Conductivity (EC)

Using a conductivity metre (CDM210, MeterLab), the electrical conductivity of the samples was ascertained, following the instructions provided by Tolulope *et al.* (2019). To summarise, the metre was turned on, and its probe was inserted into the sample that was placed within a beaker. A measurement of the electrical conductivity in μScm^{-1} was taken.

Chemical Oxygen Demand (C.O.D)

The typical closed-reflux titrimetric approach was used for this. In a culture tube, a known volume (50 ml) of water sample was combined with a solution of sulfuric acid (98% H_2SO_4) and potassium dichromate (0.01667 M $\text{K}_2\text{Cr}_2\text{O}_7$). After that, it was refluxed at 150°C for two (2) hours. When the digested solution turned from blue-green to radish-brown in colour, it was titrated with ferrous ammonium sulphate (0.025 M) using two (2) drops of ferroin indicator in a conical flask that was allowed to cool at room temperature American Public Health Association, (APHA, 2012).

Biochemical Oxygen Demand (B.O.D)

A 5-day testing approach was used to determine BOD, as stated by the American Public Health Association. (APHA, 2012). Using distilled water, two 100-ml bottles with lids were thoroughly cleaned. To create a total

amount of 100 ml, distilled water was added to each bottle after a precise volume (25 ml) of sample was placed inside. The two bottles were then sufficiently sealed with the lid.

Both bottles received two (2) milliliters of alkali azide solution and ten (10) milliliters of manganese sulfate ($MnSO_4$) solution.

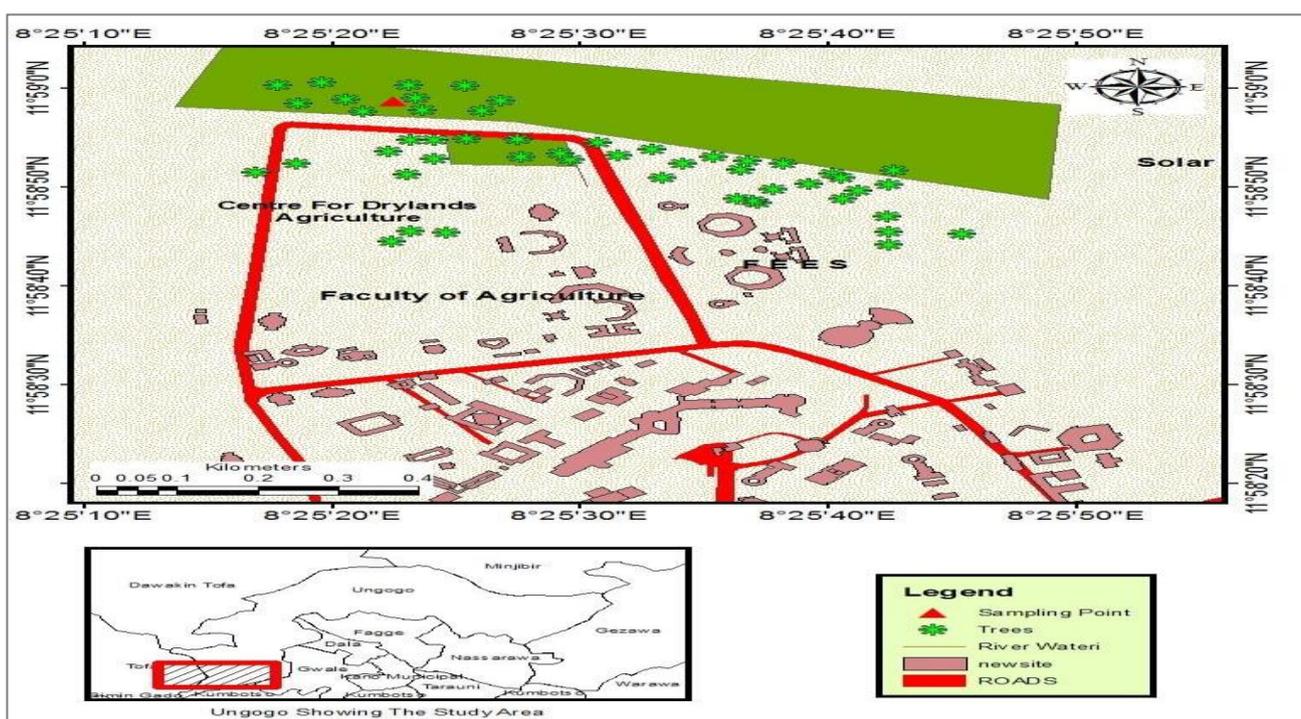
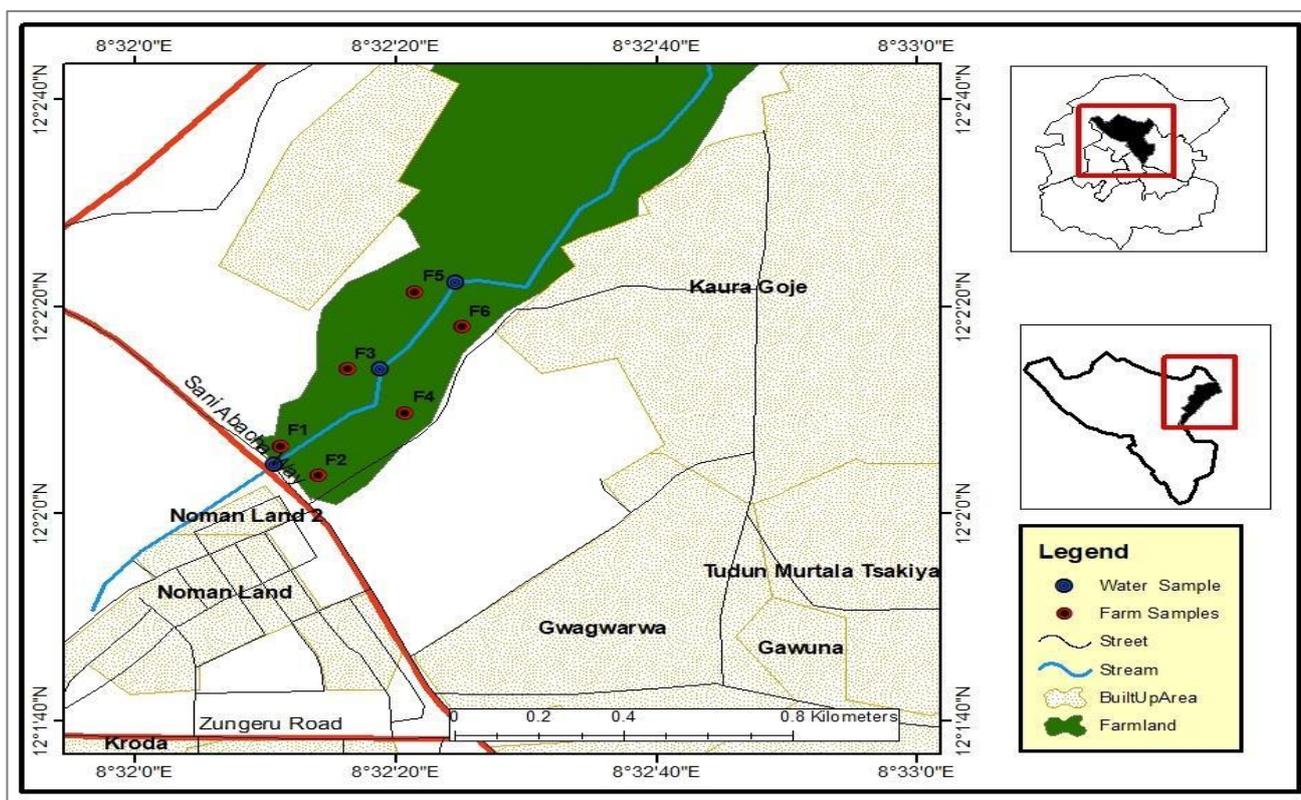


