Original Research Article

Compliance Monitoring of Microbiological and Physicochemical Parameters of Abattoirs' Effluents Discharged into Water Bodies in Owerri, Nigeria

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ABSTRACT

Aims: To assess the quality of abattoir effluents discharged into water bodies in Owerri Municipal, Nigeria using microbiological and physicochemical approaches.
Study design: The study employed microbiological and physicochemical parameters that determine effluent and water quality.
Place and Duration of Study: Sample: Abattoirs in Owerri, Imo State, Nigeria, between September 2014 and February 2016.
Methodology: Physicochemical and microbiological analyses were carried out on three abattoir effluents and their receiving water bodies. Counts of total heterotrophic bacteria, total coliform and faecal coliform, Vibrio, Salmonella and Shigella were carried using the plate count method.
Results: The bacterial isolates in the various samples included members of the genera *Bacillus, Citrobacter, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Listeria, Micrococcus, Proteus, Salmonella, Serratia, Staphylococcus, Streptococcus* and *Vibrio*. The order of increasing effluent's total coliform and faecal counts within the different abattoirs are given as Egbu abattoir > Amakohia Ikeduru abattoir > Ahiara abattoir. For the receiving streams, the order was as follows, for the total coliform count: Egbu abattoir > Ahiara abattoir > Amakohia Ikeduru abattoir while for faecal coliform: Egbu abattoir > Amakohia Ikeduru abattoir > Ahiara abattoir. About 85.7 % and 42.9% of the total bacterial isolates were found in the Egbu abattoir effluent and receiving the stream, respectively. Ahiara abattoir's effluent had 66.7 % of the bacteria while its receiving stream had 23.8 %. Over 57 % of the total bacterial isolates were distributed in the Amakohia Ikeduru abattoir with 33.3 % for its receiving stream.
Conclusion: This study revealed that pathogenic bacteria from abattoir were constantly discharged into receiving streams, thereby presenting serious health risks. The health status of residents of Owerri who have access to these water bodies should be studied to determine the health implications of unregulated practices.

Keywords: Abattoir, wastewater, faecal coliform; bacterial diversity; physicochemical quality.

1. INTRODUCTION

The abattoir industry in Nigeria is an important component of the livestock industry, providing domestic meat supply and employment opportunities to the country's teeming population [1]. Abattoir industries generate a lot of wastewater and these eventually find their way into the environment. In many states in Nigeria, facilities for the treatment of abattoir effluents are lacking [2]. A limited or complete absence of treatment facilities for abattoir effluents results

in the released of a substantial amount of abattoir effluents into receiving streams, lakes, and rivers.

Wastewaters from abattoirs comprise particularly concentrated source of oxygen-consuming waste [3] and this directly or indirectly impact on water quality. The major activities involved in the operations of an abattoir include receiving and holding of livestock, slaughtering and dressing of animals carcass, chilling of carcass products, carcass boning and packaging, freezing of finished carcass and cartooned product, rendering processes, drying of skins, treatment of wastes, and transport of processed material. These processing activities in Nigeria are mostly carried out in unsuitable buildings and by untrained personnel or butchers who are mostly unaware of sanitary principles [4].

Sherer *et al*. [5] reported that bacteria from abattoir waste discharged into water columns can subsequently be absorbed to sediments, and when the bottom stream is disturbed, may be released back into the water columns, presenting long-term health hazards. Abattoir effluents contain wastewater from the processing of animals; moreover, animal wastes are known repository of benign and pathogenic microorganisms. Consequently, microbial ecology of abattoir effluents comprise microorganisms belonging to various genera of which most may be pathogenic. Pathogens present in animal wastes may include rotaviruses, hepatitis E virus, *Salmonella* spp., *Escherichia coli* O157: H7, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium parvum*, *Giardia lamblia*, and *Listeria monocytogenes* [6-7].

In Nigeria, abattoir operations are poorly regulated. More so, abattoirs are usually sited close to water bodies to facilitate unrestricted access to water for processing [8]. This action inherently predisposes the water bodies to pollution from abattoir activities. Abattoir effluent may contain high levels of organic matter arising from manure, blood, fats, grease, hair, grit, and undigested feeds. It may also contain high levels of salts, phosphates, and nitrates. The blood and fat contents contribute mostly to organic load. Abattoir activities are aimed at optimizing the recovery of edible portions of the meat processing cycle for human consumption [9]. However, significant quantities of secondary waste materials are also generated during this process with varying degree of health implications. This study was aimed at evaluating the bacteriological and physicochemical quality of abattoir receiving water bodies in Imo State.

2. MATERIAL AND METHODS

2.1 Study Area and Sample Collection

The study was carried out on abattoirs and streams located in three local government areas (Owerri, Ahiazu Mbaise, and Ikeduru) in Imo State, Nigeria. Imo state, which is located in the Niger Delta region is one of the 36 states of Nigeria and lies in the South East of Nigeria with Owerri as its capital. It occupies between the lower River Niger and the upper and middle Imo River. The abattoir effluents were collected from Egbu abattoir (Latitude: 5^{o}28'47.7466'' N and Longitude: 7^{o}3'59.3384'' E), Oru Ahiara abattoir (Latitude: 5^{o}31'51.4812'' N and Longitude: 7^{o}17'10.2012'' E), and Amakohia Ikeduru abattoir (Latitude: 5^{o}33'16.8228'' N and Longitude: 7^{o}9'23.6268'' E) while their respective receiving stream samples were collected from Egbu River (Latitude: 5^{o}29'14.4708'' N and Longitude: 7^{o}3'58.5684'' E), Onukwu River (Latitude: 5^{o}28'27.2639'' N and Longitude: 7^{o}7'23.9365'' E) and Ikembara River (Latitude: 5^{o}34'9.7212'' N and Longitude: 7^{o}9'26.0964'' E). The coordinates were obtained using a GPS device.

From each of the abattoirs, samples were collected from five points and bulked together to make a composite sample. Samples were collected in the morning during the peak activities between 08.00 am and 09.00 am using the grab sampling method with a wide-mouthed 500 mL sterilized Pyrex glass bottles equipped with tight screw dustproof stoppers. Wastewater (effluent) samples from the abattoirs were collected from points close to the discharging points (outlet) 10 cm below the surface. Samples from receiving water bodies were collected 50 m upstream and downstream. The distance was determined using a measuring tape. The bottles were filled up to a level; with a headspace of about 2.5 cm. Samples were placed in a cooler containing ice pack and transported to the laboratory for analyses.

2.2 Physicochemical Analysis of Samples

Effluent and receiving stream samples were subjected to various laboratory analyses to determine their physicochemical characteristics. The parameters tested and the methods employed are given in Table 1.

