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Assessment and Characterization of Environmental Impact of Effluents in the Vicinity of Owerri Municipal Abattoir

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Abstract

Contamination of the ecosystem from abattoirs could cause serious environmental hazards. Continuous monitoring and assessments remain imperative to forestall such harm to the ecosystem. The present study was undertaken to assess the physicochemical and selected heavy metal contents of wastewater in the vicinity of an abattoir within Owerri Municipal, Imo State Nigeria. Samples of wastewater were collected from three different points within the abattoir under stringent control measures and analyzed for physicochemical and selected heavy metals (Pb, Cd, Fe, Ni, and Cr) using standard procedures. Results obtained showed that the values for physicochemical parameters assessed exceeded WHO permissible limits except EC. The heavy metal content of the wastewater was in the order: Fe>Cr>Cd>Pb>Ni. The values for heavy metals were well above WHO permissible limits set by the WHO except for Ni. This study underscores the need for the government to provide wastewater treatment plants within the abattoirs before discharge as this could potentially harm the ecosystem. This further implies that the environmental and health status of the inhabitants could be severely affected if nothing is done to avert the current trend.

Keywords: Pollution, Wastewater, Physicochemical, Heavy metals, Owerri

1 Introduction

Abattoirs can be defined as premises approved and registered by regulating authorities for safe and hygienic slaughtering, inspection, processing, effective preservation, and storage of meat products for human consumption [1]. Several activities carried out at abattoirs are known to generate a huge amounts of waste through the slaughtering of animals or livestock and processing of meat and byproducts (e.g. cow, goat, ram) [1]. The environmental impact of these activities is of great concern in both urban and rural arrears in Nigeria [2]. On daily basis in all the rural and urban markets in Nigeria, animals are slaughtered and the meat is sold to the public for consumption [3]. Abattoir wastes originate from killing; hide removal or dehairing, paunch handling, trimming, processing, and cleanup operations [4]. Over the years, contamination and pollution of natural water bodies have emerged as a major challenge in developing and densely populated countries like Nigeria [5]. Most of the abattoirs are sited in the vicinity of the source of water for the people and there is a possibility of the water getting polluted through runoff. The animal blood is released untreated into the flowing stream while the consumable parts of slaughtered animals are washed directly into the flowing water. Lack of proper management and supervision of abattoir activities has been identified as a major source of public health in most parts of Nigeria [3]; [4]. Water pollution makes water to become unsuitable for human use and also it becomes more expensive in treatment for acceptable quality [4]; [6]. Human activities such as indiscriminate location of abattoirs in residential areas in developing countries impact negatively on natural water sources [7]. The abattoir industry in Nigeria is an important section of the livestock industry that provides domestic meat supply to over 180 million people and also employment opportunities for a huge number of people [7]. In Nigeria, there appears to be lack of adequate waste management strategy in all public abattoirs such that large solid wastes and untreated effluents are common [8]. Abattoirs usually releases wastewaters directly into the ecosystems without adequate treatment process thereby posing serious threats to surface water quality, general environmental safety and health [4]. Water is the most essential natural resources to the existence of man on earth. Contamination of river bodies from abattoir waste could constitute significant environmental and health hazards [9]. Pollutants from the abattoir commonly include blood, paunch manure, animal feces, animal horns, bones, spent lubricants from machines like generators, etc. [10]. Also, there may be the presence of pathogenic microorganisms, such as Salmonella, Escherichia coli, Shigella, parasite eggs and amoebic cysts [8]. Research showed that effluent discharged from abattoirs has caused the deoxygenation of rivers [10]. Effluents from abattoirs have also been known to contaminate groundwater [1]. Abattoir operations produce a characteristic highly organic waste with relatively high levels of suspended solids, liquid, and fat. The liquid waste is usually composed of dissolved solids, blood, gut contents, urine, and water. Results of abattoir effluent contamination of the environment have also been reported

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affect the physicochemistry and microbiology of the ground and surface waters in Nigeria [2]. According to [12], assessing the water quality within the vicinity of abattoirs in residential areas will help to evaluate the potential risks on the health of residents who depend on shallow well waters for consumption and other domestic uses. Lack of modern facilities, hygienic environment coupled with improper siting of abattoir poses a great challenge to the environment and the people [13]. It is hoped that it will generate data that will help in the formulation of policies that will lead to the proper management of abattoirs in Imo State towards a cleaner and healthier environment. It is against this backdrop that this study was undertaken to evaluate the wastewater pollution status, by assessing the physiochemical and heavy metal contents of an abattoir in Owerri municipal, Imo state Nigeria.

