



# **Assessment of Bacterial Community Profile, Quality Impairment and Human Health Risk of Surface Water Receiving Abattoir Effluents: Evidence from Yewa and Iju Streams, Ondo State, Nigeria**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## **Article Information**

DOI: <https://doi.org/10.9734/acri/2025/v25i51207>

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/135063>

**Original Research Article**

**Received: 25/02/2025**

**Accepted: 28/04/2025**

**Published: 05/05/2025**

## **ABSTRACT**

The discharge of untreated effluents into water bodies constitutes enormous threat to ecosystem sustainability and all forms of lives dependent on surface water resource. The purpose of this research was to assess the bacterial profile, quality impairment and human health risk of surface

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water receiving abattoir effluents. Water samples were collected from Yewa and Iju streams, Ondo State, Nigeria, both of which receives abattoir effluent. The microbiological and physicochemical analyses of the samples were carried out using conventional microbiological and physical and chemical analytical techniques. The samples showed significant number of heterotrophic bacteria of  $4.6 \times 10^4$  -  $2.1 \times 10^6$  CFU/mL and  $1.8 \times 10^4$  -  $1.1 \times 10^6$  CFU/mL respectively for Yewa and Iju streams and coliform counts of 11-53 MPN/100 mL and 14-64 (MPN/100 mL). Four bacteria of the order Enterobacterales including *Escherichia coli* were isolated from the samples. The sampling points vary in their physicochemical parameters and concentration of heavy metals with pH in the range 6.58-7.27 and 6.74-8.65 for Yewa and Iju streams respectively. The biochemical oxygen demand for all the sampling points in both streams were above the 5 mg/L limit prescribed by the WHO. The water quality index of the streams was in the range 50.23-69.20 and 44.24-77.69 for Yewa and Iju streams respectively. A human health risk assessment of heavy metal exposure through dermal contact and ingestion revealed no significant hazard with hazard quotient (HQ) values of less than 1. In conclusion, the surface water receiving abattoir effluent can elicit substantial environmental and life-threatening public health concerns.

**Keywords:** *Abattoir effluent; Coliform bacteria; microorganisms; surface water; water pollution; water quality index.*

## 1. INTRODUCTION

The unending drive to increase meat production to meet the protein needs of the population has resulted in the uncontrolled establishment of abattoirs. Abattoirs, also known as slaughterhouses, are designated facilities for the slaughtering of animals, however, the locations are usually unregulated but often near streams and rivers for ease of access to water for washing and cleaning. Abattoirs play vital role in providing processed meat products and job creation to meet the needs of ever-growing population in the localities (Ire et al., 2017). Most of the abattoirs in Nigeria are ill-equipped and underdeveloped to handle wastes generated from abattoir operations of butchering, removal of the hide, intestine management, rendering, trimming, processing and equipment and floor cleaning activities. The wastewater generated from abattoir activities usually comprise of animal blood, urine, faeces, fat and oil, mineral and organic solids, salts and chemicals added during handling operations and microorganisms (Akange et al., 2016; Abasi, 2024). Abattoir effluent could significantly increase the amounts of nitrogen, phosphorus, and total solids in the receiving water body (Akange et al., 2016) leading to eutrophication. In Nigeria, many abattoirs dispose their effluents directly into water bodies such as streams and rivers without any form of treatment (Wizor and Nwankwoala, 2019) while the eviscerated consumable parts of the slaughtered animal are washed directly with the same flowing water bodies. The improper discharge of untreated wastewater into water bodies results in the accumulation of toxins and pathogens, depletion of oxygen levels, and an

increase in nutrient availability, all of which deteriorate the quality of the receiving surface water with the consequence of economic, social and environmental problems (Monroy, 2013; De la Peña, 2013; Abasi et al., 2024). The discharge of untreated wastewater into water bodies is one of the major threats to natural environment because it contributes to uncontrolled organic nutrient and microbial load of the receiving water body, thereby creating significant pollution with the attendant environmental challenges (Wizor and Nwakoala, 2019) and health risks.

Streams, lakes, rivers have diverse uses which include habitat for aquatic lives, fishing, swimming, drinking and personal and environmental hygiene such as washing and bathing. When water is contaminated by pollutants from point or nonpoint sources, it results in significant alteration of the chemical, physical and biological characteristics of receiving water bodies which in turn describe its quality usually with respect to its suitability for a defined purpose (Stupar et al., 2022). Therefore, contamination of water bodies by microorganisms and chemicals may render it unfit for aquaculture and domestic use, among others. Tyagi et al. (2013) and Britto et al. (2018) reported that the quality of any specific water body can be tested and determined using the physical, chemical and biological parameters (variables) which include pH, turbidity, temperature, concentration of dissolved oxygen, nitrate, total suspended solids, electrical conductivity (EC), biological oxygen demand, heavy metals and bacterial population. Therefore, there is the need to sustain the natural resources (water and land inclusive) for

productive agriculture (irrigation), fishing, recreation and tourism without jeopardizing fish and human health and the environment. In aquatic environments, waste can lead to oxygen depletion, eutrophication, and elevated ionic content, thereby endangering aquatic life (Abasi et al., 2024). Water plays a significant role as a vehicle for the transmission of diverse water related illnesses and therefore a major cause of morbidity and mortality. One of the critical forms of water pollution occurs when faeces enter the water supply (Steiner et al., 2006). Many diseases are perpetuated by the faecal-oral route of transmission in which the pathogens are shed only in human faeces (Adetunde and Glover, 2010). The microorganisms that are associated with water borne diseases are *Salmonella* sp., *Shigella* sp., *Escherichia coli* and *Vibrio cholera* (Adetunde and Glover, 2010). The greatest risk to public health from microbes in water is associated with the consumption of water that is contaminated with human and animal excreta (WHO, 2022). The consumption of contaminated water is associated with various illnesses and diseases, including cholera, dysentery, gastroenteritis and typhoid fever (Ire et al., 2017). In order to determine the level of pollution from abattoirs effluents and its potentials in causing environmental damage and health concerns to human and aquatic lives, there is therefore the need to focus on determining the physicochemical parameters and microbial profile in the effluent-receiving ecosystems. Therefore, this study is aimed at assessing the microbial community profile and water quality impairment of surface water receiving abattoir effluent in order to ascertain the human health risk associated with continued dependence on the water resource.

## 2. MATERIALS AND METHODS

### 2.1 The Study Area

The study sites were Yewa and Iju streams on Latitude 6.50N and Longitude 5.80E and Latitude 6.30N and Longitude 5.30E at Okitipupa and Ilutitun respectively both in Okitipupa Local Government Area of Ondo State, Nigeria. The Yewa and Iju streams coded streams A and B respectively receive abattoir effluents from Dr's Road and Oladiti abattoirs. Okitipupa town and Igbodigo are catchment communities around Yewa stream and Ilutitun for Iju stream. There is no visible industrial activity around the catchments. The communities depend on the

streams for washing, bathing and partly drinking.