Figure 1: UP: Map of Kano Metropolitan showing the sampling points in Kwakwaci (Fagge LGA). DOWN: Map of Kano Metropolitan showing the sampling points in the Centre for Dryland Agriculture (Drawn at Cartography Laboratory, Department of Geography, BUK

The bottles were correctly combined and sealed after being repeatedly inverted. The precipitate was gradually stirred again by inverting the bottles, as previously mentioned until it settled and left a clear supernatant

above the precipitate. Eight (8) milliliters of concentrated phosphoric acid were added to the suspension once the precipitate had fully settled. Once the bottles were closed, they were gently inverted and mixed until dissolution was

achieved. Ultimately, a conical flask containing 100 ml of the aqueous sample was used to titrate a 0.05M Na₂SO₄ solution until a pale-yellow solution was obtained. The conical flask was then filled with 2 ml of freshly made starch solution, and the titration was continued until a blue tint appeared. Simultaneously, a sample containing MnSO₄, alkali azide, and a phosphoric acid solution was incubated for five (5) days at 20 °C. The five-day-incubated samples were treated using the same procedure after five (5) days. Instead of using wastewater for the blank titration, distilled water was used. BOD_{5d} was computed using the following formula at 20 °C.

$$\text{BOD}_{5d} \text{ as mg of O}_2/\text{L} = 16(V_1 - V_2)$$

Where V₁=mL of Na₂SO₄ was used in the sample before incubation (at day 1) and V₂=mL of Na₂SO₄ was used in the sample after incubation (at day 5).

Dissolved Oxygen (D.O)

Winkler's technique was used to determine the samples' dissolved oxygen content. After carefully filling a 300-ml glass BOD stoppered bottle with the sample, 2 ml of manganese sulphate was added right away by placing a calibrated pipette just below the liquid's surface. To prevent bubbles, the pipette was squeezed slowly. The alkali-iodide-azide reagent was applied in an amount of about 2 ml. The sample was mixed by inverting it several times. About 2 ml of concentrated sulfuric acid was added via a pipette held above the surface of the sample and inverted several times to dissolve the floc. In a glass flask, about 20 ml of the sample was titrated with sodium thiosulfate to a pale straw color. About 2 ml of starch solution was added, and a blue colour formed. The titration continued until the sample turned clear, indicating an endpoint (United States Environmental Protection Agency, USEPA, 2018).

Total Dissolved Solids (TDS)

Using the gravimetric approach, as detailed by Tolutope *et al.* (2019), the amount of TDS in the wastewater sample was measured. A sterile Petri dish was heated up at 100 °C, allowed to cool in a desiccator, and then weighed to ensure it remained at the same weight. A pre-weighed filter paper was used to filter a certain volume (50 ml) of samples into a clean conical flask. The mixture was then transferred onto a plate and heated to 180 °C in an oven. After the residue was collected, it was weighed consistently and allowed to cool in a desiccator. The following formula was used to compute the TDS:

$$\text{TDS (mgL}^{-1}\text{)} = (A - B) \times 1000/\text{Volume of the sample (mL).}$$

Where A = weight of dried residue + evaporating dish (mg).

B = Weight of evaporating dish (mg)

Total Suspended Solids (TSS)

Using the gravimetric approach, as detailed by Tolutope *et al.* (2019), the TSS level in the samples was ascertained. In

short, a pre-weighed glass fibre filter was used to filter a known volume (50 ml) of water sample. Overnight, the filter was dried in an oven set to 105°C after removing the filter paper, the desiccator was allowed to cool to ambient temperature, and the weight was kept constant. TSS was computed using the dry filter paper's increased mass, which was noted.

$$\text{TSS (mgL}^{-1}\text{)} = (A - B) \times 1000/\text{Volume of the sample (mL)}$$

Where A = weight of the filter after filtration (mg); B = Weight of the filter before filtration (mg)

Nitrate Determination (NO₃)

Nitrate in the samples was determined using the UV spectrometric method, as described by Tolutope *et al.* (2019). A 100 mg/L standard solution of nitrate was made by dissolving 0.72g of anhydrous potassium nitrate in 1 L of distilled water. Serial dilutions from the nitrate stock solution were prepared for calibration standards of nitrate in the range of 0.1 – 1.0 mg/L. A series of reaction tubes were set up in a tube stand and placed in a cold-water bath. A specific volume (25 ml) of the sample was poured into the reaction tubes with the sequential addition of NaCl solution and sulfuric acid. A brucine-sulphanilic acid reagent was added and heated for some minutes in a boiling water bath. The samples were then allowed to cool, and the absorbance of each sample at 410 nm in a Spectrum Lab 275 UV spectrometer was measured in comparison with the reagent blank. Nitrate and nitrogen concentrations in the wastewater samples were determined by extrapolation from the calibration curve.

Phosphate Determination (P₂O₅)

Phosphate in the samples was determined by the UV spectrophotometer method, as described by Tolutope *et al.* (2019). About 20 mg/L of phosphate standard solution was made by dissolving 0.877 g of potassium dihydrogen phosphate in 80 ml of distilled water and making up the solution to 1 L. Conditional reagents were prepared by mixing appropriate quantities of sulfuric acid, potassium antimony tartrate solution, ammonium molybdate solution, and diluted ascorbic acid solution. Diluted sulfuric acid and the conditional reagent were added to 25 ml of the sample using phenolphthalein as an indicator. The absorbance of each sample at 880nm was measured using a Spectrum Lab 275 UV spectrometer and compared with the reagent blank. A calibration curve was plotted by taking various concentrations of the standard phosphate solution with specified amounts of conditional reagents.