Table 1. Physicochemical parameters analysed and the method used

Parameters	Method
pH	Hanna pocket-size pH meter
Conductivity (µS/cm)	Spectrophotometric method
Salinity (mg/L)	A refractometer (Atago Model S/Mill-E)
Nitrate (mg/L)	Buccine colorimetric method (Narayana and Sunil, 2009).
Total Nitrogen (mg/L)	Kjeldahl method
Turbidity (NTU)	Colorimetric method
Phosphate (mg/L)	Spectrophotometric method
Total Phosphorus (mg/L)	Vanado-molybdophosphoric acid colorimetric method (APHA 4500-P-B)
Total Organic Carbon (mg/L)	Rapid oxidation method (using $K_2Cr_2O_7$ and H_2SO_4)
Total Suspended Solids (mg/L)	Filter paper method (Anon, 1992)
Total Dissolved Solids (mg/L)	Instrumental method (ASTMD 1888) using HACH TDS meter
Chemical Oxygen Demand (mg/L)	Titration method (using $KMnO_4$ and dilute H_2SO_4)
Biological Oxygen Demand (mg/L)	Standard method (APPHA, 2005)
Dissolved Oxygen (mg/L)	Standard method (APPHA, 2005)
Oil and Grease (mg/L)	Liquid-liquid extraction with hexane (EPA 1664)
Sulphate (mg/L)	Spectrophotometric method (using barium chloride crystals)
Zinc (mg/L)	Copper II sulphate method (Martin *et al*.,1994)
Copper (mg/L)	Copper II sulphate method (Martin *et al*.,1994)
Iron (mg/L)	Copper II sulphate method (Martin *et al*.,1994)
Lead (mg/L)	Copper II sulphate method (Martin *et al*.,1994)
Nickel (mg/L)	Copper II sulphate method (Martin *et al*.,1994)
Temperature (^oC)	Mercury-in-glass thermometer (APHA 2550B)

2.3 Microbiological Analyses

2.3.1 Isolation and enumeration of heterotrophic bacteria, *Vibrio* spp, and *Salmonella* and *Shigella* spp

Ten-fold serial dilutions were first performed on the effluent and receiving stream samples using the method described by Jalal *et al*. [10].

To isolate and enumerate total heterotrophic bacteria (THB), one hundred microlitres (100 µl) of each of 10^{-3}, 10^{-4} and 10^{-5} dilutions were spread onto Plate Count Agar (PCA) (Himedia, India) and Nutrient agar (NA) (Merck, Germany). Salmonella-Shigella Agar (SSA)

(Himedia, India) was employed in the isolation of *Salmonella* and *Shigella* spp whereas Thiosulphate citrate bile salts sucrose agar (TCBS) was used for the selective isolation of *Vibrio cholerae* and other enteropathogenic vibrios.

All media were prepared according to the manufacturer's instruction. PCA and NA were sterilized in an autoclave at 121 ^{o}C (0.15 mPa) for 15 min whereas SSA and TCBS were not sterilized in an autoclave. The plates were incubated at 30 ^{o}C for 24 h and plates with discrete colonies ranging between 30 and 300 were selected for count utilizing the standard aerobic plate count method described by APHA [11]. Moreover, distinct colonies from selected plates were selected and sub-cultured on freshly prepared nutrient agar plates for further identification studies.

The total viable count (TVC), expressed in colony-forming units per mL (cfu/mL) was calculated using the formula below:

$$\text{TVC (cfu/mL)} = \frac{(\text{no of colonies})\ (\text{dilution factor})}{\text{volume of inoculum}} \quad \text{Eq. 1}$$

2.4 Total and Faecal Coliform Analysis

To estimate total coliform, most probable number (MPN) method using three tubes, each inoculated with 10, 1.0, and 0.1 mL of samples, was used. The test procedure involved three stages: presumptive, confirmed and completed tests. The tests were carried out according to the method described by Sutton [12]. In brief, for the presumptive test, three sets of test tubes (with a set made up of three test tubes each, resulting in a total of 9 test tubes per sample) were prepared. To the first set, 10 mL double-strength lactose broth was added to each of the three test tubes. Similarly, 5 mL of single strength lactose broth was added to each of the three tubes in the second set and finally, 5 mL single strength lactose broth was added to each of the tubes in the third set of test tubes. Thereafter, inverted Durham's tubes were placed inside all the tubes before sterilization in an autoclave at 121 ^{o}C (0.15 mPa) for 15 min. After sterilization, the tubes were allowed to cool to 40 ^{o}C before they were inoculated with the samples as follows: 10 ml of the sample was added to the test tubes containing 10 mL double-strength broth. A volume of 1.0 ml of the sample was added into each of the test tubes containing 5 mL single strength broth while 0.1 mL of the sample was added into each of the third set of test tubes containing 5 mL single strength broth. The tubes were incubated at 37 ^{o}C (for the total coliform count) or 44.5 (for the faecal coliform count) for 24 to 48 h and thereafter examined for acid (indicated by the colour change of the broth) and gas production.

The confirmed test was carried out on all positive fermentation tubes using MacConkey broth (HiMedia, India) prepared according to the manufacturer's instruction. A loopful from a positive tube was transferred into sterile MacConkey broth (containing inverted Durham's tube) and incubated at 37 ^{o}C for 48 h. A tube with evidence of gas production was considered positive.

Completed test - This stage was applied to MacConkey broth showing gas in the confirmed test. The streak plate was employed on Eosin Methylene Blue agar medium. The plates were streaked from inoculums obtained from the MacConkey broth showing gas production. The plates were incubated at 37 ^{o}C for 24 h and thereafter observed for the growth of *E. coli*.

2.5 Identification of the Bacterial Isolates

Bacterial isolates were identified using their morphological and biochemical characteristics with the guidance from Bergey's Manual of Determinative Bacteriology [13].

2.6 Statistical Analysis

The data obtained from the study were subjected to both correlational analysis and analysis of variance to determine any significant difference between experimental means at P=0.05 using SPPS version 21 (Gailly and Adler, US).

3. RESULTS

3.1 Physicochemical Analysis of Abattoir Wastewater Effluent and their Receiving Water Bodies

Physicochemical analysis of the abattoirs' wastewater effluents and that of their receiving streams are given in Tables 2 to 4. The total suspended solids (TSS) for the Egbu, Ahiara Mbaise, and Amakohia Ikeduru abattoirs effluents were 261.4, 219.4, and 185.3 mg/L whereas their respective receiving streams had TSS contents of 15.6, 13.04 and 9.42 mg/L. The respective biological oxygen demand (BOD) values for the abattoir effluents and their receiving streams were 12.3, 9.6, and 10.1 mg/L and 5.08, 3.58 and 2.6 mg/L. The heavy metals in all the samples analysed were observed to range between <0.01 and 0.33 mg/L. For the effluents, the total dissolved solids (TDS) values for Egbu, Ahiara Mbaise and Amakohia Ikeduru abattoirs were found to be 572.5, 581 and 488.1 while their respective receiving streams had TDS values of 101.3, 105 and 91.9 mg/L.

