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Research article

Physicochemical and Bacteriological Evaluation of Ogbete Market Enugu Abattoir Effluents and the Receiving Water Bodies

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ABSTRACT

The prevalence of water borne diseases is on the increase in Nigeria especially in Enugu state due to the apparent lack of portable water resulting to high mortality rate among the populace and the government`s apathetic response to these realities is even much more demoralizing. Bacteriological properties of Ogbete market abattoir wastewater and water bodies receiving the effluents were investigated using the multiple-tube most probable number (MPN) fermentation method. The results of physicochemical analysis showed high levels of BOD and nitrate-nitrogen concentrations which is an indication of high organic load in the effluents. The major bacterial isolates based on cultural and molecular analyses were Streptococcus fecaelis, Escherichia coli and Geobacillus stearothermophillus. The total Bacterial coliform count (TC) in abattoir effluents ranged from 4.8 × 106 upstream to 5.8 × 105/100 mL downstream, 8.2 × 104 to 3.2 × 104/100 mL of Fecal coliform (FC), 5.2 × 104 to 2.0 × 104/100 mL of Fecal streptococcus (FS) and 1.2 × 104 to 2.0 × 103/100 mL of Escherichia coli for abattoir effluents. The receiving water bodies had 6.2× 103 to 1.4 × 103/100 mL of TC, 2.6 × 103 to 2.2 × 102/100 mL of FC, 1.2 × 103 to 1.2 × 102/100 mL of F. streptococcus, and 4.8 × 103 to 6.6 × 102/100 mL of E. coli downstream. The total bacterial count (TC) for abattoir effluents exceeded the recommended limit for discharge into water bodies in Nigeria. The results of the studies showed that there was no significant difference (p < 0. 0 5) between bacterial counts of abattoir effluents upstream and the receiving water bodies downstream, an indication of heavy pollution of receiving water bodies by abattoir effluents and possible public and environmental health hazard implications.

Keywords: *Abattoir Effluents, Receiving Water Bodies, Total Bacterial Count, Bacteriological Evaluation*

Introduction

The abattoir industry in Nigeria is an important component of the livestock market providing domestic meat supply to over 180 million people and employment opportunities for the teaming population. It is important to note that the abattoir industries are less developed in Nigeria. Treatment facilities for abattoir effluents are lacking in Nigeria, unlike in developed nations, hence deadly pathogens can be found in water used for domestic purposes (Calmus *et al.* 1999). Major sources of contaminations of water bodies are runoff from feedlots (Coker *et al*. 2001), dairy farms (Eaton *et al*. 2005), grazed pastures (Armanda-leferse *et al*. 2005), fallow and poultry litters, grassland treated with dairy manure, and sewage sludge treated agricultural lands (Osibanjo and Achie, 2007). Such contaminations of water bodies from abattoir wastes could constitute significant environmental and public health hazards (Pepperel, 2003).

Bacteria from abattoir waste discharged into water columns can subsequently be absorbed to sediments, and when the bottom stream is disturbed, the sediment releases the bacteria back into the water columns presenting long-term health hazard. Pathogens present in animal carcasses or shed in animal wastes may include rotaviruses, hepatitis E virus, *Salmonella* spp., *E. coli* O157: H7, *Yersinia enterocolitica*, *Campylobacter* spp., *Cryptosporidium parvum*, and *Giardia lamblia*. The primary reservoir for *E. coli* O157: H7 has been reported to be healthy cattle in a study in Canada, although this bacterium is also endemic to swine and sheep. These zoonotic pathogens can exceed millions to billions per gram of feces, and may infect humans through various routes such as contaminated air, contact with livestock animals or their waste products, swimming in water impacted by animal feces, exposure to potential vectors **(**such as flies, mosquitoes, water fowl, and rodents), or consumption of food or water contaminated by animal wastes. The consequences of infection by pathogens originating from animal wastes can range from temporary morbidity to mortality, especially in high-risk individuals. Due to the difficulties in quantifying pathogens, indicators of fecal pollution, including coliform bacteria, fecal coliforms, *E. coli*, and/or Enterococci have been monitored in lieu of overt pathogens for for several years. Epidemiological evidence supports the relationship between the fecal indicator bacteria *E. coli*, Enterococci, and the incidence of gastrointestinal illness following recreational water exposure and provides the basis for water quality regulations.

Statement of the Problem

In Nigeria, abattoir wastes are sources of infections that require immediate solutions. Abattoir wastes with large quantities of animal feces are often discharged directly into water bodies and used for domestic purposes such as washing of vegetables, fruits and cooking utensils.

In Enugu area of Nigeria, abattoir effluents are discharged directly without treatment into water bodies. The bacteriological characteristics of abattoir wastewater and its possible effect on receiving water bodies which is likely to cause pollution with intensified environmental and public health hazards has not been documented in Enugu, hence the need to carry out a study on the bacterial content of the effluent water from Ogbete main market abattoir which is the biggest slaughter house in Enugu state.