Sulphate Determination (S₂O₄)

Sulphate concentration in the samples was determined by the spectrophotometric method, as described by Tolutope *et al.* (2019). A conditional reagent was prepared by mixing an appropriate amount of chloride compound, alcohol, concentrated acid, and distilled water. About 100 mg/L of standard sulphate solution was prepared by dissolving 4.438 g of anhydrous sodium sulphate in 500 ml of

distilled water and diluting the solution to 1 series of standard blank solutions. About 25 ml of the sample was prepared separately in flat bottom flasks. A known quantity (5 ml) of conditioning reagent was added to each of the flat bottom flasks and topped up to 100 ml, after which 10mg of barium chloride was added. The solutions became turbid and were then measured with a Spectrum Lab 275 UV-visible spectrometer at 420 nm. The sulphate concentration in the samples was determined with reference to the graphical representation obtained for the standard solutions.

Bicarbonate Ion (HCO_3^-)

The titrimetric approach was used to determine this. A conical flask was filled to a specified capacity (50 ml) of the sample, and after adding two to three (2 to 3) drops of phenolphthalein indicator, a pink color was produced. The mixture was titrated up to a colorless endpoint using 0.1 M HCl. The identical solution quickly turned yellow when two (2) drops of methyl orange were added. The titration was then carried out until an orange-colored endpoint was reached, at which point a reading was taken (APHA, 2005).

Total Hardness (TH)

The complexometric EDTA titration method, as reported by APHA (2012), was used to determine total hardness. To put it briefly, a clean 250 ml conical flask was filled with 50 ml of the sample. Two drops (2) of Eriochrome Black T indicator and three (3) milliliters of ammonium chloride in concentrated buffer (NH_4Cl /concentrated NH_3) were added. This was titrated till the color turned blue from violet using a 0.01 M EDTA solution.

Turbidity and Color

Turbidity levels of wastewater were measured in nephelometric units (NTUs) using a turbidity meter. The meter probe was dipped into a beaker containing 5 ml of water sample, and readings were taken directly from the meter (Olaoluwa *et al.*, 2010). Using a Lovibond disc, 5 ml of the sample was put in a sample container, and ammonia was added as a reagent to determine the color. The comparator device was filled with the Lovibond test disc. The comparator compartment was set up with the sample on the right side and a cell holding a blank of water on the left. The disc was rotated using standardized lighting until the nearest color matched the sample displayed in the comparator instrument's window (Ogundele, 2010).

Bacteriological Analysis of Water Samples

Enumeration of Aerobic Mesophilic Bacteria from Water Samples

This was carried out using the pour-plating technique. A specific volume (10 ml) of sample was transferred into a conical flask containing 90 ml of buffered peptone water using a sterile syringe and labeled 10^{-1} . About 1 ml of tube 10^{-1} was transferred after agitation into a test tube containing 9 ml of buffered peptone water (using a

separate syringe) and labeled 10^{-2} . This was repeated to obtain 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} and 10^{-9} respectively. Using another fresh syringe, 1 ml of sample 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, IOS, 2013).

Isolation of Gram-Negative 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 hours at 37°C for selective and differential identification of gram-negative bacteria (APHA, 2012; International Organization for Standardization, IOS, 2017). Colonies that appeared pinkish red or colorless and transparent on MacConkey and SSA are considered lactose and non-lactose gram-negative bacteria (Chapin and Lauderable, 2007). Representatives of the bacterial colony types with the above features were picked and subcultured on MacConkey and incubated at 37°C for 24–48 hours, respectively. 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.

Characterization and Identification of the Isolates

The colonies obtained from each isolate, were identified morphologically based on their sizes, forms, colors, margins, elevations, opacity, cell shape, organizational structure, and Gram reaction. Furthermore, biochemical characterizations were also conducted using the acceptable techniques as described by Cheesbrough (2005). While the API 20E Kit (bioMerieux) was further used to confirmed the isolates' identities.

Concentration of Residual Biocides in Water

The concentrations of biocides were analyzed using the procedure demonstrated by Yun-Feng *et al.* (2012). In detail, extraction was first carried out through filtration of the water samples using a Whatman No. 125 filter paper and acidification with 5M H_2SO_4 to keep the biocide activity to a minimum. About 0.1g of Na_2EDTA was added to release the biocides by interacting with metal cations. About 20 ml of the mixture of acetone, n-hexane, and ethyl acetate (2:1:1) was added to 40 ml of the water sample, and 0.5 gram each of MgSO_4 and NaCl was added to displace the extraction equilibrium towards the organic phase. This was ultrasonicated at 30°C for 10 minutes and centrifuged for 5 minutes at 3000 rpm. The organic phase was decanted, filtered, evaporated to dryness, and then reconstituted with 5 mL of methanol. Analysis was performed using the Agilent Intuvo 9000 GC system coupled with the detector system 5977B MSD with split and splitless injectors. A DB-5MS (5% phenyldimethylsiloxane) fused silica capillary column (30

m, 320 μm i.d., 0.25μm of film thickness) was used with helium gas (99.999% purity) as carrier gas at flow rates of 1.2 ml/min. Inlet temperature was set at 300°C, MS Source at 230°C, and MS Quad at 150°C. The oven temperature was programmed as follows: 50°C for 5 minutes, increased to 300°C at 20°Cmin-1. Data were acquired by GCMSD/Enhanced Mass Hunter Software and Processed GCMSD Data Analysis Software, incorporated with the 2017 version of the NIST Library. About 1μL of the sample 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.

RESULT

Physicochemical Properties of Water from Kwakwachi (Fagge LGA) and CDA (BUK) Study Areas

The physicochemical analysis of water from four distinct locations (W1, W2, W3, and CW) is shown in Table 1. At

a 95% confidence level, the results indicate significant associations in pH, temperature, electrical conductivity, total suspended solids, total hardness, total dissolved solids, turbidity, and color (p<0.005). There is significant variation in the parameters depending on the region, with some being lower and some higher. For instance, the pH was found to be significantly lower in W1 (6.55). In contrast, CW had significantly lower values for temperature, electrical conductivity, Total Suspended Solids, total hardness, total dissolved solids, turbidity, and color (22.84°C, 281μS/cm, 1.46 mg/ml, 41.41 mg/ml, 27.33 mg/ml, 1.54 NTU, and 2.32 Heinz respectively).

On the contrary, no significant association is shown for the BOD, COD, DO, Nitrates, Phosphates, Bicarbonate Ions, and Sulfates in any of the locations (p > 0.005). Bicarbonate ions had a value of 9.67 mg/ml in W1, whereas the other nutrients—BOD, COD, DO, Nitrates, Phosphates, and Sulfates—had values of 1.56 mg/ml, 3.05 mg/ml, 4.10 mg/ml, 16.17 mg/ml, 6.61 mg/ml, and 0.95 mg/ml that were substantially lower in CW.