Table 2. The mean value of the physicochemical analysis of wastewater Egbu abattoir

Table 3. The mean value of the physicochemical analysis of wastewater Ahiara Mbaise abattoir

Parameters	Effluent	Receiving Water (Composite)	*FMEnv maximum permissible limit
pH	9.2	5.1	6.0-9.0
Conductivity (µS/cm)	2165	83.5	1500
Salinity (mg/L)	1520	71.9	NA
Nitrate (mg/L)	40.2	0.3	20
Total Nitrogen (mg/L)	0.27	0.42	100
Turbidity (NTU)	227.1	13.4	50
Phosphate (mg/L)	5.03	1.29	3.5
Total Phosphorus (mg/L)	0.58	0.13	0.4
Total Organic Carbon (mg/L)	3.37	4.77	NA
Total Suspended Solids (mg/L)	261.4	15.6	30
Total Dissolved Solids (mg/L)	572.5	101.3	2000
Chemical Oxygen Demand (mg/L)	662	121	120
Biological Oxygen Demand (mg/L)	12.3	5.08	30
Dissolved Oxygen (mg/L)	0.21	4.76	≥ 2.0
Oil and Grease (mg/L)	32.5	5.13	10
Sulphate (mg/L)	35.0	1.31	500
Zinc (mg/L)	<0.01	<0.01	5.0
Copper (mg/L)	0.06	0.07	5.0
Iron (mg/L)	0.18	0.26	3.0
Lead (mg/L)	0.07	0.03	0.1
Nickel (mg/L)	0.05	0.03	0.5
Temperature (^{o}C)	32	29	40 ^{o}C

Parameters	Effluent	Receiving Water (Composite)	*FMEnv Maximum permissible limit
pH	9.6	4.8	6.0-9.0
Conductivity (µS/cm)	1961	72.5	1500
Salinity (mg/L)	1603	65.3	NA
Nitrate (mg/L)	29.4	0.14	20
Total Nitrogen (mg/L)	0.41	0.66	100
Turbidity (NTU)	215.6	12.8	50

Phosphate (mg/L)	3.1	0.84	3.5
Total Phosphorus (mg/L)	0.73	0.17	0.4
Total Organic Carbon (mg/L)	2.28	4.01	NA
Total Suspended Solids (mg/L)	219.4	13.04	30
Total Dissolved Solids (mg/L)	581	105	2000
Chemical Oxygen Demand (mg/L)	511	113	120
Biological Oxygen Demand (mg/L)	9.6	3.58	30
Dissolved Oxygen (mg/L)	0.12	4.22	≥ 2.0
Oil and Grease (mg/L)	28.6	3.59	10
Sulphate (mg/L)	0.04	0.05	500
Zinc (mg/L)	<0.01	<0.01	5.0
Copper (mg/L)	0.07	0.04	5.0
Iron (mg/L)	0.25	0.33	3.0
Lead (mg/L)	0.04	0.03	0.1
Nickel (mg/L)	0.05	0.02	0.5
Temperature (^{o}C)	30	28	40 ^{o}C

Table 4: The mean value of the physicochemical analysis of wastewater Amakaohia Ikeduru abattoir

Parameters	Effluent	Receiving Water (Composite)	*FMEnv Maximum permissible limit
pH	8.8	4.7	6.0-9.0
Conductivity (µS/cm)	1659	63.5	1500
Salinity (mg/L)	1427	61.1	NA
Nitrate (mg/L)	23.6	0.16	20
Total Nitrogen (mg/L)	0.33	0.39	100
Turbidity (NTU)	200.1	10.4	50
Phosphate (mg/L)	2.7	1.01	3.5
Total Phosphorus (mg/L)	0.27	0.09	0.4
Total Organic Carbon (mg/L)	4.03	3.83	NA
Total Suspended Solids (mg/L)	185.3	9.42	30
Total Dissolved Solids (mg/L)	488.1	91.9	2000
Chemical Oxygen Demand (mg/L)	428	108	120
Biological Oxygen Demand (mg/L)	10.1	2.6	30
Dissolved Oxygen (mg/L)	0.38	6.4	≥ 2.0
Oil and Grease (mg/L)	22.7	2.89	10
Sulphate (mg/L)	0.10	0.09	500
Zinc (mg/L)	<0.01	<0.01	5.0
Copper (mg/L)	0.02	0.02	5.0
Iron (mg/L)	0.09	0.17	3.0
Lead (mg/L)	<0.01	<0.01	0.1
Nickel (mg/L)	0.02	0.03	0.5
Temperature (^{o}C)	31	29	40 ^{o}C

3.2 Total Aerobic Heterotrophic Bacterial Count in the Various Samples

Figure 1 shows the mean total culturable heterotrophic bacterial (TCHB) count of the effluent and receiving the stream of the different abattoirs examined. From the table, the mean total

bacterial counts of the effluents for Egbu, Ahiara and Amakohia Ikeduru abattoirs were $7.99\pm0.23x10^6$, $2.81\pm0.39x10^6$, and $9.66\pm0.41x10^5$ cfu/mL, respectively. Meanwhile, their respective receiving streams had downstream, $2.3\pm0.09x10^5$, $2.19\pm0.14x10^2$ and $1.17\pm0.21x10^3$ cfu/mL and upstream, $1.05\pm0.39x10^4$, $6.5\pm0.37x10^2$ and $2.0\pm0.13x10^2$ cfu/mL. The order of increasing effluent's bacterial counts within the different abattoirs can be presented thus: Egbu abattoir was greater than Ahiara abattoir, which was in turn greater than Amakohia Ikeduru abattoir. However, the downstream and upstream of the respective receiving streams followed a slightly different order. The Egbu abattoir was greater than Amakohia Ikeduru abattoir, which in turn was greater than Ahiara abattoir. From the table, the downstream and upstream bacterial count of Egbu abattoir represented 2.9 and 0.13 % of its effluent count. The downstream and upstream bacterial count of Ahiara abattoir represented 0.078 and 0.023 % of its effluent count; whereas, the downstream and upstream bacterial count of Amakohia Ikeduru abattoir represented 0.12 and 0.021 % of its effluent count.