### 2.2 Sample Collection

Stream water samples were collected in sterile containers at four (4) different sampling points 50 meters apart from Yewa and Iju streams receiving abattoir effluents from Dr's Road and Oladiti abattoirs respectively. Samples from each site were neatly labeled YUSC, YDPC, YDSC, YLSC and IUSC, IDPC, IDSC, ILSC for Yewa and Iju streams respectively. Water samples for physicochemical and metal analyses were collected in 1L acid-prewashed plastic sample bottles, labeled appropriately and transported in an ice chest to the Laboratory. Samples were collected between 7-10 am considered to be the activity hours of abattoir and to enable the effluent from the abattoir reach the stream.

### 2.3 Microbiological Analysis of Water Samples

#### 2.3.1 Sterilization of glassware and preparation of media

The glassware used in this study were thoroughly washed with detergents and then oven sterilized at 160 °C for 2 hours. All the media and diluents used were accurately weighed and dissolved in appropriate volume of distilled water according to manufacturers' specifications and then stirred with a magnetic stirrer. Twenty milliliter (20 ml) of the media solution was measured and poured into McCartney bottles, autoclaved at 121° C for 15 minutes and maintained at 45°C.

#### 2.4 Enumeration of Total Heterotrophic Bacterial Population

The stream water samples were serially diluted to the 6<sup>th</sup> dilution in normal saline (8.5 g/l (NaCl solution). Exactly 1mL of each dilution was pour plated in triplicate unto labelled Petri dishes and overlaid with pre-sterilized nutrient agar. The culture plates, having allowed the agar medium to set, were incubated at 37 °C for 24 hr. The plates were observed for growth and selected for count after the expiration of the incubational period. Culture plates in which the number of colonies was 30-300 and its triplicates for each sample was selected and counted. The average count was then multiplied by the dilution factor at that dilution and expressed as colony forming unit (CFU/mL).

## 2.5 Enumeration of Total Coliform

Enumeration of total coliform was done using the three- tube assay of the standard multiple tube fermentation also referred to as Most Probable Number (MPN) index technique. The MacConkey broth (LAB M) used was prepared according to manufacturer's specification as double and single strength. Ten milliliter (10 ml) of double strength was dispensed into the first three sets of test tubes while the remaining two sets of three test tubes each also received 10 ml of single strength of the prepared medium. A Durham tube was inverted in each of the tubes containing broth, all the tubes were plugged with cotton wool and sterilized at 121°C in an autoclave. This procedure was repeated for each of the assayed water samples. Ten milliliter (10 ml) of a sample was inoculated into each tube of the double strength broth, 1.0 ml into first three tubes of the single strength and 0.1 ml of the same sample was inoculated into each of the other set of three tubes of the single broth. The culture tubes were carefully agitated to mix the inoculum with the broth medium. They were incubated at 35°C for 48 hours and each tube bearing Durham tube were observed for growth and acid and gas production. Appearance of gas in the first 24- hr period is a positive presumptive test, appearance of gas in the next 24-hour period is a doubtful test while absence of gas after the 48-hour period is a negative test indicating that sample does not have coliform bacteria. From the combined numbers of positive tubes in each set, the most probable number (MPN) of coliform bacteria present in the original water sample was appropriately determined using a standard MPN table developed by McCrady to obtain the estimated number of coliform cells present in 100 ml of original sample. The positive presumptive tubes were followed by the confirmed and completed tests to determine the presence of *Escherichia coli* as indicators of faecal contamination in the samples.

## 2.6 Identification of Bacterial Isolates from Assayed Stream Water Receiving Effluents

The pure cultures of the bacterial isolates obtained by repeated streaking on nutrient agar plates and stored on nutrient agar slants at 4°C were identified by using colonial and cellular morphology as well as biochemical reactions according to Sneath et al. (2009). Different tests carried out on isolates include Gram reaction, spore staining, coagulase, motility, oxidase, indole production, methyl red, Voges Proskauer,

sugar fermentation, citrate utilization, catalase test and H<sub>2</sub>S gas production.

## 2.7 Determination of Physicochemical Parameters

Water temperature was measured *in situ* using a mercury-in-glass thermometer, while dissolved oxygen, salinity, and pH were assessed on-site using a Horiba Water Checker (Model U-10). Water samples were analyzed for other quality parameters including electrical conductivity, turbidity, hardness, total dissolved solid (TDS), total suspended solid (TSS), total solids (TS), biochemical oxygen demand (BOD), chemical oxygen demand (COD) by conventional physical and chemical analytical methods described by APHA (2017). Phosphate, sulfate, and nitrate levels were analyzed following the American Public Health Association (2017) guidelines, while heavy metals (As, Mn, Cd, Pb, Cr, Cu, Zn, Co, Ni, and Fe) were assessed using an ACCUSYS 211 Atomic Absorption Spectrometer (AAS), based on the method described by Thomas and Mohaideen (2015).

## 2.8 Water Quality Index (WQI)

The water quality of the streams was evaluated using the Water Quality Index (WQI), which was calculated based on the drinking water standards recommended by the World Health Organization (WHO). In the WQI analysis, each examined parameter was assigned a weight (AW<sub>i</sub>) ranging from 1 to 4, depending on its significance (Shetaia et al., 2020). The Water Quality Index (WQI) was then determined using the following formula:

$$WQI = \sum_{i=1}^n Sli \quad (1)$$

Where;  $Sli$  of each parameter was calculated as:

$$Sli = RW \times Qi \quad (2)$$

Where;  $RW$  (Relative Weight of each parameter) was calculated as:

$$RW = \frac{AW_i}{\sum_{i=1}^n AW_i} \quad (3)$$

Where;  $AW$  = assigned weight of each parameter,  $n$  = number of parameters (Table 2).

While;  $Qi$  (quality rating for each parameter) was determined as:

$$Qi = \frac{Ci}{Si} \times 100 \quad (4)$$

Where;  $C_i$  = value of the parameter,  $S_i$  = the recommended value obtained from WHO (2003) and FEPA (2003). (Shetaia et al., 2020).

The computed WQI values were classified based on the criteria outlined by Shetaia et al. (2020): *Excellent* (WQI < 50), *Good* (WQI 50–100), *Poor* (WQI 101–200), *Very Poor* (WQI 201–300), and *Bad* (WQI > 300).

## 2.9 Pollution Indices of Water

### 2.9.1 The single-factor Pollution Index (Pi)

The Single-Factor Pollution Index ( $P_i$ ) was calculated according to Yan et al., (2015) as:

$$P_i = \frac{C_i}{S_i} \quad (5)$$

Where:  $P_i$  = pollution index of pollution indicator  $i$ ,  $C_i$  = concentration of the pollution indicator in water (mg/l).  $S_i$  = permissible limit for the pollution indicator in water.