2 Materials and methods

2.1 Study Site

The Owerri Municipal abattoir (plate 1) was commissioned in July 1997. It is located behind Owerri Mall along Egbu road and lies between Latitude 5^0 28.449" North and 7^0 03:192" East on an elevation of 234.9ft. The abattoir is situated on about 4 hectares of land. It is divided into different sections; the pen, where the animals await slaughter or sale, the butchering section, where the animals are rendered; the rinsing section, where the parts of the animals are rinsed, the dung pit, where the intestines are emptied and the burning section where hairs on their skin are burnt. On average, about 35-48 cows and 62-73 goats/sheep are slaughtered on daily basis. Generally, it is a red meat slaughterhouse. The wastes from the abattoir are collectively discharged directly into the *Otamiri* river which is about 100m away at an elevation of 172.6f and the surrounding soil without treatments.

Currently, the abattoir is grossly dilapidated and sandwiched by a highbrow upcoming residential estate (Plate 2). Owerri Municipality (Fig. 1) which houses the abattoir is approximately located between Latitude 5^0 28’35.6” North and Longitude $7^0 1^1$ 0.6” East of the Greenwich Meridian on an average elevation of 75 meters (246ft) above sea level. It has a tropical climate with significant rainfall most months, with a short dry season. The average annual temperature is 26.4°C. Precipitation averages 2219mm with a relative humidity of 80%. It has an area of 58km^2 and a population of 127,213 according to the 2006 Census. The Municipality sits at the intersection of roads from Port Harcourt, Onitsha, Aba, and Umuahia. It is drained by *Nwaorie* and *Otamiri* rivers. It is also the trade center for palm produce, maize, yam, and cassava. *Eke Ukwu Owere* is the Main Market in the Municipality. Historically, before the advent of the British in 1901-2, *Owere* town (Anglicised Owerri) was and still is today made up of five villages namely-*Umuororonjo, Amawom, Umuonyeche, Umuodu*, and *Umuoyima* (collectively known locally as *Owere Nchi Ise*). With British influence and colonization in the early 1900s, Owerri became the headquarters for Owerri Division and later Owerri Province. Also, when Imo State was created on 3rd February 1976, Owerri was chosen as its Capital. On the 15th of December, 1996, Owerri attained Municipal status (WOPC, 2017). The Municipality has continued to witness phenomenal growth as a result of its location and being an administrative, business, and hospitality center (This Study, 2017).