Aims and Objectives of the Study

The aim of the study is to carry out bacteriological evaluation of the waste water from Ogbete main market abattoir and the receiving water body, the Akwata stream linked to other sources of domestic water supplies in Enugu, Nigeria.

Specific Objectives

The specific objective of this study is

- **i.** To investigate the bacteriological characteristics of abattoir wastewater.
- **ii.** To determine its strength and relationship with receiving water bodies and possible public and environmental health hazards in Enugu, Nigeria.

iii. The results will be based on the mean concentrations of total coliform (TC), fecal coliform (FC), *Fecal streptococcus and Escherichia coli* in samples of abattoir wastewater and receiving water bodies in Enugu, Nigeria.

iv. To investigate the presence of other contaminants that may constitute health hazards.

Materials and Methods

Samples and data were collected from five major abattoirs in Abuja and water bodies receiving abattoir wastewater. Abuja is the federal capital of Nigeria with a population of about 1.857 million people and one of the fastest growing cities in the world [23]. It is located in the north central part of Nigeria, with coordinates of 9°4′0′′N 7°29′0′′E and a total land area of 713 km^2(275.3 sq m). The 5 major abattoirs serving the city and its environs are the Deidei, Gwagwalada, Karu, Kubwa, and Kuje abattoirs. Animals slaughtered at the 5 major abattoirs

Sampling Procedure

Five (5) effluent samples (1000ml each) were collected aseptically from five locations in Enugu metropolis prior to analysis, these include: Ogbete abattoir effluent at the point of entry into Akwata stream, Akwata receiving water body, coal camp water body, Zik Avenue water body and Uwani receiving water body

Physicochemical Analysis

Determination of pH

The pH of each sample of abattoir and receiving water body effluent was determined using JENWAY pH meter (model 3510, Bibby Scientific Ltd, U.K) following the method of Manyi-loh *et al.* (2015)

Procedure: The electrode of the standardized pH meter was dipped into 50ml of each sample of effluent in 50ml glass beaker. The pH reading was taken on the appearance of the indicator slope and was recorded against each substrate in the test.

Determination of the Total Dissolved Solids

The total dissolved solid (TDS) of each sample of fishpond effluent was measured using CRISON multiple meters (model: CRISON MM40, Made in EU).

The electrode of the meter was dipped into 50ml of the sample and reading was taken after one (1) minute.

Determination of Total Suspended Solids

Total suspended solid was determined by fitering a 300 ml sample on a pre-weighed and pre-dried Whatman GF/A glass micro-fiber filter. The filter was then oven dried at 70°C for 24 hours and reweighed after cooling.
The difference in weight after drying was used in calculating the Tss as described by Manyi-loh et al. (2015).

$$X/W+X \times 100/1$$

Where x= weight of sample after drying

W=weight of filter paper

Determination of Total Nitrate-Nitrogen

The nitrogen content of the effluent samples was determined based on the method described by Dioha *et al.* (2013).

Extraction of Nitrate

Nitrate was extracted from 10.0gram of effluent sample in a 50ml beaker using 50ml 1M NH_4Cl_2 solution for 30 minutes, stirring every 10 minutes interval. During extraction, the nitrate was reduced to nitrite and forms a red-azo dye. The intensity of the red colour produced is proportional to the nitrate level in the sample. The nitrate level was determined using Palintest photometer.

Procedure
A round glass test tube was filled to 10 ml mark with the extract. One (1) nitricol N-tablet was ground in a motar and mixed with the extract solution to dissolve. The solution was allowed to stand for 10 minutes to develop full colour. The nitrate nitrogen was determined at a wave length of 570 nm using the photometer. The nitrate calibration chart was used to find the nitrate nitrogen concentration in the sample.

Determination of Biological Oxygen Demand (BOD) of Abattoir and Receiving Water Effluent
Biological oxygen demand is the measure of effluent strength in terms of the dissolved oxygen utilized by microorganisms to degrade organic matter in a waste water or effluent. The higher the organic matter content, the higher the BOD. The BOD of different effluent samples were determined using the method described by Sharma (2007).

Procedure
One hundred 100ml (composite sample) of each abattoir effluent collected at different discharge dates at the point of entry to Akwata stream was first diluted five (5) times with distilled water of known dissolved oxygen (DO). The dissolved oxygen of the diluted sample (250ml) was measured with dissolved oxygen meter before incubation and recorded as CO. The diluted sample was incubated together with 250ml of diluent for 5 days in the dark after which their dissolved oxygen was measured with dissolved oxygen meter and recorded as C1 and D1 respectively. The BOD was calculated as follows using Sharma`s method (2007):

BOD = A× Dilution factor, dilution factor in this case = 5
Where A= (CO-C1) - (DO-D1)
CO = dissolved oxygen of diluted sample before incubation
C1= dissolved oxygen of diluted sample after incubation
DO = dissolved oxygen of diluent before incubation
D1= dissolved oxygen of diluents after incubation

Bacteriological Analysis

Isolation and Identification of Microorganisms

Isolation Techniques
The bacterial groups in the abattoir and receiving water body samples were isolated according to the methods described by Sharma (2007), Gopinath *et al*. (2014), Pandian *et al*. (2012) and Mezes *et al*. (2015). One (1.0) ml of effluent sample was suspended in 99ml distilled water each and serial dilutions with distilled were made up to 10^{-8} folds. An inoculum from 10^{-8} fold dilution was inoculated on nutrient agar medium by streak plate technique and incubated at 35^{o}C for 24 hours. After incubation, different colonies were subcultured onto fresh nutrient agar medium to purify the isolates.