Table 1: Physicochemical Parameters of Water samples collected at Fagge LGA and CDA

Physicochemical parameters	Water				P-value	WHO	NSDWG	FAO
	W1	W2	W3	CW				
pH (H ₂ O)	6.55±0.47	7.89±0.30	8.07±0.30	7.29±0.55	0.000*	6.5-8.5	6.5-8.5	6.5-8.5
Temp °C	26.23±0.61	26.67±0.44	26.86±0.44	22.84±0.71	0.000*	22-30	22-30	-
EC (US/CM)	2036±1.0	1357±1.0	2774.60±4.6	281±1.1	0.001*	300	1400	-
BOD (mg/mL)	3.50±0.21	3.82±1.88	3.86±1.54	1.56±0.85	0.060	5	5	-
COD (mg/mL)	6.29±3.85	6.30±3.81	6.09±3.68	3.05±0.72	0.259	10	-	250
DO (mg/mL)	9.76±0.1	6.23±0.10	6.14±0.10	4.10±0.10	0.102	4-6	5-7	2
TSS (mg/mL)	113.83±0.76	104.33±1.53	117±1.0	1.46±0.72	0.011*	250	500	100
TH (mg/mL)	120.67±6.81	134±1.0	107.05±1.0	41.41 ±0.96	0.000*	500	200	-
TDS (mg/mL)	255±5.0	330±10	207±2.0	27.33±1.53	0.000*	0.5	500	40
NO ₃ (mg/mL)	39.47±0.84	19.50±0.5	31.33±1.53	16.17±0.76	0.096	50	50	30
Turbidity (VTU)	19.37±7.80	23.52±8.71	25.51±13.12	1.54±1.08	0.000*	0.5-5	5.0	5.0
Phosphate(mg/mL)	16.42±13.31	8.79±6.52	14.64±8.81	6.61±4.34	0.093	0.4-5.9	-	-
HCO ₃ (mg/mL)	9.67±6.97	10.38±5.99	15.52±0.50	35.66±1.55	0.216	12.5	350	-
Sulphate(mg/mL)	14.12±12.61	10.86±8.31	14.14±11.20	0.95±0.85	0.058	150	100	20
Color (Heinz)	4.75±2.11	5.18±2.05	5.40±1.99	2.32±1.07	0.019*	5	-	-

Key: ANOVA Test, ≤ 0.05* Significant Associations at 95% Confidence Interval. Mean ± SD, W1 = upstream, W2 = midstream W3 = downstream, and CW = borehole water pH = potential of hydrogen, EC = electrical conductivity, BOD = biological oxygen demand, COD = chemical oxygen demand, DO = dissolved oxygen, TDS = total dissolved solids , WHO. (2017) = World Health Organization, FAO = Food and Agricultural Organization, NSDWG (2017) = National Standards for Drinking Water Quality, TSS = Total Suspended Solids, TH = Total Hardness , HCO₃ = Bicarbonate Ion, NO₃ = Nitrates, S₂O₄ = Sulphates, P₂O₅ = Phosphate

Qualitative Analysis of Biocides in Water from Fagge (LGA) and Cda (BUK) Study Areas

A bar chart titled "Qualitative Analysis of Biocides in Water from Fagge (LGA) and Cda (BUK)" can be found in Figure 2 below. The horizontal axis of the picture shows the distribution of two biocides, triclosan, and cypermethrin, over several water sources and locales (W1, W2, W3, and CW). Every biocide is represented by a distinct color: red (triclosan) and blue (cypermethrin). The frequency of biocides is shown by the vertical axis, where each centimeter on the scale corresponds to 0.2 units.

The findings demonstrated that cypermethrin was not present in borehole water but was found in wastewater samples on multiple occasions, with the highest frequency

occurring in W1 (2 occurrences), followed by W2 and W3 (1 occurrence each). On the other hand, none of the samples (W1, W2, W3, and CW) had any Triclosan.

Quantitative Analysis of Biocides in Water from Kwakwaci Fagge (LGA) and CDA (BUK) Study Areas

A bar chart titled Quantitative Analysis of Biocides in Water from Kwakwaci Fagge (LGA) and CDA (BUK)) is shown in Figure 3 below. The horizontal axis of the picture shows the presence of two biocides: Triclosan and Cypermethrin, in several water samples and locations (upstream, midstream, downstream, and borehole water). Two distinct colors are used to indicate each biocide: red (Triclosan) and blue (cypermethrin). Each centimeter on

the scale corresponds to 0.05 units of biocide concentration, which is displayed on the vertical axis and expressed in mg/ml. The findings demonstrated the presence of cypermethrin in wastewater samples (upstream: 0.226 mg/mL, midstream: 0.071 mg/mL, and

downstream: 0.065 mg/mL) but at different quantities in each of these locations. It was not found in the borehole water, though. In contrast, no trace of Triclosan (0 mg/mL) was found in any of the investigated areas.