The single-factor pollution index ( $P_i$ ) is classified into five grades, according to Lin et al., (2010) (Table 2).

### 2.9.2 The Comprehensive Pollution Index (CPI)

The Comprehensive Pollution Index (CPI) was calculated according to Tao et al., (2011), as:

$$CPI = \frac{1}{n} \sum_{i=1}^n \frac{C_i}{S_i} \quad (6)$$

Where: CPI = the comprehensive pollution index,  $C_i$  = concentration of the pollution indicator  $i$  (mg/l),  $S_i$  = permissible limit for the pollution indicator  $i$  in water,  $n$  = the number of analyzed pollution indicators.

CPI is classified according to Tao et al. (2011) into five water quality levels (Table 3).

## 2.10 Human Health Risk Assessments

Inhalation, ingestion, and dermal absorption of surface water are key pathways through which trace metals enter the human body. To evaluate potential health risks, the dermal exposure, ingestion pathways, and hazard quotients (HQs) associated with heavy metals were assessed using a risk assessment model. The exposure dose was calculated following the guidelines of USEPA (2005) using the following formula:

$$D(\text{ingestion}) = \frac{C_w \times IRW \times EF \times ED}{BW \times AT} \quad (7)$$

and

$$D(\text{dermal}) = \frac{C_w \times SA \times K_p \times ET \times EF \times ED \times CF}{BW \times AT} \quad (8)$$

Where: IRW is drinking water ingestion rate (2 L/day).  $C_w$  represents average concentration of heavy metals in water. EF indicates exposure frequency (350 days/year). ED stands for exposure duration (54 years equivalent to the average age lifetime in Nigeria). AT stands for average time for carcinogens and non-carcinogens (19710 day/year).

**Table 1. Assigned weight (AW) and relative weight (RW) of the water quality parameters**

Parameters	WHO/EPA Standards	Assigned weight (AW)	Relative weight (RW)
Temperature	30	4	0.09
Ph	6.5	3	0.07
Turbidity	5	3	0.07
Dissolved Oxygen	6	3	0.07
BOD	5	4	0.09
COD	10	4	0.09
TDS	500	2	0.04
Nitrate	50	2	0.04
Phosphate	0.05	1	0.02
Fe	0.3	3	0.07
Cu	2	2	0.04
Cd	0.003	3	0.07
Cr	0.05	3	0.07
Pb	0.01	4	0.09
Mn	0.08	3	0.07
Zn	3	2	0.04
Total		46	1

Key: BOD; Biological Oxygen Demand, COD; Chemical Oxygen Demand, TDS; Total Dissolved Solid

**Table 2. Standards for single-factor pollution index (Pi)**

Pi	Pollution grades
< 0.4	Non-pollution
0.4 - 1.0	Slight pollution
1.0 - 2.0	Medium polluted
2.0 - 5.0	Heavy polluted
>5.0	Serious polluted

**Table 3. Classification of surface water quality based on CPI**

Values	Water Quality Grades
< 0.2	Cleanness
0.21 - 0.4	Sub-cleanness
0.41 - 1.0	Slight pollution
1.01 - 2.0	Moderate pollution
> 2.01	Severe pollution

BW represents body weight (60 kg). SA represents exposed skin area (18000 cm<sup>2</sup>). *Kp* represents dermal permeability of the last constant in cm/hr, (Co is 0.0004, Ni is 0.0002, Zn is 0.0006, Cr is 0.002 while Cd, Cu, Fe, Mn, and Pb is 0.001). ET is exposure time 0.6 h/day. CF is unit conversion factor; for water, it is equal to 1 L/1000 cm<sup>3</sup>.

The hazard quotients (HQs) were therefore calculated as:

$$HQ_s = \frac{D}{RfD} \quad (9)$$

Where: RfD is the reference dose for different heavy metals (mg/kg/day), which is based on US risk-based assessment. Reference Dose (Oral) was 0.04, 0.30, 0.70, 0.004, 0.001, 1.50, 0.02, 0.14 and 0.02 for Cu, Zn, Fe, Pb, Cd, Cr, Co, Mn and Ni respectively while Reference Dose (Dermal) was Cu (0.012), Zn (0.06), Fe (0.045), Pb (0.42), Cd (0.005), Cr (0.003), Co (0.005), Mn (0.8) and Ni (0.005) according to USEPA (2005) and Olawusi-Peters et al. (2019).

### 3. RESULTS

#### 3.1 Load and Identity of Bacteria in the Assayed Stream water Receiving Abattoir Effluent

The sampling points of each stream receiving effluent vary in the load and type of bacteria as shown in Table 4. The results revealed that the population of heterotrophic bacteria were in the range  $4.6 \times 10^4$ -  $2.1 \times 10^6$  CFU/mL and  $1.8 \times 10^4$ -  $1.1 \times 10^6$  CFU/mL and coliform of 11-53 (MPN/100mL) and 14-64 (MPN/100mL) respectively for Yewa and Iju streams respectively. All the sampling points for both

streams indicated the presence of *Escherichia coli* with the effluent discharge points having the highest number of coliform bacteria per 100 mL. The identity of bacteria revealed in Table 5 were *Bacillus* sp., four genera of coliform bacteria, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Staphylococcus aureus* with *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella* sp. having the highest percentage occurrence of 87.5% among the sampling points of the two streams. The results in Fig. 1 also revealed that among the coliforms *Salmonella* sp. had the highest percentage occurrence of 75% and 100% respectively in the Yewa and Iju streams respectively.

#### 3.2 Physicochemical Analysis

The results of the physicochemical analysis for Streams A and B are presented in Tables 5 and 6. In Stream A, most physicochemical parameters fell within the WHO/EPA recommended permissible limits, except for dissolved oxygen (DO) ( $4.05 \pm 0.02$  to  $4.61 \pm 0.01$  mg/L), biological oxygen demand (BOD) ( $7.77 \pm 0.03$  to  $8.06 \pm 0.04$  mg/L), and chemical oxygen demand (COD) ( $14.14 \pm 0.02$  to  $15.87 \pm 0.07$  mg/L). A significant difference was observed across all parameters at the sampling stations ( $p < 0.05$ ), as determined by Duncan's post hoc analysis (Table 6). Similarly, in Stream B, all parameters except DO ( $4.00 \pm 0.00$  to  $6.00 \pm 0.00$  mg/L), BOD ( $7.00 \pm 0.00$  to  $8.00 \pm 0.00$  mg/L), and COD ( $13.00 \pm 0.00$  to  $16.00 \pm 0.00$  mg/L) were within the WHO/EPA permissible limits. A significant variation was also observed across the sampling stations ( $p < 0.05$ ) when subjected to Duncan's post hoc analysis (Table 7).