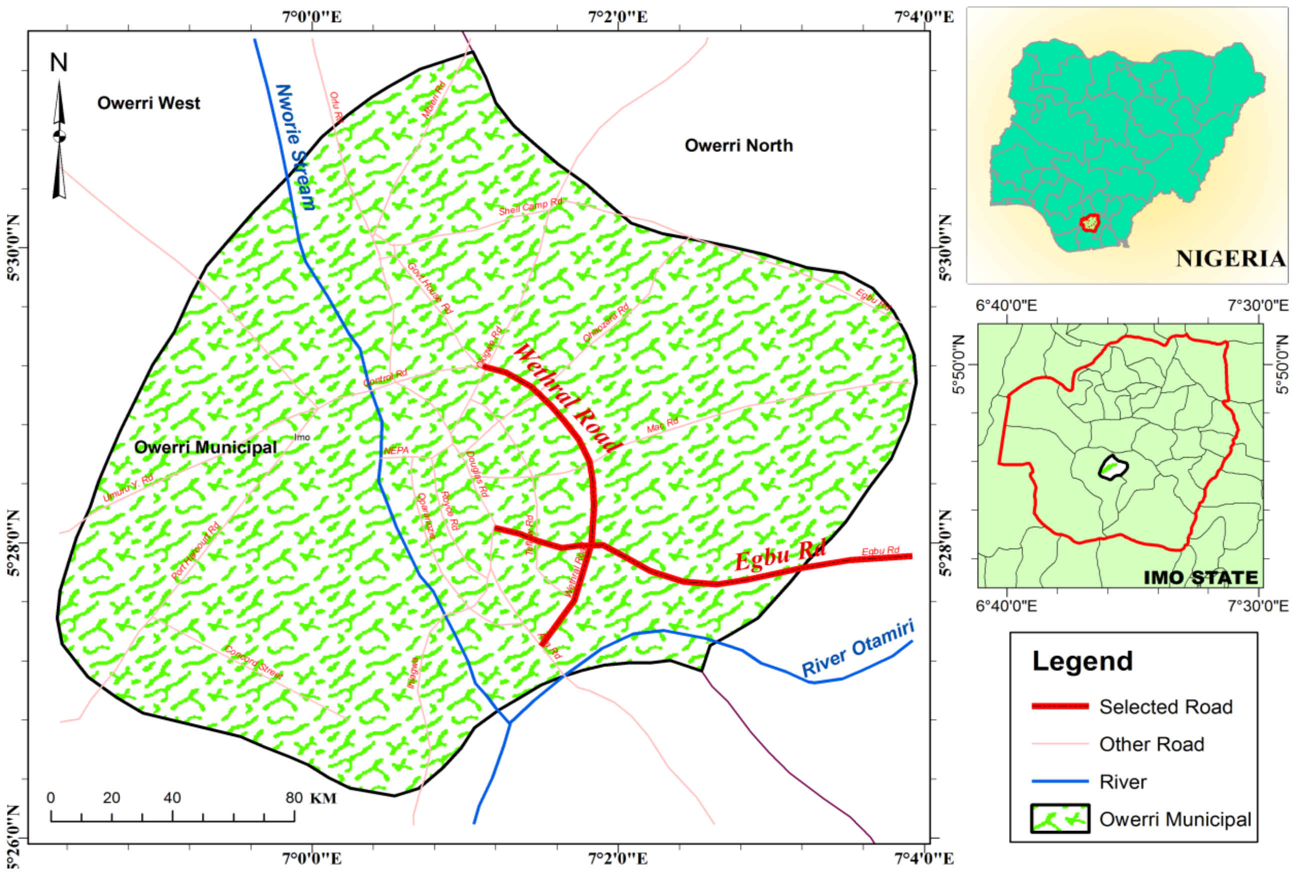

Figure 1: Map of Owerri Municipal showing the Abattoir

Plate 1: Owerri Municipal Abattoir

Plate 2: Effluent drain into Otamiri River

2.2 Estimation of Abattoir Waste

Data on the number of cows and goats/sheep slaughtered daily was collected through direct observation, abattoir records, and interviews. Supplementary data were obtained from records of abattoir operations. Additional information was also collected through interviews with the veterinary officers and meat inspectors. The waste materials generated from abattoir operations were calculated according to estimations by Aniebo *et al.* (2009) based on the average size of animals. The computations were done using average data on body weight for the cows and carcass weight per 1,000kg (Fearon *et al*., 2014) as shown in Table 1.

2.3 Evaluation of methods of handling and disposal of abattoir waste

The type of abattoir waste generated and the methods of disposal in the abattoir and the challenges of waste management were also investigated and characterized through regular visits and observation.

2.4 Method of sample collection

Wastewater samples were collected from a total of 9 sampling locations, made up of 3 each to make a composite sample per location, around the abattoir and coded AWS1, AWS2, AWS3, and AWC (Abattoir Wastewater samples 1-3 and control respectively) under stringent control. Wastewater samples used for physicochemical parameters were collected into 2 liter pre-cleaned polyethylene bottles while wastewater samples for heavy metals determination were collected into 1 liter pre-cleaned polythene bottles and preserved by the addition of 2 mL Analar grade concentrated trioxonitrate (v) acid (HNO_3).

2.5 Physicochemical analysis of wastewater samples

The physicochemical properties of the wastewater samples were determined under the American Public Health Association (APHA) series of Standard Methods of Examination of Water and Effluent (APHA, 1998). The physicochemical properties determined include Temperature and pH which were done *in-situ*, Turbidity, Dissolved Oxygen (DO), Total alkalinity, Conductivity, Total hardness, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Solids (TS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Salinity (Chloride ion test), Nitrate, and $PO4^{3-}$.