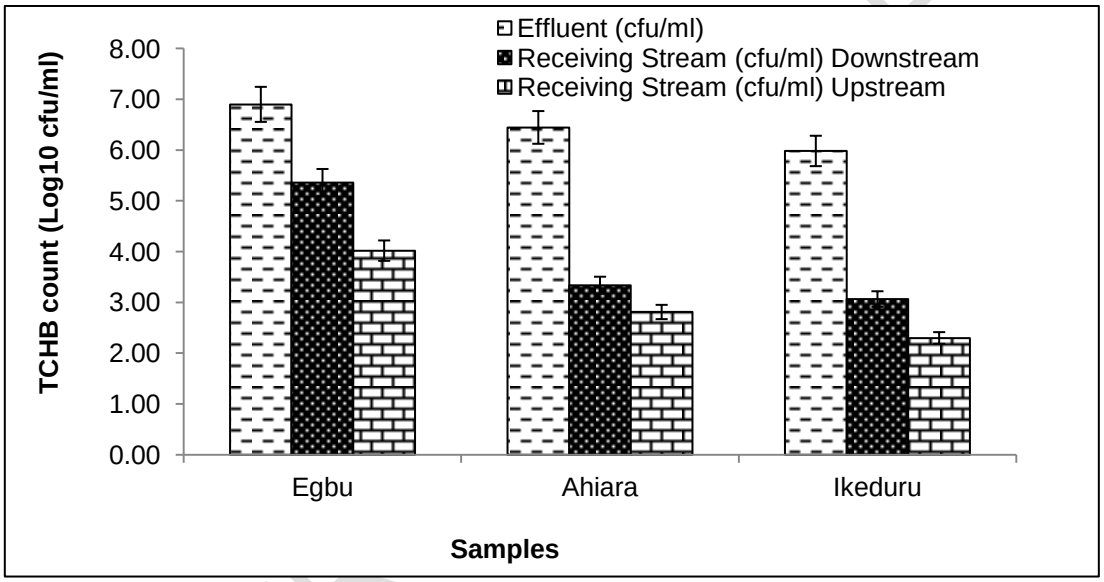

Figure 1. Count of total culturable heterotrophic bacterial (TCHB) for abattoir effluents and their receiving streams.

3.3 Salmonella and Shigella Count in the Various Samples

Salmonella and *Shigella* counts of the effluents from Egbu, Ahiara and Amakohia Ikeduru abattoirs are given in Figure 2. Effluents from Egbu, Ahiara and Amakohia Ikeduru abattoirs had *Salmonella* and *Shigella* counts of $2.85\pm0.17x10^4$, $3.1\pm0.05x10^3$, and $3.5\pm0.23x10^3$ cfu/mL while their receiving streams had down and upstream counts of 3.0±0.18x10, 5.0±0.8x10, and $1.01\pm0.15x10^2$ cfu/mL and 2.30±0.61x10, 2.1±0.32x10, and 7.2±0.11x10 cfu/mL. The order of increasing effluent's *Salmonella* and *Shigella* counts within the different abattoirs can be presented thus: Egbu abattoir was greater than Amakohia Ikeduru abattoir, which was in turn greater than Ahiara abattoir. The downstream and upstream of the respective receiving streams followed an entirely different order. Amakohia Ikeduru abattoir was greater than Ahiara abattoir, which in turn was greater than Egbu abattoir. From the table, the downstream and upstream *Salmonella* and *Shigella* count of Egbu abattoir represented 0.11 and 0.081 % of its effluent count. The downstream and upstream *Salmonella* and *Shigella* count of Ahiara abattoir represented 1.61 and 0.68 % of its effluent

count. While the downstream and upstream *Salmonella* and *Shigella* count of Amakohia Ikeduru abattoir represented 2.89 and 0.21 % of its effluent count.

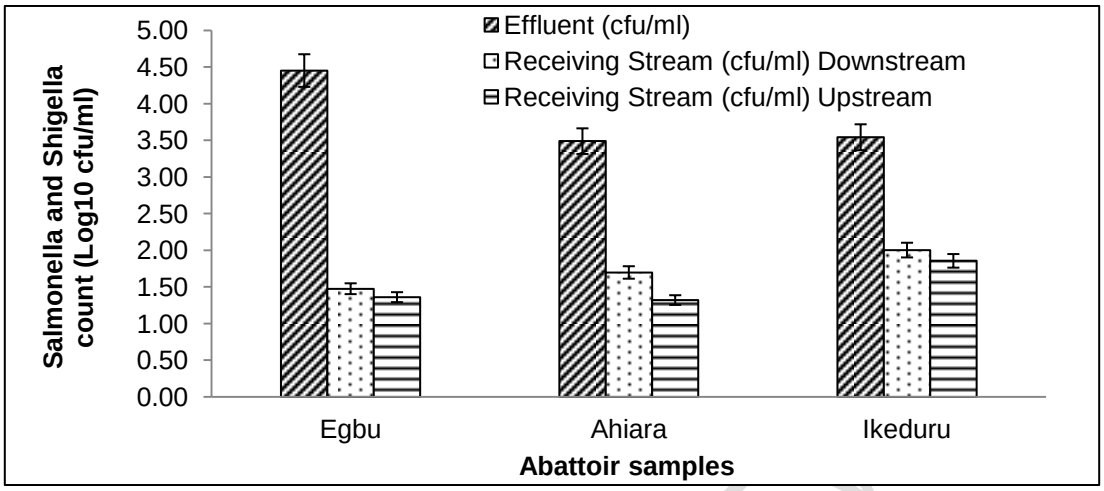

Figure 2. Count of Salmonella-Shigella for abattoir effluents and their receiving streams.

3.4 Mean Vibrios Count of Effluents and their Receiving Streams

Figure 3 shows the mean *Vibrio* counts of the effluents from Egbu, Ahiara, and Amakohia Ikeduru abattoirs. The respective mean *Vibrio* counts of the effluents from Egbu, Ahiara, and Amakohia Ikeduru abattoirs were $7.0\pm0.37x10^4$, $1.7\pm0.07x10^2$, and $1.08\pm0.23x10^2$ cfu/ml while the downstream and upstream of their receiving streams showed no counts. From the table, the *Vibrio* counts of the effluents from Egbu abattoir was higher than that of Ahiara abattoir, which in turn was higher than Amakohia Ikeduru abattoir. From the results, the effluent with the highest *Vibrio* count is effluents emanating from Egbu abattoir.