**Table 4. Load of heterotrophic and coliform bacteria in yewa and iju streams receiving abattoir effluent**

Sample code	THB (CFU/ml)	Presumptive Test (MPN/ 100 MI)	Confirmed/ Completed Tests
AUSC	$8.3 \times 10^4 \pm 1.2$	28	ECNP
ADSC	$4.6 \times 10^4 \pm 1.2$	53	ECP
ADS1	$2.1 \times 10^6 \pm 0.1$	15	ECP
ADS2	$5.3 \times 10^4 \pm 3.5$	11	ECP
BUSC	$2.4 \times 10^5 \pm 0.28$	15	ECNP
BDSC	$1.8 \times 10^4 \pm 0.1$	44	ENP
BDS1	$1.1 \times 10^6 \pm 0.1$	64	ECP
BDS2	$1.3 \times 10^5 \pm 0.4$	14	ECP

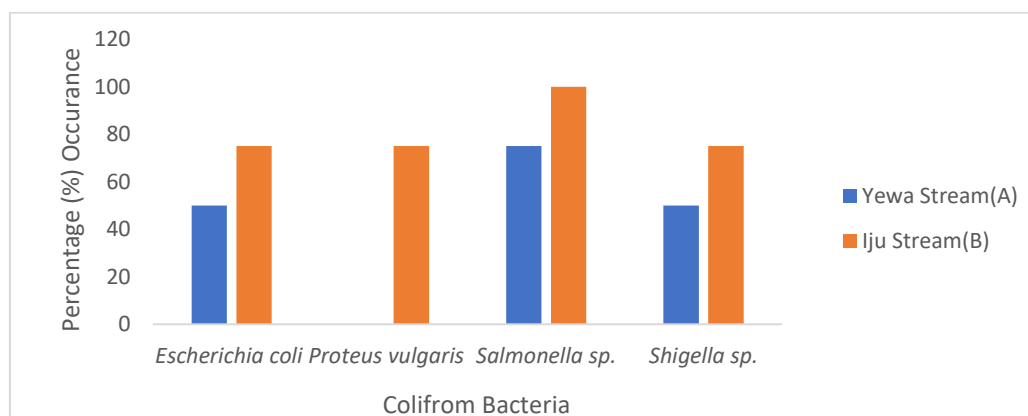
Values above are expressed as mean  $\pm$  Standard Error.

Legend: THB; Total Heterotrophic Bacteria, AUSC; Yewa Upper Stream, ADSC; Yewa Discharge Stream Catch, ADS1; Yewa Down Stream 1, ADS2; Yewa Down Stream 2, BUSC; Iju Upper Stream, BDSC; Iju Discharge Stream Catch, BDS1; Iju Down Stream 1, BDS2; Iju Down Stream 2, ECNP; E. coli not Present, ECP; E. coli Present

**Table 5. Identity and percentage occurrence of bacterial isolates from yewa and iju streams receiving abattoir effluents**

Isolate	Yewa Stream Samples				Iju Stream Samples				% Occurrence
	AUSC	ADSC	ADS1	ADS2	BUSC	BDSC	BDS1	BDS2	
<i>Bacillus cereus</i>	-	-	-	-	-	+	+	+	37.5
<i>Bacillus subtilis</i>	+	+	+	+	+	-	+	+	87.5
<i>Enterococcus faecalis</i>	+	-	+	+	+	+	+	+	75
<i>Escherichia coli</i>	-	+	+	+	-	+	+	+	75
<i>Micrococcus luteus</i>	+	-	+	+	+	-	-	+	62.5
<i>Proteus vulgaris</i>	-	-	-	-	+	-	+	+	37.5
<i>Pseudomonas aeruginosa</i>	+	-	+	+	+	+	+	+	87.5
<i>Salmonella sp.</i>	+	-	+	+	+	+	+	+	87.5
<i>Shigella sp.</i>	+	-	+	+	+	+	+	-	75
<i>Staphylococcus aureus</i>	+	+	+		+		+		62.5

Key: AUSC; Yewa Upper Stream, ADSC; Yewa Discharge Stream Catch, ADS1; Yewa Down Stream 1, ADS2; Yewa Down Stream 2, BUSC; Iju Upper Stream, BDSC; Iju Discharge Stream Catch, BDS1; Iju Down Stream 1, BDS2; Iju Down Stream 2



**Fig. 1. Percentage (%) occurrence of coliform bacteria in the assayed surface water receiving abattoir effluent**



**Table 6. Physicochemical characteristics of Yewa (A) stream receiving abattoir effluent**

Parameters	Sampling stations				WHO/EPA Limit
	AUSC	ADPC	ADS1	ADS2	
Temp. (°C)	25.93±0.15 <sup>c</sup>	26.17±0.15 <sup>c</sup>	25.57±0.15 <sup>b</sup>	25.27±0.12 <sup>a</sup>	30
pH	6.58±0.00 <sup>a</sup>	7.27±0.00 <sup>d</sup>	6.86±0.02 <sup>b</sup>	6.91±0.01 <sup>c</sup>	6.5
Turbidity (NTU)	4.37±0.00 <sup>c</sup>	2.74±0.02 <sup>a</sup>	5.61±0.01 <sup>d</sup>	3.95±0.01 <sup>b</sup>	5
Salinity (mg/L)	0.07±0.00 <sup>a</sup>	0.09±0.00 <sup>b</sup>	0.13±0.00 <sup>d</sup>	0.10±0.00 <sup>c</sup>	120
Hardness (mg/L)	116.38±0.03 <sup>c</sup>	75.14±0.02 <sup>a</sup>	124.63±0.02 <sup>d</sup>	114.09±0.16 <sup>b</sup>	150
Alkalinity (mg/L)	82.51±0.00 <sup>c</sup>	30.58±0.03 <sup>a</sup>	92.33±0.00 <sup>d</sup>	80.68±0.04 <sup>b</sup>	200
Chloride (mg/L)	10.34±0.03 <sup>d</sup>	5.22±0.01 <sup>a</sup>	8.85±0.02 <sup>b</sup>	10.01±0.07 <sup>c</sup>	250
DO (mg/L)	4.61±0.01 <sup>c</sup>	4.05±0.02 <sup>a</sup>	4.60±0.01 <sup>c</sup>	4.37±0.01 <sup>b</sup>	6
BOD (mg/L)	7.85±0.02 <sup>b</sup>	8.22±0.02 <sup>d</sup>	7.77±0.03 <sup>a</sup>	8.06±0.04 <sup>c</sup>	5
COD (mg/L)	14.14±0.02 <sup>a</sup>	15.87±0.07 <sup>d</sup>	15.38±0.02 <sup>c</sup>	14.82±0.03 <sup>b</sup>	10
TDS (mg/L)	58.23±0.03 <sup>c</sup>	36.19±0.02 <sup>a</sup>	52.63±0.04 <sup>b</sup>	75.18±0.08 <sup>d</sup>	500
TSS (mg/L)	3.85±0.02 <sup>b</sup>	2.64±0.00 <sup>a</sup>	5.27±0.00 <sup>c</sup>	6.15±0.01 <sup>d</sup>	25
TS (mg/L)	62.09±0.04 <sup>c</sup>	38.82±0.01 <sup>a</sup>	57.9±0.05 <sup>b</sup>	81.32±0.08 <sup>d</sup>	30
Conductivity µS/cm	106.37±0.03 <sup>b</sup>	78.58±0.03 <sup>a</sup>	107.22±0.02 <sup>c</sup>	156.47±0.03 <sup>d</sup>	1000
Sulphate (mg/L)	0.26±0.02 <sup>a</sup>	0.21±0.01 <sup>a</sup>	0.37±0.02 <sup>b</sup>	2.14±0.05 <sup>c</sup>	250
Nitrate (mg/L)	0.64±0.01 <sup>d</sup>	0.36±0.02 <sup>c</sup>	0.23±0.06 <sup>b</sup>	0.14±0.01 <sup>a</sup>	50
Phosphate (mg/L)	0.03±0.00 <sup>c</sup>	0.01±0.00 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.05
P(ppm)	3.45±0.03 <sup>d</sup>	0.23±0.01 <sup>b</sup>	0.19±0.00 <sup>a</sup>	0.32±0.01 <sup>c</sup>	12