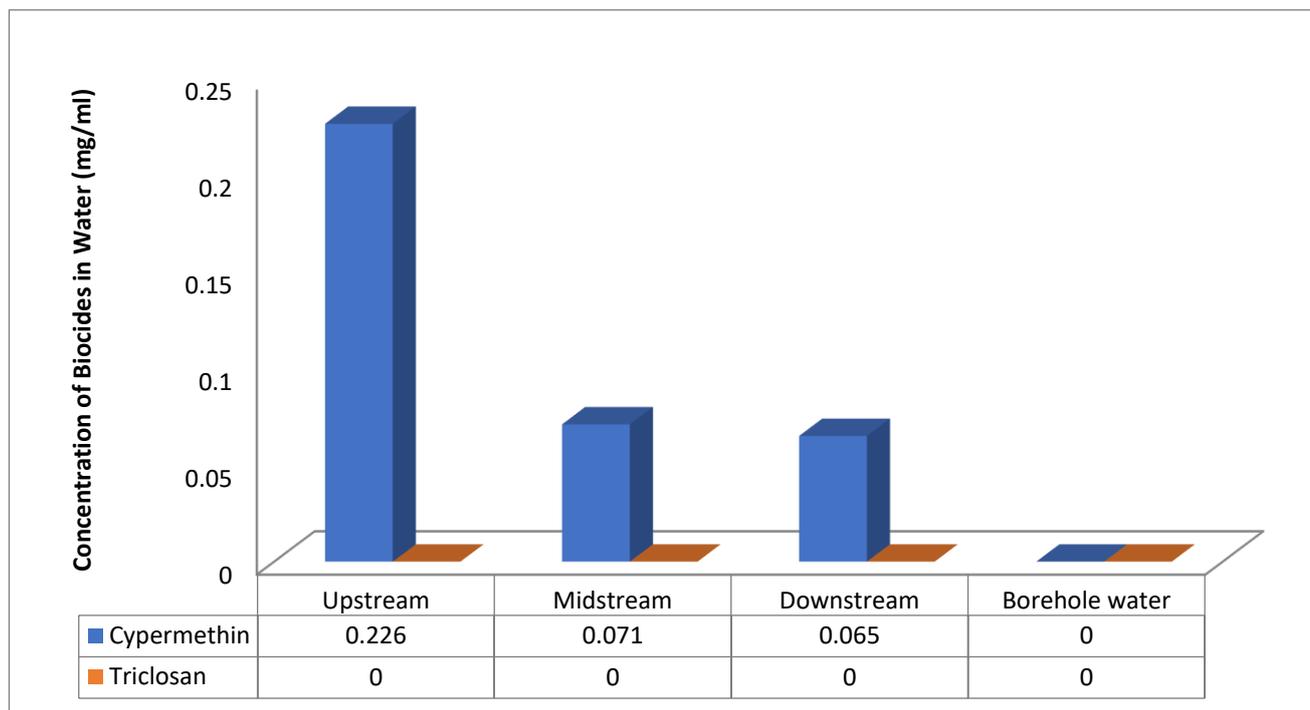


Figure 2: Quantitative Analysis of Biocide in Water Obtained from Kwakwaci (Fagge LGA) and CDA Bayero University

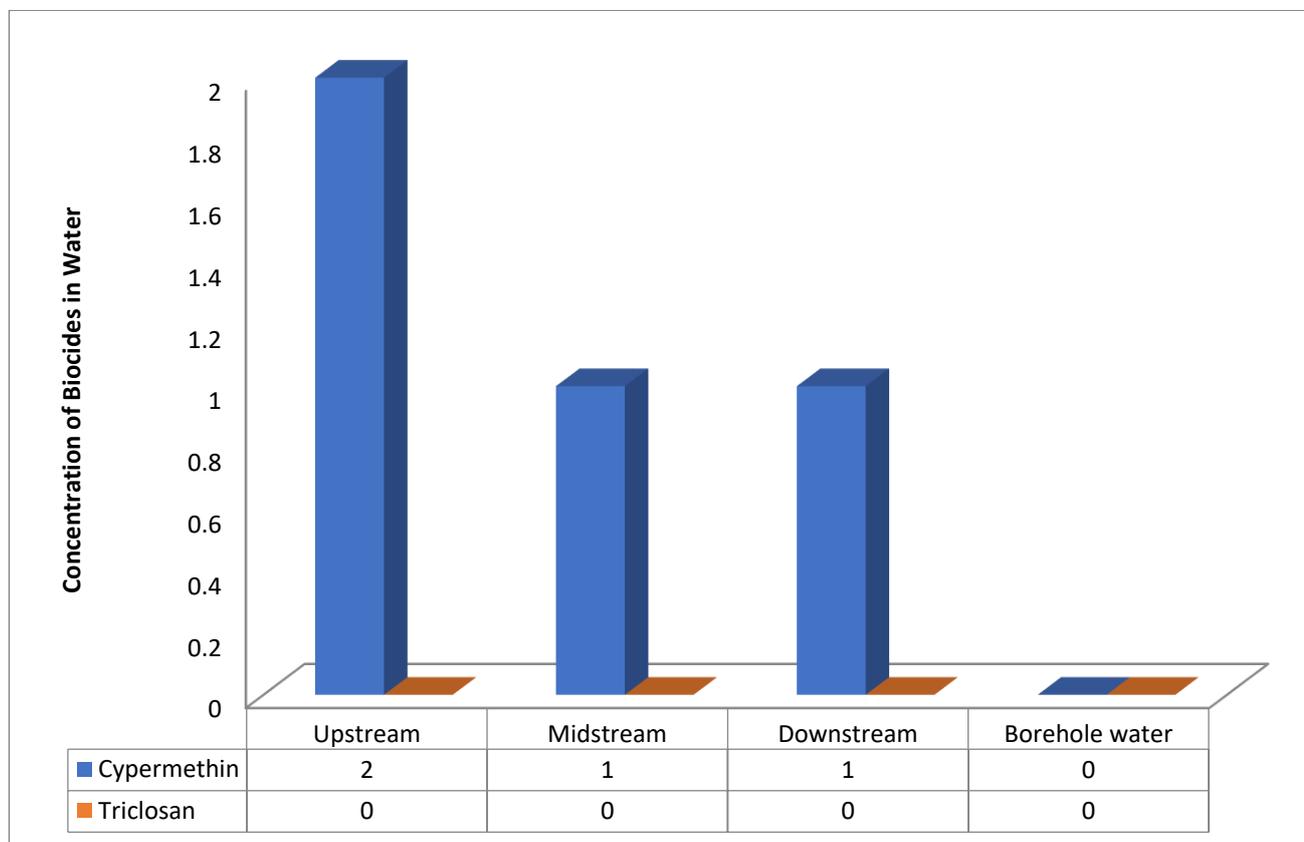


Figure 3: Qualitative Residual Analysis of Biocides in Water Obtained from Kwakwaci (Fagge LGA) and CDA Bayero University, Kano

Distribution of Bacteria in Wastewater from Fagge (LGA) Study Area

Table 3 displays the distribution of gram-negative bacteria in Fagge (LGA) wastewater. This table summarises the findings from the Gram-negative bacterial analysis of 36 wastewater samples.

Distribution of Bacteria in Water from CDA (BUK) Study Area

Table 5 shows the distribution of gram-negative bacteria from CDA (BUK) in water. The table summarises the

findings from the examination of twelve (12) control samples of water for gram-negative bacteria. Two (2) samples of water tested positive for Gram-negative bacteria.

Total Bacterial Counts of Water from Fagge (LGA) and CDA (BUK) Study Areas

The total bacterial counts in cell forming units per milliliter (CFU/ml) in borehole water (CW) and wastewater samples from three sources (W1, W2, and W3) are shown in Table 2.

Table 2 Aerobic Bacterial Count of Water Samples

S. No.	Sample code	Bacterial Count (x10 ⁸ CFU/ml)	Standards		
			WHO	NSDWG	FAO
1	W1	104±1	100cfu/ml	100cfu/ml	<500cfu/ml
2	W2	103±0.6			
3	W3	77±1.7			
4	CW	6.0 ±0.13			

Key: W1 =upstream, W2 =midstream, W3 = downstream, and CW = borehole water. WHO = World Health Organization, FAO = Food and Agriculture Organization, NSDWG (2017) = National Standards for Drinking Water Quality.