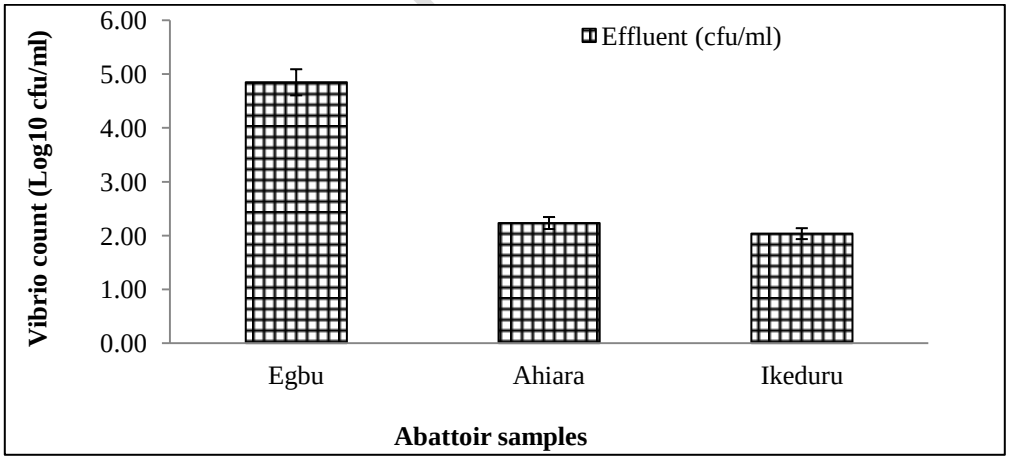

Figure 3. Vibrio counts of the various abattoir effluents and receiving streams

3.5 Total and Faecal Coliform Count Of Effluents and Their Receiving Streams

Figure 4 shows the mean total coliform and faecal coliform count of the effluent and the receiving stream of the different abattoirs examined. From the table, the mean total coliform

counts of the effluents from Egbu, Ahiara and Amakohia Ikeduru abattoirs were $2.16\pm0.15x10^6$, $1.78\pm0.29x10^5$, and $2.00\pm0.31x10^5$ MPN/100 ml, respectively while the respective faecal coliform counts were $1.76\pm0.51x10^4$, $1.16\pm0.28x10^3$, and $2.39\pm0.66x10^3$ MPN/100 ml. Meanwhile, their respective receiving streams had total coliform counts for downstream of, $1.24\pm0.04x10^2$, $1.04\pm0.01x10^2$, and $1.01\pm0.03x10^2$ MPN/100 ml and upstream of, 1.38±0.11x10, 1.21±0.20x10 and 1.04±0.31x10 MPN/100 ml. The respective receiving streams had faecal coliform counts for downstream of, $2.15\pm0.14x10^2$, $1.09\pm0.11x10^2$, and 1.75±0.14x10 MPN/100 ml and upstream of 2.41±0.22x10, 1.01±0.05x10 and 1.35±0.37x10 MPN/100 ml.

The order of increasing effluent's total coliform and faecal counts within the different abattoirs can be presented thus: Egbu abattoir > Amakohia Ikeduru abattoir > Ahiara abattoir. For the receiving streams, the order was as follows, for the total coliform count, Egbu abattoir > Ahiara abattoir > Amakohia Ikeduru abattoir while for faecal coliform, Egbu abattoir > Amakohia Ikeduru abattoir > Ahiara abattoir.

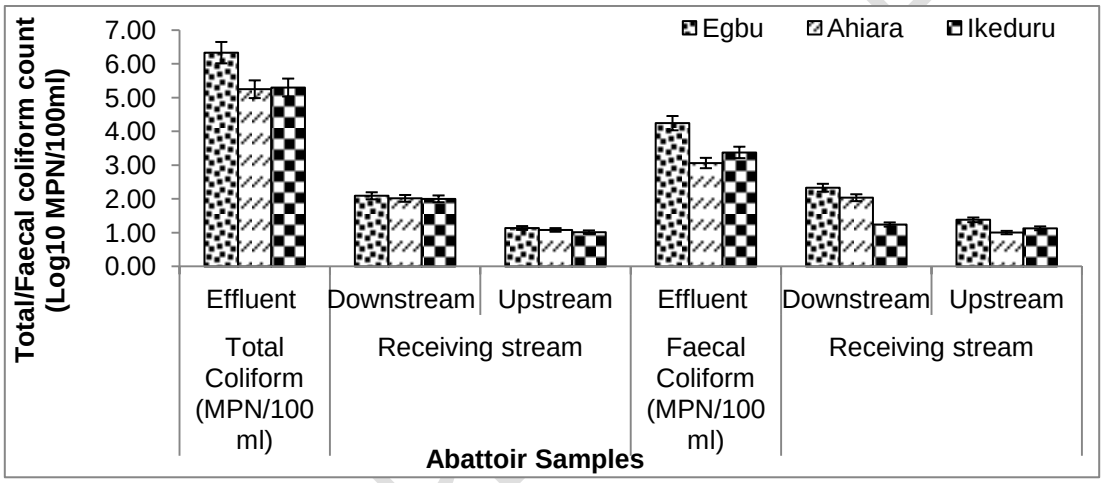

Figure 4. Total coliform and faecal coliform counts of the various abattoir effluents and receiving streams.

3.6 Identification of bacterial isolates

Table 5 shows the biochemical characteristics of the bacterial isolates from the different abattoir effluents and their receiving streams.

Table 5. Biochemical characteristics of the bacterial isolates from the different abattoir effluents and their receiving streams

Isolate code	Gram stain	Citrate	Motility	Oxidase	Catalase	Indole	Urease	Methyl Red	VP	TSI			Starch	Gelatin	Sugar fermentation					Probable bacterial
										Slant	Butt	H_2S			Glucose	Maltose	Sucrose	Lactose	Mannitol	
ECO-1	+ (rod)	+	+	-	+	-	-	-	+	K	A	-	+	+	+/A	+/A	+/A	-	-	*Bacillus cereus*
ECO-2	+ (rod)	+	+	+	+	-	-	-	-	K	A	-	+	-	+/A	-	-	+/A	+/A	*Bacillus megaterium*
ECO-3	+ (rod)	+	+	+	+	-	-	-	-	K	A	-	+	-	+/A	-	+/A	+/A	-	*Bacillus* sp.
ECO-4	+ (rod)	-	+	-	+	-	+	-	+	K	A	-	+	+	+/A	+/A	+/A	-	+/A	*Bacillus subtilis*
ECO-5	- (rod)	+	+	-		-	+	+	-	A	A	-	+	-	+/A	+/A	-	+/A	+/A	*Citrobacter* sp.
ECO-6	- (rod)	+	+	-	+	-	-	+	+	K	A	-	-	+	+/A	+/A	+/A	-	+/A	*Enterobacter aerogenes*
ECO-7	- (rod)	+	+	-	+	-	-		+	K	A	-	-	+	+/A	+/A	+/A	-	+/A	*Enterobacter faecalis*
ECO-8	- (rod)	-	+	+	+	+	-	+	-	A	A	-	+	-	+/A	+/A	+/A	+/A	+/A	*Escherichia coli*
ECO-9	- (rod)	+	-	-	+	-	+	-	+	A	A	-	+	-	+/A	+/A	+/A	+/A	+/A	*Klebsiella pneumoniae*