Key: AUSC; Yewa Upper Stream Catch, ADPC; Yewa Discharge Stream Catch, ADS1; Yewa Down Stream 1, ADS2; Yewa Down Stream 2, DO; Dissolved Oxygen, BOD; Biological Oxygen Demand, COD; Chemical Oxygen Demand, TDS; Total Dissolved Solid, Total Suspended Solid

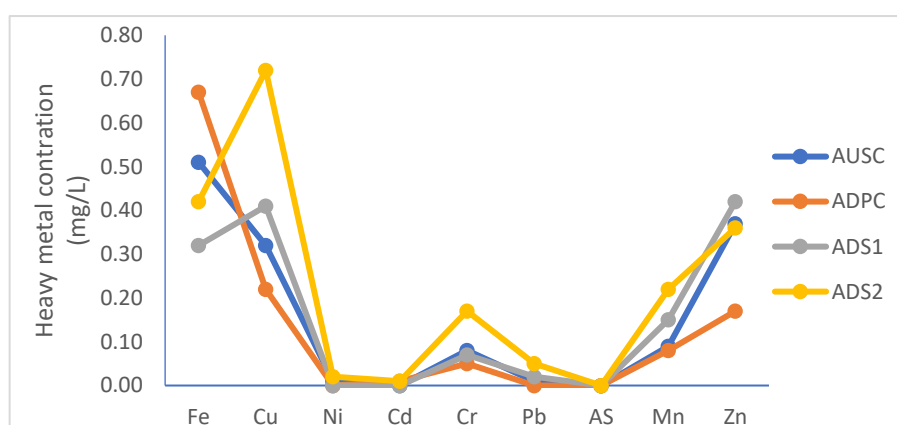
**Table 7. Physicochemical characteristics of iju stream (B) receiving abattoir effluent**

Parameters	Sampling stations				WHO/EPA Limit
	BUSC	BDPC	BDS1	BDS2	
Temp,	26.43±0.12 <sup>b</sup>	26.20±0.10 <sup>a</sup>	26.13±0.12 <sup>a</sup>	26.47±0.06 <sup>b</sup>	30
pH	8.65±0.02 <sup>d</sup>	6.74±0.01 <sup>a</sup>	6.97±0.01 <sup>b</sup>	7.35±0.02 <sup>c</sup>	6.5
Turbidity (NTU)	7.71±0.00 <sup>d</sup>	4.41±0.01 <sup>c</sup>	2.13±0.01 <sup>a</sup>	3.10±0.03 <sup>b</sup>	5
Salinity (mg/L)	1.24±0.01 <sup>d</sup>	0.32±0.01 <sup>c</sup>	0.08±0.00 <sup>b</sup>	0.03±0.00 <sup>a</sup>	120
Total Hardness (mg/L)	205.24±0.01 <sup>d</sup>	90.81±0.01 <sup>c</sup>	80.62±0.01 <sup>b</sup>	56.27±0.03 <sup>a</sup>	150
Alkalinity (mg/L)	171.78±0.03 <sup>d</sup>	30.43±0.01 <sup>b</sup>	34.11±0.18 <sup>c</sup>	20.93±0.03 <sup>a</sup>	200
Chloride (mg/L)	12.82±0.02 <sup>d</sup>	5.31±0.00 <sup>b</sup>	4.65±0.00 <sup>a</sup>	7.15±0.03 <sup>c</sup>	250
DO (mg/L)	4.13±0.06 <sup>a</sup>	5.82±0.02 <sup>d</sup>	5.40±0.02 <sup>c</sup>	5.14±0.02 <sup>b</sup>	6
BOD (mg/L)	8.34±0.02 <sup>d</sup>	7.20±0.02 <sup>a</sup>	7.51±0.00 <sup>b</sup>	7.69±0.00 <sup>c</sup>	5
COD (mg/L)	15.64±0.02 <sup>d</sup>	12.92±0.03 <sup>a</sup>	13.24±0.03 <sup>b</sup>	13.71±0.01 <sup>c</sup>	10
TDS (mg/L)	64.36±0.01 <sup>d</sup>	27.37±0.14 <sup>b</sup>	22.13±0.04 <sup>a</sup>	61.40±0.01 <sup>c</sup>	500