Table 3. Distribution of Gram-Negative Bacteria in Wastewater from Fagge (LGA) and CDA Study Area

Sampling Area	Kwakwachi (Fagge LGA)	CDA	Total
No. samples collected	36	12	48
No. Gram Negative	17	2	19

Key: LGA = Local Government Area, CDA = Center for Drylands Agriculture, Bayero University Kano

Table 4: Frequency and Percentage Occurrence of Gram-Negative Bacteria in Wastewater Samples from Fagge (LGA) Study Area

S. No.	Isolates	Water			Total
		W1	W2	W3	
1	<i>Bordetella</i> sp.	0 (0.0)	1(5.88)	0 (0.0)	1 (0.7)
2	<i>Bulkholderia cepacia</i>	0 (0.0)	0 (0.0)	0 (0.0)	0(0.0)
3	<i>Citrobacter freundii</i>	0 (0.0)	2 (1.4)	2 (1.4)	8 (5.5)
4	<i>Citrobacter youngae</i>	0 (0.0)	0 (0.0)	1 (5.88)	1 (0.7)
5	<i>E. coli</i>	5(29.41)	1 (5.88)	1 (5.88)	11 (7.6)
6	<i>Enterobacter cloacae</i>	0 (0.0)	1 (5.88)	0 (0.0)	2 (1.4)
7	<i>Klebsiella pneumonia</i>	2(11.76)	2(11.76)	2(11.76)	11 (7.6)
8	<i>Kluyvera</i> species	0 (0.0)	0 (0.0)	1 (5.88)	1 (0.7)
9	<i>Pantoea</i> sp.	0 (0.0)	2(11.76)	0 (0.0)	13 (9.0)
10	<i>Proteus mirabilis</i>	0 (0.0)	0 (0.0)	1 (5.88)	7 (4.8)
11	<i>Providencia stuartii</i>	1 (5.88)	1 (5.88)	0 (0.0)	2 (1.4)
12	<i>Pseudomonas fluorescens</i>	1 (5.88)	2(11.76)	3 (2.1)	26 (17.9)
13	<i>Pseudomonas oryzae</i> habitans	3(17.64)	0 (0.0)	0 (0.0)	14 (9.7)
14	<i>Rabnella aquatilis</i>	1 (5.88)	0 (0.0)	0 (0.0)	1 (0.7)
15	<i>Serratia liquefaciens</i>	0 (0.0)	0 (0.0)	0 (0.0)	0(0.0)
16	<i>Stenotrophomonas maltophilia</i>	1 (5.88)	0 (0.0)	1 (5.88)	5 (3.5)
17	<i>Vibrio fluvialis</i>	1 (5.88)	0 (0.0)	0 (0.0)	1 (0.7)
	Total	15 (10.3)	12 (8.3)	12 (8.3)	145 (100.0)

Key = Values Outside the Bracket are Frequency of occurrence, while those within are the Percentage W1 = upstream, W2 = midstream, W3 = downstream, and LGA = local government area

Table 5: Frequency and Percentage Occurrence of Gram-Negative Bacteria in Water from CDA (BUK) Study Area

S/N	Isolates	CW	Total
1.	<i>Aeromonas hydrophilia</i>	1(20)	2(40)
2.	<i>Pantoea</i> sp.	-	1(20)

To be continued next page

Table 5 continued

S/N	Isolates	CW	Total
3.	<i>Myroides</i> sp.	1(20)	1(20)
4.	<i>Pseudomonas fluorescens</i>	-	1(20)
	Total	2(40)	5(100)

Key = Values Outside the Bracket are Frequency of occurrence, while those within are the Percentage, CW = borehole water, and CDA (BUK) = Center for Dryland Agriculture Bayero University Kano.

DISCUSSION

The average pH of the irrigation water was between 6.55 ±0.47 to 8.07 ±0.30 from upstream to downstream, which is within the range that the [FAO \(1985\)](#), [WHO \(2017\)](#), and [NSDWQ \(2017\)](#) consider suitable for irrigation water. The presence of more organic matter in the effluents downstream, however, may have contributed to the much higher pH in downstream (W3). According to [Tunc and Sahin \(2015\)](#) and [Lu et al. \(2016\)](#), this organic debris may decompose into other basic compounds that would raise the pH downstream. According to [Thorvat et al. \(2012\)](#), who studied the physico-chemical features of the Panchaganga River in Kollhapur City, India, from upstream to downstream, the pH values in the current study were within that range.

Monitoring the pH value is essential because it impacts the quality of the water and is greatly impacted by its chemical and biological makeup as well as the existence of dangerous materials ([Minhas et al., 2022](#)). The turbidity level was determined to be greater than the [FAO \(1985\)](#), [WHO \(2017\)](#), and [NSDWQ \(2017\)](#) allowed limits of 5 VTU. The suggested limit of 1.54±1.08 was exceeded by the turbidity measurement at the control site, which is borehole water. Turbidity is the property of the water that affects its capability to scatter light or its transparency. The main contributors to murky or turbid water are silt, clay, fine organic material, and microscopic organisms, mostly algae, according to [Suleiman \(2018\)](#).

This significantly elevated turbidity downstream (W3) (25.51±13.12) may be due to the river's flow rate, human and animal activity close to the water source, and the high amount of organic matter that promotes microbial growth downstream ([Gil et al., 2015](#)). Upstream had the highest turbidity in the current study, followed by midstream and downstream. On the other hand, [Daniel et al.'s 2023](#) study showed that the Man-man River Jabi in Abuja, Nigeria, has high levels of turbidity in the middle, downstream, and upstream.

The control location exhibited minimal turbidity because of the properties of the borehole water and the decline in animal and human activity. This is consistent with the findings of [Suleiman \(2018\)](#) and [Unique et al. \(2016\)](#). They found low turbidity levels in borehole water extracted from specific locations in the states of Niger and Kano, respectively.

Another important measure of water quality is electrical conductivity (EC), which indicates that there are more ions present than recommended ([Ayers and Westcot, 1985](#)). In a normalized measurement of water's electrical

conductivity (EC), dissolved salts such as potassium and sodium chloride have the biggest impact. Wastewater EC (W1, W2, and W3) was over the [WHO \(2017\)](#) permissible limit of 300 µs/cm but below the [NSDWQ \(2017\)](#) standard of 1400 µs/cm.

This study's results are in contrast to those of [Daniel et al. \(2023\)](#), who found that the Man-made Jabi River in Abuja, Nigeria, had higher conductivity upstream, then downstream, and midstream. Water with high conductivity levels is not suitable for irrigation or human consumption. The downstream region in the current investigation had the highest conductivity value, followed by the upstream and midstream regions. The high EC value may have been caused by high amounts of dissolved salts caused by inadequate irrigation, minerals from agricultural runoffs, or other discharges downstream ([Unique et al., 2016](#)).

One possible explanation for the low EC readings at the control site is the low concentration of dissolved salts in the borehole water, which is consistent with the findings of [Unique et al. \(2016\)](#), who assessed the quality of a subset of borehole water at the Federal Polytechnic Bida in Niger State, Nigeria.