ECO 10	+ (rod)	-	+	-	-	-	+	+	+	A	K	-	-	-	+/A	+/A	+/A	+/A	+/A	*Lactobacillus plantarum*
ECO-11	+ (rod)	+	+	-	+	-	-	+	+	K	A	-	-	-	+/A	-	+/A	+/A	-	*Listeria* sp.
ECO-12	+ (cocci)	+	-	+	+	-	-	+	-	K	A	-	-	-	-	-	-	-	-	*Micrococcus* sp.
ECO-13	- (rod)	+	+	-	+	-	+	+	+	K	A	+	-	+	+/A	-	-	-	-	*Proteus mirabilis*
ECO-14	- (rod)	+	+	-	+	-	-	+	-	K	A	+		+	+/A	+/A	-	-	+/A	*Salmonella* sp.
ECO-15	- (rod)	+	+	-	+	-	-		+	A	A	-	-		+/A	+/A	+/A	-	+/A	*Serratia* sp.
ECO-16	+ (cocci)	+	-	-	+	-	+	+	+	A	A	-	+	+	+/A	+/A	+/A	+/A	+/A	**Staphylococcus aureus*
ECO-17	+ (cocci)	+	-	-	+	-	+	+	+	A	A	-	+	+	+/A	+/A	+/A	+/A	+/A	*Staphylococcus saprophyticus*
ECO-18	+ (cocci)	+	-	-	-		-	-	+	A	A	-	-	-	+/A	+/A	+/A	+/A	+/A	$^{\beta}$ *Streptococcus agalactiae*
ECO-19	- (rod)	+	+	+	+	+	-	-	+	A	A	-	+	+	+/A	+/A	+/A	-	+/A	*Vibrio cholerae*
ECO-20	- (rod)	+	-	+	+	+	-	+	-	A	A	-	+	+	+/A	+/A	+/A	+/A	+/A	*Vibrio parahaemolyticus*

Legend: + = positive; - = negative; K = alkaline; A = acid; VP = Vogues Proskauer; TSI = triple sugar iron; * = tested positive to coagulase; β = tested positive to beta-haemolysis.

3.7 Bacterial ecology of the various abattoirs' effluents and their receiving streams

Table 5 presents bacteria isolated from the various abattoirs and their receiving streams. Phenotypic and biochemical characterization of the isolates placed the bacteria into fourteen (14) genera namely: *Bacillus*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Micrococcus*, *Proteus*, *Salmonella*, *Serratia*, *Staphylococcus*, *Streptococcus* and *Vibrio*. A total of 20 bacteria were isolated from all the samples. The Egbu abattoir effluent and its receiving water had the highest number of bacterial isolates. Eighteen (18) isolates were obtained from the Egbu effluent while nine (9) isolates were obtained from its receiving stream. From Ahiara abattoir, 14 bacterial isolates were obtained from the effluent while 5 isolates were gotten from the receiving stream. Meanwhile, the Amakohia Ikeduru abattoir had the least number of bacterial isolates for the effluent with twelve bacterial isolates while from the receiving stream 8 bacteria were isolated.

In terms of percentage distribution, 85.7 % of the total bacterial isolates were found in the Egbu abattoir effluent while 42.9 % of the total bacterial isolates were found in the receiving stream. Ahiara abattoir's effluent had 66.7 % of the total bacterial isolates while its receiving stream had 23.8 %. Over 57 % of the total bacterial isolates were distributed in the Amakohia Ikeduru abattoir with 33.3 % for its receiving stream (Figure 6).

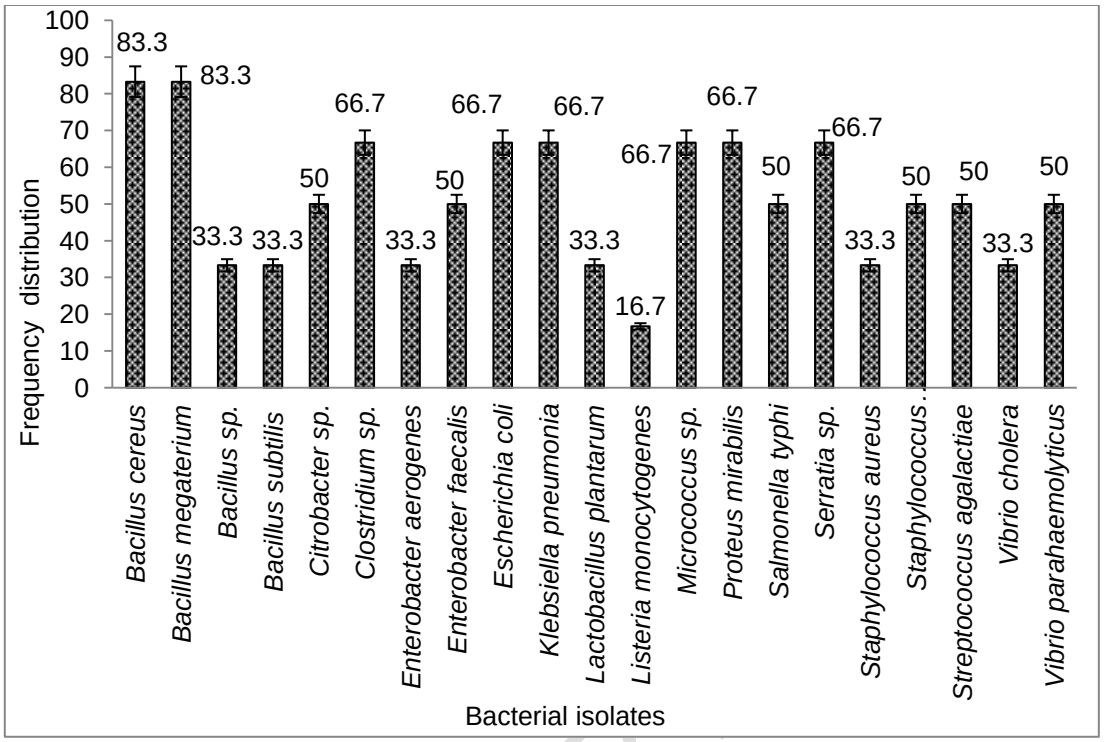

Figure 6. Percentage frequency distribution of the bacterial isolates in the three abattoir samples

4. DISCUSSION

This study investigated bacterial diversity and physicochemical quality of abattoir effluent receiving streams in Imo state using rivers Onuku, Egbu and Ikembara as references. Physicochemical analysis of the various abattoirs and their receiving streams revealed the following: mean pH values of 9.2 and 9.6 were observed for Egbu and Ahiara Mbaise effluents, respectively. These pH values were alkaline and above the upper limit of the maximum permissible limit 6 - 9. Meanwhile, for Amakohia Ikeduru abattoir effluent, a mean pH of 8.8 was observed. This value is within the maximum permissible limit. The pH of the receiving streams of the entire abattoir under investigation was outside the permissible limit. While the pHs of the effluent were all alkaline, the receiving streams all had slightly acidic values. pH is the measure of acidity or alkalinity of water. Discharging pH values outside the permissible limit into water bodies could be detrimental to the ecology of such aquatic habit as most of the metabolic activities of aquatic organisms are pH-dependent [14-15]. The finding of the research is similar to the report of Raheem and Morenikeji [16] for the effluent and the report of Hassan *et al*. [17] for the receiving streams.