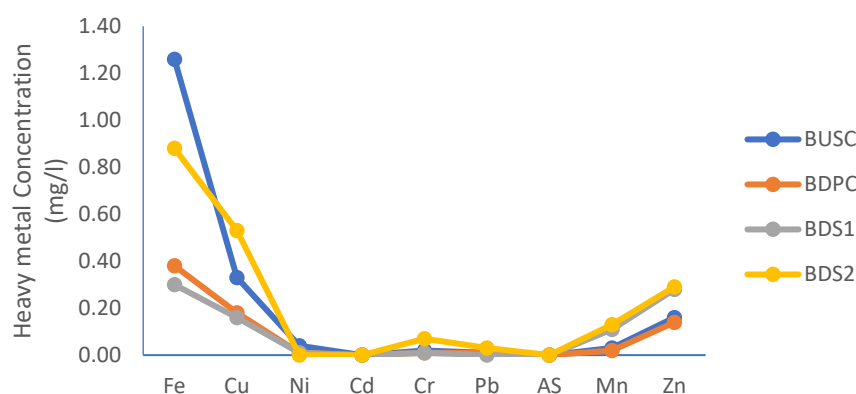
Parameters	Sampling stations				WHO/EPA Limit
	BUSC	BDPC	BDS1	BDS2	
TSS (mg/L)	5.33±0.01 <sup>d</sup>	1.16±0.01 <sup>a</sup>	1.85±0.00 <sup>b</sup>	4.26±0.02 <sup>c</sup>	25
TS (mg/L)	69.70±0.03 <sup>d</sup>	28.53±0.14 <sup>b</sup>	23.98±0.03 <sup>a</sup>	65.66±0.03 <sup>c</sup>	30
Conductivity μS/cm	115.67±0.25 <sup>d</sup>	48.13±0.21 <sup>b</sup>	45.37±0.06 <sup>a</sup>	113.73±0.03 <sup>c</sup>	1000
Sulphate (mg/L)	1.40±0.01 <sup>d</sup>	0.21±0.00 <sup>b</sup>	0.19±0.01 <sup>a</sup>	0.41±0.00 <sup>c</sup>	250
Nitrate (mg/L)	0.58±0.03 <sup>c</sup>	0.10±0.00 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	50
Phosphate (mg/L)	0.02±0.01 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.05
P(ppm)	2.36±0.00 <sup>d</sup>	2.14±0.02 <sup>c</sup>	1.85±0.01 <sup>b</sup>	1.21±0.00 <sup>a</sup>	12

Key: BUSC; Iju Upper Stream, BDPC; Iju Discharge Stream Catch, BDS1; Iju Down Stream 1, BDS2; Iju Down Stream 2, DO; Dissolved Oxygen, BOD; Biological Oxygen Demand, COD; Chemical Oxygen Demand, TDS; Total Dissolved Solid, Total Suspended Solid



**Fig. 2. Heavy metal concentrations in Yewa Stream**

Key: AUSC; Yewa Upper Stream, ADPC; Yewa Discharge Stream Catch, ADS1; Yewa Down Stream 1, ADS2; Yewa Down Stream 2



**Fig. 3. Heavy metal concentrations in Iju Stream**

Key: BUSC; Iju Upper Stream, BDPC; Iju Discharge Stream Catch, BDS1; Iju Down Stream 1, BDS2; Iju Down Stream 2

### 3.3 Heavy metal Concentration of Yewa Stream (A) and Iju Stream (B) Receiving Abattoir Effluent

The results of heavy metal concentrations in Stream A and Stream B are presented in Figs. 2

and 3, respectively. In Stream A, the mean concentrations of Cu, Ni, Cd, and Zn were within the WHO/EPA permissible limits. However, Fe ( $0.32 \pm 0.00$  to  $0.67 \pm 0.02$  mg/L), Cr ( $0.05 \pm 0.00$  to  $0.17 \pm 0.00$  mg/L), Pb ( $0.01 \pm 0.00$  to  $0.05 \pm 0.00$  mg/L), and Mn ( $0.08 \pm 0.00$  to  $0.22 \pm 0.01$  mg/L)

exceeded the recommended limits. The concentrations of these metals varied significantly across the sampling stations ( $p < 0.05$ ), as determined by Duncan's post hoc analysis (Fig. 2). Similarly, in Stream B, the mean concentrations of Cu, Ni, Cd, and Zn was within permissible limits across all sampling stations, except for Cr ( $0.07 \pm 0.00$  mg/L) and Pb ( $0.03 \pm 0.00$  mg/L) at the LSC station. Furthermore, Fe ( $0.30 \pm 0.00$  to  $1.26 \pm 0.00$  mg/L) and Mn ( $0.03 \pm 0.00$  to  $0.13 \pm 0.00$  mg/L) exceeded the permissible limits. Significant variations in metal concentrations were observed across the sampling stations ( $p < 0.05$ ), as indicated by Duncan's post hoc analysis (Fig. 3).

### 3.4 Water Quality Index

The overall Water Quality Index (WQI) values ranged from 50.23 to 69.20 in Stream A (Table 8) and from 44.24 to 77.79 in Stream B (Table 8). Based on these values, the overall quality of both streams suggests that the water is safe for drinking with respect to the physicochemical variables since the WQI values fall within the 50–100 range indicated as good water quality in Table 1.

### 3.5 Single-Factor and Comprehensive Pollution Index

The single-factor pollution index of heavy metals in the two streams is presented in Tables 9. In Stream A, the single-factor index values for Cu (0.11–0.36), Ni (0.01–0.06), and Zn (0.06–0.14) fell within the non-pollution category ( $P_i < 0.4$ ).

However, Fe (1.05–2.22) and Mn (1.06–2.74) indicated medium pollution ( $1 < P_i \leq 2$ ). Additionally, Cd (0.00–4.56), Pb (0.00–5.00), and Cr (0.90–3.47) showed heavy pollution levels ( $2 < P_i \leq 5$ ) at certain sampling stations (Table 9).

In Stream B, the single-factor index values for Cu (0.08–0.10), Ni (0.01–0.14), and Zn (0.05–0.10) were within the non-pollution range ( $P_i < 0.4$ ). However, Cd (0.00–0.44), Cr (0.19–1.39), Pb (0.00–2.70), and Mn (0.22–1.64) showed slight pollution levels ( $0.4 < P_i \leq 1.0$ ) at some stations (Table 9).

The Comprehensive Pollution Index (CPI) values for Stream A were: OAE (0.71), USC (1.11), DPC (0.83), and DSC (1.92). For Stream B, the CPI values were: IAE (0.82), USC (0.31), DPC (0.34), and LSC (1.13). These values indicate that both streams fall within the classification of slight pollution ( $0.41 < CPI \leq 1.0$ ) to moderate pollution ( $1.01 < CPI \leq 2.0$ ).

### 3.6 Risk Assessment of Heavy Metals through Dermal and Oral Routes

The hazard quotients (HQs) of heavy metals through dermal and oral (ingestion) routes were presented in Table 9 to Table 10. At both streams, the levels of HQs of heavy metal (Fe, Cu, Ni, Cd, Cr, Pb, As, Mn and Zn) through dermal and ingestion (oral) route were found to be less than one ( $HQs < 1$ ), which showed that the dermal absorption and the ingestion of metals may have little or no health threat.