Our results indicate that the mean nitrate concentration of irrigation water ranged from 16.62±22.70 to 39.90±32.95, which was within the [WHO \(2017\)](#) and [NSDWQ \(2017\)](#) permissible limits. However, W1 and W3 had much higher nitrate concentrations than the [FAO's \(1985\)](#) allowable limit of 30 mg/ml due to the presence of organic matter from human and animal activity, as well as runoff from farms (which use manure and nitrate fertilizers). [Mohammad et al. \(2015\)](#) evaluated the variability of irrigation water quality in the Kano River Irrigation Project, and [Suleiman \(2018\)](#) found that irrigation water from a few areas in Kano, Nigeria, had lower nitrate contents.

According to [WHO \(2007\)](#), the recommended nitrate level of 50 mg/ml is based on the incidence of methemoglobinemia or blue baby syndrome in bottle-fed neonates caused by excessive nitrate exposure in infants up to approximately 3-6 months of age. Despite having greater soil nitrogen concentrations than control farms, wastewater-irrigated farms all remained within the FAO-recommended range.

The dissolved oxygen levels in W2, W3, and CW satisfied the [WHO \(2017\)](#) and [NSDWQ \(2017\)](#) tolerable criteria for dissolved oxygen. The upstream (W1) DO levels exceeded all permitted limits set by the FAO, NSDWQ, and WHO. According to the study, the area upstream

(W1) has the highest DO wastewater, which tends to decrease downstream (W2 and W3). High numbers of organisms that use oxygen to survive in irrigation water may be the cause of the DO drop from upstream to downstream (Suleiman, 2018). According to Kale (2016), another explanation would be the proportionally reduced or slow flow rate in the midstream and downstream. The findings of Ramdhiani and Suharyanto (2021) and Daniel *et al.* (2023) are in agreement with this one. They found that the Man-Made Jabi Lake in Abuja, Nigeria, and the upper Citarum River in Indonesia had high levels of DO upstream that decreased downstream.

In addition to determining the water's freshness, dissolved oxygen is essential for aquatic species to survive (Suleiman, 2018). The higher the DO, the better the water. A sufficient amount of dissolved oxygen in the water is required for proper water quality because oxygen is an essential component of all life. Decreased concentration leads to increased stress. Unique *et al.* (2016) state that if oxygen levels drop below 1-2 mg/l for a few hours, fish may die.

The BOD levels in wastewater were low upstream and increased downstream, measuring 3.55 ± 2.13 , 3.82 ± 1.88 , and 3.86 ± 1.54 (w1, w2, and w3). The biological oxygen demands in all locations were below the FAO, NSDWQ, and WHO acceptable levels. This is consistent with research by Ramdhiani and Suharyanto (2021), who found that the BOD concentration increased downstream in Indonesia's upper Citarum River but contradicts findings by Daniel *et al.* (2023) and Fatusin *et al.* (2020), which found a decline in BOD concentration downstream of the Oko-oba River in Agege, Lagos, and the Man-Made Jabi River in Abuja, Nigeria.

The concentrations of BOD and DO are clearly correlated, with higher BOD suggesting lower DO concentrations (Hidup and Bandung, 2017). This aligns with what is being investigated. According to Unique *et al.* (2016), SIT ranks BOD at 1-2 mg/ml as highly good, with less organic matter, 3-5 mg/ml as moderately clean, and 6-9 mg/ml as poor, somewhat polluted, indicating the presence of organic matter and the presence of bacteria that are breaking down the waste. According to the previously stated classification, the wastewater samples used in this study are deemed to be reasonably clean and appropriate for irrigation.

Meanwhile, the control (borehole) is regarded as excellent and safe for irrigation and drinking. The BOD levels found in the present investigation were lower than Suleiman's (2018) findings.

The COD levels discovered during this analysis were within the permitted ranges set by the FAO and WHO. Midstream has significantly higher COD levels than upstream and downstream. The control site had a low COD value (3.05 ± 0.72). Its remarkably high COD value may be the result of pollutants that have been collected in the midstream, including deposited residential and municipal sewage, organic fertilizers from agricultural

runoff, and industrial effluents. This is in line with research conducted by Fatusin *et al.* (2020) and Randhiani and Suharyanto (2020), which found higher COD levels in the middle of the upper Citarum River in Indonesia and the Oko-oba River in Agege, Lagos, Nigeria, followed by upstream and downstream. The COD was lower than what Suleiman (2018) had stated for irrigation water management and wastewater.

The total suspended particles in wastewater from the current study exceeded the FAO, NSDWQ, and WHO permissible limits for irrigation water. The TSS for W3 was significantly higher than those of W1 and W2. Microorganisms, silt and fine salt content, landslides, and the movement of material from the river due to water flow are some of the natural and man-made causes of the high total dissolved solids (TSS) downstream (Randhiani and Suharyanto, 2021). The findings of this study corroborate those of Fatusin *et al.* (2020), who found that TSS was higher downstream of the Oko-oba River in Agege, Lagos, Nigeria, followed by upstream and midstream. The increased runoff from domestic effluents and farmlands into the water may also be a contributing factor to the high TSS in wastewater (Suleiman, 2018).

The overall hardness at all four sites (W1, W2, W3, and CW) was between 200 and 500 mg/ml, which is the permitted range set by the WHO and NSDWQ. The highest overall hardness value was found in W2, which W1 and W3 followed. The control location was the one with the lowest TH value. The TH in W2 is substantially increased, which could be due to midstream domestic and industrial effluent.

However, the subsurface nature of the borehole water may be the cause of the low TH value seen in CW (Suleiman, 2018). The results of Daniel *et al.* (2023), which showed that the TH value was higher upstream of the artificial Jabi Lake, are supported by this. The research also contradicts Suleiman's (2018) results that groundwater and wastewater utilized for irrigation in Kano State, Nigeria, have lower levels of TH.

With the exception of the control site, which was within the FAO permissible limit of 40 mg/ml, the total dissolved solids at every location were within the NSDWQ permitted limit but beyond the WHO and FAO permissible limits. TDS was highest in the midstream, upstream, and downstream. The midstream's elevated dissolved salt and industrial and household wastewater levels may be the cause of this (Suleiman, 2018). This investigation contradicts the findings of Daniel *et al.* (2023), who found a decrease in the TDS value from upstream to downstream of the artificial Jabi River in Abuja, Nigeria.