Conductivity (µS/cm) of the entire abattoir effluents examined exceeded the maximum limit set by FMEnv. Meanwhile, their respective receiving streams had conductivity values much lower than the maximum permissible limit. Lower conductivity levels for abattoir effluent receiving stream have been reported by Hassan *et al*. [17]. However, the effluents' result reported in this study is higher than the conductivity of Lairage effluent as reported by Eze *et al*. [18]. High salinity values were observed within the samples. The various abattoir effluents had higher salinity values when compared to their respective receiving streams. The receiving streams had <5 % of the salinity values of the effluents.

Nitrate and total nitrogen (mg/L) were determined all through the different abattoir effluents and their receiving streams. The nitrate values for all the effluents (23.6 – 40.2 mg/L) were higher than the FMEnv maximum permissible limit of (20 mg/L). Whereas the nitrate levels (0.14 - 0.3 mg/L) of the receiving streams were found to be lower than the maximum permissible limit. In surface water, nitrification and denitrification may also occur, depending on the temperature and pH. The uptake of nitrate by plants, however, is responsible for most of the nitrate reduction in surface water [19]. The total nitrogen levels in the entire effluents and the receiving stream samples under investigation were very low concerning the maximum permissible limit. The faeces of the animals would have contributed to the high nitrate level observed in the various effluents.

The temperature of the effluent and that of the receiving streams were all observed to be lower than the permissible limit; conforming to the standard of the effluent discharge limit and thus that of effluent receiving water bodies.

Turbidity levels (200.1 – 227.1 %) observed in the various abattoir effluents were higher than the maximum permissible effluent discharge limit. However, the receiving streams turbidity levels were below the recommended permissible limit. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through a water sample [20]. Turbidity in water is caused by the presence of suspended matter such as clay, silt, finely divided organic and inorganic matter, plankton, and other microscopic organisms. Turbidity units are supposed to correspond to TSS concentrations, but this correlation is only approximate. Water bodies with turbidity above 50 NTU are quite cloudy, and water bodies with turbidity levels exceeding 500 NTU are very muddy. Suspended sediment is a ubiquitous water pollutant, with a multitude of environmental impacts on water bodies, including transport of other pollutants such as adsorbed nutrients and toxic materials.

Phosphate and total phosphorus levels were determined in the entire effluents and receiving stream samples examined. The phosphate levels observed in the Ahiara Mbaise and Amakohia Ikeduru abattoir effluents were below the permissible limits (3.5 mg/L) while the effluent from Egbu abattoir exceeded the permissible limit. Meanwhile, in the entire receiving stream samples the phosphate levels observed were within the permissible limit. For total phosphorus, the values obtained from Egbu and Ahiara Mbaise exceeded maximum limit while that of Amakohia Ikeduru abattoir effluent fell with the permissible limit. Atuanya *et al.* [21] reported abattoir effluent greater than the permissible limit set by FMEnv. While Raheem and Morenikeji [16] reported a less than and greater permissible limit values for two abattoir effluent phosphates levels.

Sulphate levels for the entire samples (effluents and their receiving streams) were within permissible limits across the three abattoirs examined. The sulphate levels obtained in the Ahiara Mbaise effluent (0.04 mg/L) and the receiving stream (0.05 mg/L) were higher than that reported by Atobatele [22] for Ogunpa River (13 – 59.5 mg/L). However, Atobatele [22] maximum sulphate value of 59.5 mg/ml exceeded the maximum value obtained in this study across all the abattoir samples. The sulphate level of Egbu abattoir effluent observed in this study is higher than the range (2.333 ± 0.751 mg/L and 15.303 ± 0.389 mg/L) reported by Raheem and Morenikeji [16].

Biochemical oxygen demand (BOD) and dissolved oxygen (DO) levels in the effluents and their receiving streams were determined throughout the three abattoirs examined in this study. The BOD for all the samples (effluents and receiving streams) was below the FMEnv maximum permissible limit for effluent discharge. The result obtained is lower than the report of Atuanya *et al.* [21]. If effluent with high BOD levels is discharged into a stream or river, it

will accelerate bacterial growth in the river and consume the oxygen levels in the river [23]. Meanwhile, the DO observed in all the abattoir effluent samples was lower than the ≥ 2.0 mg/L permissible limit. However, the DO of the entire receiving streams was within the acceptable range. The DO findings observed in this study is in line with the report of Hassan *et al*. [17].

Oil and grease levels obtained in this study revealed higher than permissible limit values for the effluent. The highest oil and grease 32.5 mg/L was observed in Egbu abattoir effluent followed by the Ahiara Mbaise effluent and then the Amakohia Ikeduru abattoir effluent. The oil and grease levels (22.7 - 32.5 mg/L) of the effluent observed in this study are lower than the 2500-12590 mg/L reported by Osibanjo and Adie [24]. However, the result is similar to the reported 34±9 mg/L oil and grease value for a cattle slaughter by Bazrafshan *et al*. [25]. Osibanjo and Adie [24] suggested that high oil and grease value could be as a result of fat released during roasting and dressing of goats and sheep carcass. Meanwhile, the entire receiving stream had oil and grease values lower than the permissible limit.

Chemical oxygen demand (COD) and Total organic carbon (TOC) of the entire samples were examined in this study. COD levels in the three abattoir effluents and their receiving streams were determined in this study. For the effluents, the CODs (428 – 662 mg/L) was higher than the permissible limit (120 mg/L) in all the abattoir effluents. As with most parameters, maximum COD (662 mg/L) was observed with Egbu abattoir effluent. However, the entire receiving streams' COD values were within the permissible limit. The effluent's COD values observed in this study were all higher than those reported by Atuanya *et al.* [21]. Total organic carbon (TOC) of the effluents was lower than in the receiving streams for the entire abattoirs examined.

Total suspended solids (TSS) range (185.3 – 261.4 mg/L) observed in the abattoir effluents were higher than the permissible limit by FMEnv of 30 mg/L. However, the values for the receiving streams were within the approved limit. The TSS effluent values obtained in the study are lower than the reported 1028 mg/L by Hassan *et al*. [17]. The values are higher than the 56 and 61 mg/L reported for government and privately owned abattoirs in Benin City, Edo State, Nigeria [21]. Slaughterhouse wastewater contains a high concentration of suspended solids (SS) including pieces of fats, hair, feathers, fresh manure, grits and undigested feeds [26]. These substances can contribute to TSS content in abattoir effluents.

Total dissolved solids (TDS) values observed in the effluents and their receiving streams in this study were below the FMEnv permissible limit. Atuanya *et al*. [21] reported the highest mean value (2363.5 mg/L) for TDS in a privately owned abattoir. The result obtained in this study was lower than the total dissolved solids (TDS) reported for some rivers in Ibadan metropolis by Oloruntoba [27] and Atobatele [22].