**Table 8. Water Quality index of streams A and B receiving abattoir effluent**

Stream A		Stream B	
Stations	WQI	Stations	WQI
AUSC	62.11	BUSC	77.79
ADPC	69.2	BDPC	46.25
ADS1	50.26	BDS1	44.24
ADS2	50.23	BDS2	70.67

Key: AUSC; Yewa Upper Stream, ADSC; Yewa Discharge Stream Catch, ADS1; Yewa Down Stream 1, ADS2; Yewa Down Stream 2, BUSC; Iju Upper Stream, BDSC; Iju Discharge Stream Catch, BDS1; Iju Down Stream 1, BDS2; Iju Down Stream 2

**Table 9. Single-factor and comprehensive pollution index of streams A and B receiving abattoir effluent**

Parameters	Stream A				Stream B			
	Sampling stations				Sampling stations			
	AUSC	ADPC	ADS1	ADS2	BUSC	BDPC	BDS1	BDS2
Fe	1.71	2.22	1.05	1.4	4.21	1.28	1.01	2.95
Cu	0.16	0.11	0.2	0.36	0.16	0.09	0.08	0.26

Parameters	Stream A				Stream B			
	Sampling stations				Sampling stations			
	AUSC	ADPC	ADS1	ADS2	BUSC	BDPC	BDS1	BDS2
Ni	0.03	0.01	0.01	0.06	0.14	0.04	0.02	0.01
Cd	0	4.56	0	2.22	0.44	0	0	0
Cr	1.53	0.9	1.35	3.47	0.49	0.29	0.19	1.39
Pb	1.07	0	2.03	5	0.6	0.53	0	2.7
Mn	1.09	1.06	1.89	2.74	0.43	0.22	1.33	1.64
Zn	0.12	0.06	0.14	0.12	0.05	0.05	0.09	0.1
CPI	0.71	1.11	0.83	1.92	0.82	0.31	0.34	1.13

Key: AUSC; Yewa Upper Stream, ADSC; Yewa Discharge Stream Catch, ADS1; Yewa Down Stream 1, ADS2; Yewa Down Stream 2, BUSC; Iju Upper Stream, BDSC; Iju Discharge Stream Catch, BDS1; Iju Down Stream 1, BDS2; Iju Down Stream 2

**Table 10. Dermal health risk of heavy metal in stream A and stream B receiving abattoir effluent**

Stream	Stations	Fe	Cu	Ni	Cd
A	AUSC	1.97E-03	4.63E-03	5.75E-04	0.00E+00
	ADPC	2.56E-03	3.10E-03	1.38E-04	4.72E-04
	ADS1	1.21E-03	5.84E-03	2.76E-04	0.00E+00
	ADS2	1.61E-03	1.04E-02	1.27E-03	2.30E-04
	Total	1.84E-03	5.98E-03	5.64E-04	1.75E-04
B	BUSC	4.85E-03	4.74E-03	2.85E-03	4.60E-05
	BDPC	1.47E-03	2.56E-03	7.36E-04	0.00E+00
	BDS1	1.17E-03	2.36E-03	4.14E-04	0.00E+00
	BDS2	3.39E-03	7.56E-03	1.61E-04	0.00E+00
	Total	2.72E-03	4.31E-03	1.04E-03	1.15E-05
Stream	Station	Cr	Pb	Mn	Zn
A	AUSC	8.78E-03	4.38E-06	1.88E-05	3.17E-03
	ADPC	5.18E-03	0.00E+00	1.83E-05	1.45E-03
	ADS1	7.79E-03	8.36E-06	3.26E-05	3.59E-03
	ADS2	1.99E-02	2.05E-05	4.73E-05	3.13E-03
	Total	1.04E-02	8.32E-06	2.92E-05	2.84E-03
B	BUSC	2.80E-03	2.47E-06	2.93E-06	1.34E-03
	BDPC	1.69E-03	2.19E-06	3.81E-06	1.17E-03
	BDS1	1.11E-03	0.00E+00	9.18E-06	2.40E-03
	BDS2	7.98E-03	1.11E-05	2.83E-05	2.51E-03
	Total	3.39E-03	3.94E-06	6.24E-06	1.85E-03

Key: AUSC, Yewa Upper Stream Catch, ADPC; Yewa Discharge Point Catch, ADS1; Yewa Downstream 1, ADS2; Yewa Downstream 2, BUSC, Iju Upper Stream Catch, BDPC; Iju Discharge Point Catch, ADS1; Iju Downstream 1, ADS2; Iju Downstream 2

**Table 11. Oral Health Risk of Heavy Metal in Abattoir Effluent and A Streams receiving effluent**

Stream	Stations	Fe	Cu	Ni	Cd
A	AUSC	3.65E-04	8.57E-04	5.33E-05	0.00E+00
	ADSC	4.74E-04	5.74E-04	1.28E-05	8.74E-05
	ADS1	2.24E-04	1.08E-03	2.56E-05	0.00E+00
	ADS2	2.98E-04	1.92E-03	1.17E-04	4.26E-05
	Total	3.40E-04	1.11E-03	5.22E-05	3.25E-05
B	BUSC	5.77E-05	2.63E-04	6.61E-05	4.26E-05
	BDSC	1.75E-05	1.42E-04	1.70E-05	0.00E+00
	BDS1	1.39E-05	1.31E-04	9.59E-06	0.00E+00
	BDS2	4.04E-05	4.20E-04	3.73E-06	0.00E+00
	Total	3.24E-05	2.39E-04	2.41E-05	1.07E-05

Stream	Stations	Cr	Pb	Mn	Zn
A	AUSC	8.13E-04	8.12E-07	3.48E-06	9.78E-04
	ADSC	4.79E-04	0.00E+00	3.38E-06	4.48E-04
	ADS1	7.21E-04	1.55E-06	6.03E-06	1.11E-03
	ADS2	1.85E-03	3.81E-06	8.76E-06	9.67E-04
	Total	9.65E-04	1.54E-06	5.41E-06	8.75E-04
B	BUSC	5.19E-07	4.79E-05	7.76E-06	1.65E-05
	BDSC	3.13E-07	4.26E-05	4.03E-06	1.44E-05
	BDS1	2.06E-07	0.00E+00	2.43E-05	2.97E-05
	BDS2	1.48E-06	2.16E-04	3.00E-05	3.10E-05
	Total	6.29E-07	7.66E-05	1.65E-05	2.29E-05

Key: AUSC, Yewa Upper Stream Catch, ADPC; Yewa Discharge Point Catch, ADS1; Yewa Downstream 1, ADS2; Yewa Downstream 2, BUSC, Iju Upper Stream Catch, BDPC; Iju Discharge Point Catch, ADS1; Iju Downstream 1, ADS2; Iju Downstream 2