2.6 Determination selected Heavy Metals in Wastewater

Heavy metal of the samples was done by measuring 5 mL of conc. HNO_3 and 200mL of water sample in a 250 cm^3 beaker. The solution was evaporated to near dryness (less than 25 mL). After cooling, the solution was made up to 25 mL with conc. HNO_3 and transferred into the sample bottle before analysis in line with the method reported by Ogunlade *et al*., (2021). The heavy metal concentration was subsequently determined with Atomic Absorption Spectrophotometer (AAS, Model GFA-EX7i, Shimadzu Corporation, Japan). All results are expressed in mg/kg.

.7 Microbial analyses

10g of the soil samples were diluted in 90ml of sterile deionized water, while 10ml of the water samples were diluted in 90ml of the same water instead. 1ml of each soil and water sample was pipetted into a test tube containing 9mls of distilled water. 10-fold serial dilution of the samples was prepared using sterile distilled water as the diluents. Aliquots (01ml) of each (10^{-2}, 10^{-3}, 10^{-4}, or 10^{-5}) of the test tubes were incubated in a nutrient agar plate, sabouraud dextroxe agar, and mineral salt agar by spread plate technique and incubated at 37^{o}c for 24hrs for bacteria and 25^{o}c for 78hrs – 96hrs for fungi on the incubator. After incubation, the number of colonies on the plates with distinct growth was estimated and recorded as colony-forming units per milliliter (cfu/ml). Subculturing was done until distinct colonies were obtained. In identifying fungi, microscopic and macroscopic examinations including staining for morphological characteristics were carried out on fungal isolates, and identification was done based on the characteristics. For bacteria, pure isolates were isolated and identified using biochemical tests according to Cappucino and Sherman (1998).

Table 1: Basis for estimating abattoir waste

S/No	Waste Category	Cow	Goats/sheep
1	Blood per head (kg)	12.6	0.72
2	Intestinal content/head (kg)	8.0	1.25
3	Waste tissue per head (kg)	6.4	0.80
4	Bone per head (kg)	11.8	2.06

Source: Aniebo *et al*., 2009.

2.8 Analysis of Data

All data collected were subjected to descriptive statistics (mean, median, and mode) and one-way Analysis of Variance using Statistical Package for Biological and Social Sciences (SPSS) Incident 20.0 Software. All data were represented in mean ± standard deviation (M±SD). Confident level of determination (P=0.05).

3 Results and discussion

3.1 Animals Slaughtered at Owerri Abattoir

The findings of the study showed that the daily slaughter of animals ranged between 35-48 cows and 62-73 goats/sheep (Table 3.2). The number of animals slaughtered varied according to the season. More animals were killed in January and April which could be attributed to the Christmas/New Year and Easter celebrations. The least number slaughtered was in February (957cows and 1,882 goat/sheep).

3.2 Waste generation, Components, and quantities

The slaughter of animals daily at the abattoir led to the generation of about 2,094.0 kg of blood, 1,546.0 kg of intestinal content, 2,339.6 kg of bones, and 2,712.0 kg of waste tissues. These figures translate into a total of 8,691.6 kg of the waste directly discharged into the surrounding environment and the *Otamiri* river within one month (Table 3.3). Greater portions of the 584.9kg of bones were sold off for bone meal preparation. Intestinal contents constituted the least quantity (386.5kg) whereas waste tissues presented the highest generation (678.08 kg).

3.3 Abattoir Waste Generation and Disposal

Table 4 shows the type of abattoir wastes generated and their method of disposal in the study area. The blood waste is collected for blood meal preparation and animal feed production and is also seen draining into surrounding areas and the river. Intestinal contents are composted, heaped within premises, and also washed into the river. Bone wastes are stockpiled within the premises of the Abattoir for animal feed preparation in feed mills and also burnt. Waste tissues are disposed into depressions on the premises and also burnt.