The heavy metals analysed in this study included zinc (Zn), copper (Cu), iron (Fe), lead (Pb) and nickel (Ni). The concentration of Zn (mg/L) observed in the three abattoir effluents and their receiving streams was <0.01 much with the maximum permissible limit (5 mg/L) set by FMEnv. This result is consistent with other reports. Raheem and Morenikeji [16] reported Zn level of 0.307±0.020 mg/L from abattoir effluents in Ibadan. Hassan *et al*. [17] reported 0.0169 mg/L values for Zn in governor road abattoir at Ikotun, Lagos state. The Cu concentration range for the entire effluents was 0.02 – 0.07 mg/L, which was below the maximum permissible limit of 5 mg/L. The finding of this research is comparable to the report of Siyanbola *et al*. [28], who observed 0.1 mg/L in one of the industrial effluents examined in Lagos state. Additionally, 0.14 mg/L Cu concentration was observed by Osibanjo and Adie [23] Bodija abattoir in Ibadan, Oyo state. Other heavy metals examined in this study such as

Fe, Pb and Ni showed levels lower than the maximum permissible limit for the respective metals.

The bacterial ecology of the entire effluents and their receiving streams examined comprised bacteria belonging to 15 genera vis., *Bacillus*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Micrococcus*, *Proteus*, *Salmonella*, *Serratia*, *Staphylococcus*, *Streptococcus* and *Vibrio*. The isolation of these bacterial genera is consistent with the other researchers' findings [21, 29-30]. A similar finding has been reported by Bala [31] from faecally contaminated water sources in Jimeta-Yola.

Rabah *et al*. [30] (2010) isolated the following bacteria from soil polluted with abattoir wastewater, *Pseudomonas aeruginosa, Bacillus anthracis, Staphylococcus epidermidis, Bacillus polymyxa, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Streptococcus faecalis* and *Staphylococcus aureus*. Atuanya *et al*. [21] isolated *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Enterobacter* sp. from the effluent samples of government and private abattoirs in Benin City, Edo State, Nigeria. While they isolated *Escherichia coli, Streptococcus faecalis, Staphylococcus aureus* and *Klebsiella* sp. from Ikpoba River water samples. These findings conform to the reports of Adesemoye *et al*. [29] and Ogbonna and Igbenijie [32]. When these organisms are present in wastewater discharged into water bodies they could affect the ecological balance in the water bodies [33].

The microbiological parameters examined in this study included total culturable heterotrophic bacterial (TCHB), total fungal (TF), salmonella and shigella, total vibrio, total coliform and faecal coliform counts. The TCHB count showed high bacterial presence, especially in the Egbu samples.

The TCHB counts obtained in this study showed variation between the different abattoir effluents as well as their receiving streams. The results obtained in this study are consistent with the results of other researchers. TCHB range of 9.66 – 79.9 $x10^5$ cfu/ml obtained for the abattoir effluents is higher than the reported 4.1 – 9.3 $x10^5$ cfu/ml and 4.8 – 9.8 $x10^5$ cfu/ml for government and private-owned abattoirs respectively, in Benin, Edo State, Nigeria by Atuanya *et al*. [21]. The TCHB for the receiving streams also varied between the different abattoirs and from upstream to downstream. In this study, TCHB counts ranged from $2.19\pm0.14x10^2$ - $2.3\pm0.09x10^5$ cfu/ml downstream and from $2.0\pm0.13x10^2$ - $1.05\pm0.39x10^4$ cfu/ml upstream. This result is similar to the TCHB count of 1.10 – 1.45 $x10^4$ cfu/ml for upstream and 1.68 – 2.20 $x10^4$ cfu/ml downstream reported by Atuanya *et al*. [21].

The respective TC and FC count obtained in this study ranged from 1.78±0.29 - 21.6±0.15 $x10^6$ MPN/100 ml and 1.16±0.28 – 17.6±0.51 x103 MPN/100 ml for effluents, 1.01±0.03 - 1.24 ± 0.04 $x10^2$ MPN/100 ml for downstream and 1.04±0.31 - 1.38±0.11 x10 MPN/100 ml for upstream. The TC and FC for effluents were higher than the respective 4.0 x 10^2 and 0.0 x 10 MPN/100 ml TC and FC effluent limit for discharge into surface water in Nigeria as contained in FEPA [34]. This work is similar to Nafarnda *et al*. [1] report of TC and FC values higher than FMEnv limit for effluent discharge into surface water in Nigeria. Atuanya *et al*. [21] also reported values for TC and FC counts higher than the effluent limit for discharge into surface water in Nigeria. These findings show negligence to the guidelines stipulated by the federal ministry of environment in Nigeria. However, the values obtained for the downstream and upstream of the receiving streams were lower than the reports of Atuanya *et al*. [21] and Nafarnda *et al*. [1].

The salmonella and shigella count of the abattoir effluents revealed the presence of either *Salmonella* or *Shigella* in the effluent. However, only *Salmonella* was isolated from the

effluents. However, neither *Salmonella* nor *Shigella* was isolated from the receiving water bodies. This might be as a result of dilution of the effluent samples. In contrast, however, total vibrio count showed high population *Vibrio* species in the abattoirs effluent and their receiving water bodies. This is of concern considering the role of *Vibrio* species especially *V. cholerae* in the disease outbreak. Discharge of *V. cholerae* into water bodies used for agricultural, recreational or domestic purposes can lead to the widespread of cholera in with the human population.

Analysis of Variance (ANOVA) and Post Hoc Multiple Comparison assuming equal variances (p=0.05) was carried out on the different abattoir effluent and their receiving stream counts to determine whether there is a significant difference between the counts observed in the effluents and those in the downstream and upstream of the receiving streams. There was a significant difference between the effluent samples and the receiving streams for the samples considered. Meanwhile, no significant difference was observed with the TCHB, salmonella and shigella, TC and FC count between the receiving streams upstream and downstream for the three abattoirs under examination.

4. CONCLUSION

This study had evaluated the microbial ecology and physicochemical quality of abattoir effluent receiving streams in Imo state using rivers Onuku, Egbu and Ikembara as references. The bacterial isolates in the various samples included members of the genera *Bacillus*. *Citrobacter*, *Clostridium*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Micrococcus*, *Proteus*, *Salmonella*, *Serratia*, *Staphylococcus*, *Streptococcus* and *Vibrio*. The result obtained showed that effluents discharged into the selected abattoirs were not treated and in most instances contained chemical above the maximum permissible limit by the federal ministry of environment. Also, the presence of pathogenic bacteria in the effluents and their receiving stream indicated the likelihood of health risk.

ETHICAL APPROVAL

As per international standard and university standard, written approval of the Ethics committee has been collected and preserved by the authors.

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