#### 4. DISCUSSION

The indiscriminate and frequent discharge of untreated effluent into surrounding streams and rivers may compromise the quality of receiving water bodies. Abattoirs are critical for providing well-prepared and quality meat to meet human protein requirements, but also contribute to environmental impairment through the large amounts of waste produced, and frequently disposed untreated into surrounding water and soils. The microbial load and physicochemical indicators are important considerations for water quality monitoring in order to guarantee environmental sustainability and human safety. In addition to the physical and chemical variables, the biological characteristics of water are also critical for consideration of its quality (Tyagi et al., 2013; Britto et al., 2018). The assayed surface water samples showed significant number and diverse genera of heterotrophic and coliform bacteria. The values were higher than the WHO prescribed limit of zero coliform for drinking water. The microbial species identified in this study were among those that have been identified in similar studies and implicated in various food and waterborne diseases causing gastroenteritis, typhoid fever among other diseases. The high bacterial load and presence of coliform bacteria in the assayed samples implies the unwholesomeness of the stream water for human consumption. Human activities in different forms including indiscriminate disposal of untreated waste, can result in surface water and groundwater contamination. Both streams at various points showed the presence of *E. coli* which suggests faecal contamination. The immediate traceable sources of *E. coli* in these streams are the abattoir effluents which usually contain faeces and *E. coli* bacterium as normal flora of lumen of cow and other herbivorous animals. However,

this assertion does not foreclose other potential sources of bacterial contamination of surface water due to runoff and other anthropogenic activities. The presence of coliform bacteria in these water samples establishes that a possibility of pathogenic organisms exists in the streams. However, Ikuesan and Ediagbonya (2024) stated the consumption of these natural resource with evidence of faecal contamination poses danger to human health. Ikuesan and Ediagbonya ((2024) reported that the consumption of contaminated or poor-quality water can elicit diseases which could lead to death among different age groups.

The physicochemical properties of the streams exhibited significant spatial variation across the sampling stations. Water temperature, which plays a crucial role in aquatic ecosystem health, was within the optimal mean range of  $25.27 \pm 0.12$  °C to  $26.17 \pm 0.15$  °C (stream A) and  $26.13 \pm 0.12$  °C to  $26.47 \pm 0.06$  °C (stream B). These temperature ranges support maximal growth rates, efficient food conversion, and increased resistance to toxins and diseases in aquatic organisms, corroborating previous studies by Loto and Ajibare (2021) and Loto et al. (2023). However, while most physicochemical parameters remained within WHO/FEPA (2003), permissible limits, high BOD, COD, and TS values suggested organic pollution in the environment.

The BOD levels across the stations exceeded the recommended 5 mg/L limit set by the International Environmental Protection Agency (IEPA, 2001) for unpolluted natural waters. According to Adakole et al. (1998), BOD concentrations between 2 and 9 mg/L indicate moderate pollution, while values above 10 mg/L signify heavy pollution. Similarly, the Department of Petroleum Resources (DPR, 2002) and WHO

(2005) established permissible limits of 10 mg/L and 5 mg/L, respectively. Based on these standards, the study area exhibited moderate organic pollution, likely due to abattoir activities and other anthropogenic influences along the streams.

The pH of the streams ranged from 6.50 to 8.65, slightly exceeding WHO-recommended values but aligning with APHA (2017), assumptions that natural waters typically range from 4 to 9. The slight basicity of the water may be attributed to the presence of bicarbonates and carbonates of alkali and alkaline earth metals.

Dissolved oxygen (DO), a key indicator of water quality, reported as 4.92 mg/L, is slightly below the recommended threshold of 5.00 mg/L by the WHO. A further decline in the DO could negatively affect aquatic organisms, as oxygen is essential for their survival (Iqbal et al., 2019). Additionally, water with low DO levels tends to emit an unpleasant odour, whereas higher DO levels enhance drinking water quality.

The concentrations of nitrates, phosphates, and sulfates were relatively low, likely due to the minimal agricultural activity in the study area. This finding aligns with the results of Iwegbue et al. (2023), who reported similar observations in a comparable aquatic ecosystem. Heavy metal analysis showed that Ni, Zn, Cu, and Cd were within permissible limits, whereas Fe, Cr, Pb, and Mn slightly exceeded safe levels. Elevated concentrations of these metals can pose long-term environmental and health risks if not properly managed.

Water Quality Index (WQI) values ranged from 50.23 to 69.20 in Stream A and 44.24 to 77.79 in Stream B, suggesting that the water is generally safe for drinking concerning the physicochemical parameters examined. These results align with findings by Khatita et al. (2017) and Anweting et al. (2024). However, these findings are not in agreement with Iwegbue et al. (2023), who reported that the water in Bomadi Creek, Niger Delta, Nigeria, was unsuitable for drinking (WQI > 300), a conclusion further supported by the water quality classifications provided by Shetaia et al. (2020).

The single-factor pollution index (Pi) indicated varying levels of pollution across the streams, ranging from non-pollution to heavy pollution. Specifically, Fe and Mn exhibited heavy pollution levels in Stream A, consistent with findings by Olawusi-Peters et al. (2021) and Loto et al.

(2023). The comprehensive pollution index (CPI), which evaluates overall water quality, indicated slight to moderate pollution, with values ranging from 0.41 to 2.0. These findings align with Yan et al. (2015), who assessed water quality in the Honghe River watershed, China, and Loto and Ajibare (2021), who examined Lagos Lagoon pollution levels.

A human health risk assessment of heavy metal exposure through dermal contact and ingestion revealed no significant hazard, as all hazard quotient (HQ) values were below 1. This suggests minimal immediate health risks, corroborating the findings by Loto et al. (2023). However, continued monitoring is essential to prevent long-term environmental and health consequences.

## 5. CONCLUSION

The biological indicators (heterotrophic and coliform count) of the assayed streams receiving abattoir effluent suggested that all the streams were contaminated by faeces with the abattoir effluent being the main source of contamination. The bacteriological variables of these samples did not conform with international standards and therefore considered unfit for human consumption in order to reduce the burden of waterborne diseases and improve human health.

The study revealed spatial variations in physicochemical parameters and pollution indices in the two streams. While most parameters were within acceptable limits, elevated BOD, COD, and TS levels indicated organic pollution. DO levels were slightly below the recommended threshold, and certain heavy metals, such as Fe, Cr, Pb, and Mn, exceeded permissible limits. Despite this, the WQI suggested that the water remained suitable for drinking. The pollution indices (Pi and CPI) highlighted varying degrees of pollution, largely influenced by anthropogenic activities. Human health risk assessments showed no immediate concerns, but proactive management is essential to prevent future environmental and health risks. The study concludes that surface water receiving effluent can elicit substantial environmental and life-threatening public health concerns.

## 6. RECOMMENDATION

Strengthened pollution control measures and periodic water quality assessments are recommended to safeguard water resources for beneficial users. In the foregoing, regulatory agencies concerned with the establishment of

slaughter facilities should ensure that such facilities are well equipped to appropriately manage and treat wastewater generated from abattoirs before discharge into the surrounding water bodies and soil. Also, environmental awareness campaign should be organized to educate communities and residents of the potential health risk associated with surface water as drinking water resources.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## ACKNOWLEDGEMENT

Authors acknowledge the management of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria for providing the conducive environment for this research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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