With the exception of the control site, which was within the FAO permissible limit of 40 mg/ml, the total dissolved solids at every location were within the NSDWQ permitted limit but beyond the WHO and FAO permissible limits. TDS was highest in the midstream, upstream, and downstream. The midstream's elevated

dissolved salt and industrial and household wastewater levels may be the cause of this (Suleiman, 2018). This investigation contradicts the findings of Daniel *et al.* (2023), who found a decrease in the TDS value from upstream to downstream of the artificial Jabi River in Abuja, Nigeria. As for Suleiman's (2018) reference to Binnes, as salts comprise a sizable amount of water, TDS is a crucial indicator for assessing the salinity level of that water. The quality of irrigation water can be greatly impacted by the kind and concentration of dissolved salts in the water. There is a little but discernible amount of salt in irrigation water. They are caused by the weathering and dissolution of rocks and soil, which includes the dissolution of soil-based mineral salts that dissolve slowly, such as gypsum and lime (Belan, 1985). One possible explanation for the control water's low TDS concentration is that it contains less industrial and home wastewater.

The reported temperatures at all study sites were within the allowed ranges set by the NSDWQ (2017) and WHO. The notably high temperature in W3 may be caused by a number of reasons, including the standing nature of the water downstream, the weather, the time of year when the sample was taken, and the frequency of human activity in the area (Suleiman, 2018). The temperature values were greater than those seen upstream, midstream, and downstream of Jabi Lake by Daniel *et al.* (2023), but they were within the range reported by Ogbu *et al.* (2016) for the Ajah River in Udi, Enugu State, with brewery effluent. The low temperature at the control site could be due to the properties of the groundwater. As the temperature decreases, gases such as O₂, CO₂, and CH₄ become more soluble in water (Oyem *et al.*, 2014).

Bicarbonate ion concentrations in irrigation water were within the NSDWQ (2017), and WHO (2017) allowed limits, with the exception of the control site, which had values above the WHO (2017) recommendation of 12.5 mg/ml. One possible explanation for the notably elevated HCO₃ at the control site is the amount of carbon dioxide that dissolved in rainfall and precipitated as bicarbonate ions in groundwater (Rajkumar *et al.*, 2010). When Segaran *et al.* (2022) examined the principal ion concentration analysis and evaluation of groundwater derived from Malaysia for drinking water, they found lower HCO₃ than the current inquiry did.

Except for the control site, which had phosphate concentrations below the WHO guideline (4.61±4.34), all of the study locations had phosphate concentrations over the WHO's (2017) permitted limit of 0.4-5.9 for irrigation water. The effluents possibly containing phosphoric compounds may have contributed to the notably elevated phosphate concentration (16.42±13.31) upstream (Suleiman, 2018). Compared to the findings of Alghobbar and Surasha (2016), the phosphate level of the wastewater used for irrigation in the western part of Mysore City, Karnataka, India, was higher in the current study. The phosphorus level of CW irrigation water, on the other hand, was consistent with the authors' findings for

groundwater, treated wastewater, and untreated wastewater, ranging from 0.061 to 5.11 mg/l.

In every site, the color was within the WHO's (2017) authorized limit of 5 Heinz, with the exception of W2 and W3, which were slightly beyond the legal limit. The hue of the wastewater was twice that of the groundwater (CW). This suggests that apart from organic compounds, the wastewater might have also been contaminated by colorants such as dye and other business and residential effluents. Arimoro (2009) noted a comparable instance of high color values at the Adofi River's industrial wastewater discharge location in Delta State, Nigeria. Additionally, Suleiman (2018) noted that irrigation water exhibited high color values in various parts of Kano State, Nigeria.

According to this study, triclosan might not have existed due to a variety of processes, including microbial degradation, adsorption to soil and sludge particles, chemical oxidation and transformation, photodegradation, and enhanced treatment methods from wastewater treatment facilities (Chen *et al.*, 2018; Zhang *et al.*, 2021). During the wastewater treatment process, it could also be the consequence of a successful elimination or a very slight presence. Bakare and Adeyinka's (2022) study on the fate mechanisms of parabens, triclocarban, and triclosan during wastewater treatment: assessment using field measurements and model simulations found high quantities of triclosan in wastewater, ranging from 1.732 to 6.980µg/L, which is in contrast to our findings. Triclosan is a well-known broad-spectrum antiseptic biocide that has many applications in both medicine and the home. It belongs to the bisphenol family. It enters the ecosystem through soil and water bodies because of its high prevalence and ability to accumulate (Jablonska-Trypuc, 2023).

In contrast to other insecticides used in soil habitats, where frequent and extensive usage might render it hazardous to non-target organisms, cypermethrin is a pyrethroid that is believed to be less harmful, according to studies by Braganca *et al.* (2019a, 2019b). According to Farina *et al.* (2018), the organic matter's highly hydrophobic properties allow it to permeate groundwater and a range of crops. In the present investigation, Cypermethrin was found at 2-0.226 mg/ml in W1, 1-0.071 mg/ml in W2, and 1-0.065 mg/l in W3, with a detection rate of 11%. The control water, however, did not contain it. Nonetheless, a detectable 8% cypermethrin rate was found in soil that was irrigated with effluent. Surface runoff following crop application, loss from hard stands on farms after treating sheep and animals, domestic sources from home and garden use, and industrial activities like wool processing are some of the potential causes of this concentration (EA, 2019).

The wastewater and control water, or W1, W2, W3, and CW, have a significant connection ($p < 0.005$). There is statistical significance in this association. Borehole water (CW) consistently demonstrated a lower aerobic bacterial count ($6.0 \pm 0.13 \times 10^8$ CFU/ml) than wastewater

samples. Additionally, there is a statistically significant difference ($p < 0.005$) in the number of bacteria in the wastewater samples. At a 95% confidence level, W1's bacterial count ($104 \pm 1 \times 10^8$ CFU/ml) is significantly greater than both W2 and W1.

CONCLUSION

This study highlights the significant presence of residual biocide-resistant Gram-negative bacteria in wastewater samples from irrigated farms in Kano State, Nigeria. The findings demonstrate that industrial and agricultural activities contribute to the pollution of water bodies, leading to elevated levels of contaminants that adversely affect water quality. Major physicochemical parameters such as pH, electrical conductivity, total suspended solids, and dissolved oxygen exhibited significant variations, indicating the impact of wastewater on these metrics.

The presence of biocides, specifically Cypermethrin, in the wastewater samples further underscores the issue of chemical pollution, although Triclosan was not detected. The identification of various Gram-negative bacteria resistant to biocides in the wastewater samples but not in the control borehole water suggests a selective environment fostering resistant strains due to the presence of antimicrobial substances in the wastewater.

These findings underscore the urgent need for improved waste management and treatment infrastructure in developing regions. Effective policies and practices are essential to reduce the introduction of harmful pollutants into water bodies, ensuring safe water for agricultural and domestic use. Additionally, regular monitoring and stringent regulations are imperative to control and mitigate the spread of biocide-resistant bacteria, protecting public health and sustaining agricultural productivity.

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