The result of the physicochemical properties of wastewater collected in the vicinity of the Abattoir is presented in Table 5. Results obtained showed that the temperature of the wastewater ranged from 26.20 – 29.09 within the sampling locations. As opined by [14], Temperature is an essential environmental parameter in waste water as it controls the behavioral characteristics of organisms, and the solubility of gases, and salts in water. The results obtained for temperature were similar to the report of previous studies [15] and [16] which reported a range of 27.8–28.3 °C. However, the temperature across the sampling sites was within the benchmark of 25-29 ^{0}C by the WHO. the pH of the wastewater revealed an acidic medium. Although the value is in concordance with an earlier report [14] and within the optimum limit of the WHO, it may likely cause undesirable ailments such as acidosis [12]. The acidic nature could be attributed to the type of waste which includes dung, blood, fat, intestines, and urine which are generated from the abattoir which reduced anaerobic activities [17]. Conductivity is an indication of the amount of dissolved salts present in water. The conductivity measured in wastewater ranged from 38.00 – 108.00 µS cm^{-1} with the control having the least value. The mean value of the EC was within the tolerance limit of 1200 µS cm^{-1} by the WHO. Values of EC obtained in this study compare well with that of [18] but are lower than values reported by [14].

Table 2: Animals slaughtered at Owerri abattoir (January-May, 2017)

S/N	Month	Male cattle	Female cattle	Total cattle	Male goat	Female goats	Male sheep	Female sheep	Total goat/sheep
1	Jan.	1,387	101	**1448**	1,583	21	604	56	**2,264**
2.	Feb.	903	54	**957**	1,334	18	480	50	**1,882**
3.	March	1001	71	**1,072**	1721	26	307	170	**2,204**
4.	April	1,129	39	**1,168**	1,006	49	919	82	**2,056**
5.	May	1,007	41	**1,048**	1,083	37	689	118	**1,927**

Source: Veterinary Department, Ministry of Agriculture, Environment and Natural Resources, Owerri.

Table 3: Projected Abattoir Waste Generation/Month (kg)

S/N	Waste Type	Cattle	Goat & sheep	Total/month
1	Blood	474.2	49.3	523.5
2	Intestinal content	301.1	85.4	386.5
3	Bones	444.2	140.7	584.9
4	Waste tissues	240.9	437.12	678.0

Source: This Study, 2017

Table 4: Type and method of waste disposal

S/N	Waste Type	Method of Disposal
1	Blood	Collected for a blood meal and animal feed production and also drain into surrounding areas and the river
2	Intestinal contents	Composting and heaped within premises, washed into the river
3	Bones	Stockpiled for preparation of organic fertilizer, animal feed preparation, and burning.
4	Waste tissues	Disposed into depressions in the premises and also burning

Source: This Study, 2017

The value for turbidity obtained in this study ranged from 5.98 – 15.27 with 4.27 gotten from the control site. the result further revealed that the turbidity of the wastewater was higher than the WHO benchmark of 5. The turbidity values recorded in the study are lower than 115 as earlier reported [15] and higher than the 2 reported in another study [18]. The values of TDS mg/l fall within the acceptable limit of WHO. The implication of the high value of TDS among others is that the water can corrode containers used for water storage [17]. Total solids (mg/l) content of the wastewater ranged from 83.00 – 153.00 while TSD ranged from 49.70 – 106.70 respectively. Values of TS and TSD obtained in this study compares well with that of [12] and [14]. Results further revealed that the dissolved oxygen (DO), Biochemical oxygen demand (BOD) and COD is above the accepted standard for wastewater by the WHO. High Biochemical Oxygen Demand values at the discharge point could be attributed to the low Dissolved Oxygen level since low Dissolved Oxygen will result in high Biochemical Oxygen Demand which is a strong indication of pollution [12]. Both BOD and COD are important water quality parameters and are very essential in water quality assessment [19]. Therefore, the more organic material present in the abattoir wastewater, the higher the BOD and COD. The values for Total chloride ranged from 257.40 - 285.90, Nitrate 15.20 - 24.55, Nitrate-Nitrogen 4.36 - 8.55 Phosphate 3.50 - 5.85, and Sulphate 327.50 - 540.55 respectively.

The levels of chloride in the wastewater sample ranged from (15.20 - 27.40 mg/L) which fell within the tolerance limit of 45.0 – 69.0 mg/L. The mean Nitrate levels exceeded the WHO limits of 45 mg/L in wastewater, while the mean Sulphate level was below the WHO limit of 500mg/L. The mean Phosphate level ranged from 3.5 - 4.850 which is lower than the WHO limit of 5mg/L for the discharge of wastewater into the river. The levels of nitrate in the abattoir wastewater may give rise to methaemoglobinemia, also the levels of nitrate reported in this study in addition to phosphate levels can cause eutrophication and may pose a problem if discharged into river or stream.

Table 6 depicts the concentration of selected heavy metals in the abattoir wastewater samples. Results obtained showed that the concentration of Pb ranged from 0.11 – 0.16 with no value obtained at the control site. The values were well above the 0.03 mg/L permissible limit by WHO (2013). Lead could get into the human body's consumption of food, water, and air [20]. In exposed individuals, lead impacts the central nervous system leading to delayed mental and physical growth in children and could also affect the attention span and learning abilities of children [21]. Chromium concentration within the vicinity of the abattoir ranged from 0.21 – 0.91 exceeding the 0.05 mg/L permissible limit by the WHO (2013). The iron concentration of the wastewater ranged from 1.88 – 2.81 which is above the 0.30 mg/L benchmark by the WHO. The high iron content observed in this study can be attributed to the influx of waste blood that is carried by runoff and deposited into the shallow wells and nearby water sources. The concentration of nickel in the wastewater samples ranged from 0.09 – 0.39 which fell within the permissible limit of 6.70 mg/L. The concentration of Arsenic in all the sampling locations was observed to be above the permissible limit of WHO. According to [22] Arsenic contamination poses a risk on consuming farm products (vegetables) and kinds of seafood in such vicinity.

Table 5: Physicochemical Parameters of Wastewater Samples

Parameters	AWWS1	AWWS2	AWWS3	Control	WHO (2011)
Temperature (°C)	29.05±0.6	29.20±0.8	26.20±0.9	28.20±0.9	25-29
pH	6.35±0.2	6.22±0.3	6.67±0.2	6.67±0.2	6.5-8.5
Conductivity (µs/cm)	108.00±68.0	38.00±0.0	55.50±13.4	48.50±13.4	1200
Turbidity (NTU)	7.90±1.6	5.98±0.7	15.27±2.0	4.27±2.0	5
Total dissolved solid (mg/l)	64.80±40.8	22.80±0.0	33.30±8.1	30.30±8.1	500
Total solids (mg/l)	153.00±47.0	129.50±50.5	83.00±17.0	72.00±17.0	-
Total suspended solid (mg/l)	88.20±8.8	106.70±50.5	49.70±16.0	99.70±50.5	NA
Dissolved oxygen (mg/l)	7.55±2.9	6.15±3.1	5.95±0.1	5.15±3.1	7.5
BOD (mg/l)	69.35±0.4	51.85±1.5	47.05±1.7	29.05±1.7 40	
COD (mg/l)	501.36±0.6	233.36±1.7	687.48±3.7	103.48±3.7	120
Total chloride (mg/l)	27.40±41.9^{a}	23.95±59.2	25.90±11.6^{b}	21.90±11.6b	250
Nitrate (mg/l)	56.15±0.8	57.55±1.8	73.20±2.8	31.20±2.8	50
Nitrate-Nitrogen (mg/l)	4.36±0.2	5.55±0.4	8.55±6.6	1.85±1.8 500	
Phosphate (mg/l)	3.50±1.5	3.85±1.8	4.85±2.8	3.85±2.8 5	
Sulphate (mg/l)	440.55±80.5	179.45±67.5	327.50±32.5	108.45±67.5	500

Data are mean±S.D of triplicate determinations. Values bearing superscript alphabets "a, b" show a significant difference in total chloride concentration of upstream and downstream water. **Note:** NTU= Nephelomentry turbidity unit; µs/cm = microsiemens per centimeter.

Table 6: Heavy metal concentration of wastewater in the vicinity of abattoir

Sampling locations	**Heavy metals**				
	Pb	**Cr**	**Fe**	**Ni**	**As**
AWWS 1	0.11±0.03^{a}	0.91±0.06^{a}	2.81±0.10^{a}	0.09±0.01^{b}	0.30±0.10^{a}
AWWS 2	0.15±0.05^{b}	0.13±0.04^{b}	1.88±0.11^{b}	0.21±0.00^{a}	0.31±0.14^{a}
AWWS 3	0.16±0.07^{b}	0.21±0.03^{c}	2.04±0.12^{a}	0.39±0.03^{a}	1.02±0.19^{b}
Control	ND	0.01±0.00^{d}	0.91±0.09^{b}	ND	ND
FAO/WHO (2013)	**0.03**	**0.05**	**0.30**	**6.70**	**0.20**

Values are presented as mean ± S.D triplicates samples. Values with the same superscript letter(s) along the same row are not significantly different (P<0.05) using Duncan multiple range test. ND=Not Detected. AWWS 1-3=Abattoir waste water samples 1-3.

Table 7: Total Counts of Bacteria and Fungi in the soil Samples of the Abattoirs (cfu/g)

Parameters	**Control**	**Soil 1**	**Soil 2**	**FEPA (1999)**
Total *E. coli* count	$17.5^{a}\pm005$	$2.5^{a}\pm001$	$2.9^{c}\pm002$	NS
Total *S. shigella* Count	$8.0^{b}\pm0.03$	$2.1^{a}\pm004$	$2.0^{b}\pm001$	NS
Total fungi count	$4.3^{c}\pm0.07$	$1.1^{b}\pm001$	$5.5^{a}\pm003$	NS

Data are presented as mean ± S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05) using Duncan multiple range test.

Table 8: Total Counts of Bacteria and Fungi in the Wastewater Samples of the Abattoirs (CFU/ml)

Parameters	**Upstream**	**Midstream**	**Downstream**	**FEPA (1999)**
Total *E. coli* count	$2.65^{a}\pm002$	$1.15^{c}\pm001$	$1.65^{a}\pm001$	0.00
Total *S. Shigella* Count	$2.90^{a}\pm001$	$3.70^{b}\pm005$	$2.15^{a}\pm001$	0.00
Total fungi count Control	$1.35^{b}\pm002$	$6.50^{a}\pm004$	$6.10^{c}\pm005$	0.00

Data are presented as mean ± S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05) using Duncan multiple range test.

The results of the microbial analysis of soil samples are represented in Table 7. The table showed that total E. coli counts (17.5±005, 2.9±002) cfu/g were the highest whereas total *S. shigella* counts (8.0±0.03, 2.0±001) cfu/g were the least microbe in the Control and Soil 2 samples. Similarly, the total E. coli count (2.5±001) cfu/g was highest in Soil 1 and the total fungi count (1.1±001) cfu/g was the least microbe in Soil 1. The results of the microbial population of water samples are as in Table 8. The table showed that total *S. shigella* counts (3.70±005) cfu/ml were the highest while total fungi counts (1.35±002) cfu/ml were the least microbe in the upstream, midstream, and downstream waters. The result indicated that the three sampling points had higher counts of microbial populations and were significantly higher ($p<0.05$) when compared to the permissible limits (FEPA, 1999).

4 Conclusion

The study was undertaken to assess the physicochemical and selected heavy metal concentration of wastewater in the vicinity of abattoirs in Owerri Municipal, Imo State. The results obtained showed that the concentrations of Pb, Cd, Fe, and Cr were above the WHO permissible limit in contrast with the concentrations of Ni which fell below the permissible limit of the used standard in this study. This implies that if the wastewater should be discharged to water bodies without proper treatment could be a source of environmental health risk to humans. It is therefore recommended that such wastewater is treated before being discharged into water bodies. This underscores the need for urgent steps to be taken toward treating the wastewater now in other to avoid a health crisis in the foreseeable future. This is because heavy metals accumulate in the body for a long time before constituting health risks.

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Ethical issue

Authors are aware of and comply with, best practices in publication ethics specifically about authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that the submitted work is original and has not been published elsewhere in any language. Also, all procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures performed in this study involving animals were following the ethical standards of the institution or practice at which the studies were conducted.

Competing interests

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution to data collection, data analyses, and manuscript writing.

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