Selective cultivation using selective media was applied for fastidious organisms. Eosin-methylene blue agar was used for members of *Enterobacteriaceae*, pseudomonas Selective medium was used for *Pseudomonas* species, streptococcus agar for *Streptococcus* species,

All the plates containing the different bacterial inocula were incubated at 35^{o}C for 24 to 48 hours.

Morphological and Biochemical Characterization of the Isolates
The morphological and biochemical characteristics of the isolates from the effluents were determined according to the methods of Sharma (2007) and Norrell and Messley (2003).

Gram Staining of the Isolates
The isolates were subjected to Gram staining test to determine their Gram reaction.

Gram Staining Reagents: Crystal violet solution, lugol iodine solution, ethanol (95%) and dilute carbofuchsin.

Gram Staining Procedure

A loopful of the colony of an isolate was picked with a sterile wire loop and a smear of it was made on a clean glass slide with a drop of distilled water.

The smear was heat-fixed on the slide by flaming.

Two drops of crystal violet solution were poured on the smear and allowed to react for one (1) minute with the cell after which it was rinsed-off with running water.

Two drops of lugol iodine were poured on the smear and allowed to react for one minute and rinsed-off with water

The smear in inclined position was flooded with 95% ethanol solution to decolourize the stain and rinsed-off with water

Two drops of dilute carbofuchsin were poured on the smear as counter stain and allowed to react for 30 seconds after which the excess stain was rinsed-off with water.

The smears were air-dried on a rack for microscopy.

Microscopic examination of stained cells

The stained cells were examined to ascertain their gram reaction using oil immersion objective lens (×100) of a light binocular microscope.

Molecular Identification of the Isolates

The processes adopted in molecular identification of the isolates were extraction of the organism genomic DNA, Polymerase chain reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), sequencing of the PCR products (amplicons) and BLAST (Basic local alignment search tools) following the methods of Stephen et al. (1997) and Mullis (1990)

Extraction of Organism Genomic DNA

The genomic DNA of each of the isolate was extracted following the boiling method as described by Maria *et al.* (2008).

Procedure

Four hundred microlitres (400µl) of sterile distilled water was measured into 1.5ml Eppendorf tube each and 3 loopfuls of each of the isolate was added into the tube, covered and vortexed to dissolve. The tubes containing the isolates were put into the wells of Accublock heater (Dri-Block Techne, model: FDBO3DD R, Mbby Scientific Ltd. USA) and boiled at 100^{o}C for 10 minutes. After boiling, the tubes were cooled in ice and centrifuged at 15,000 rpm for 5 minutes. The supernatant of each isolate was decanted into fresh eppendorf tubes and used as clean DNA extracts which were stored at -20^{o}C for PCR (polymerase chain reaction) amplification.

Polymerase Chain Reaction

The 16S rRNA target region of the organism was amplified using Dream Taq^{tm} DNA polymerase (Thermo Scientific^{tm}), Universal bacterial primers 16S-27F (5`-AGAGTTTGATCMTGGCTCAG-3`) and 16S-1492R (5`-CGGTTACCTTGTTACGACTT-3`)

Reagents and Materials: Mastermix (containing DNA polymerase, dntps and $Mgcl_2$), nuclease-free water and DNA template (DNA extract), 1.5ml Eppendorf tubes, PCR tubes and Thermal cycler,

Procedure

A cocktail solution was first prepared by measuring 12µl of the mastermix, 1µl of forward and reverse primer each, 6µl nuclease-free water each (according to the number of DNA extract samples) into 1.5ml Eppendorf tube. After preparing the cocktail solution, 5µl of DNA template (isolate) and 20µl of cocktail solution were added into each Pcr tube. The pcr tubes containing the reagents were then loaded into the thermal cycler.

The thermal cycler was then programmed for polymerase chain reaction which runs in cycles starting with the initial heating at 94^{o}C for 3 minutes to activate the DNA polymerase, denaturation at 93^{o}C for 30 seconds to disrupt the hydrogen bonds between complimentary bases yielding single- stranded DNA, annealing at 58^{o}C for 1 minute to bind the primers to the complimentary part of the DNA template, initial elongation at 72^{o}C for 7 minutes for DNA polymerase to synthesize a new DNA strand complementary to DNA template strand by adding dntps that are complimentary to the template in 5` to 3` direction, final enlongation at 72 ^{o}C for 7 minutes to ensure that any

remaining single-stranded DNA is fully extended and final hold at 4°C for indefinite time for short storage of the reaction.

As a rule-of-thumb, the DNA polymerase at its optimum temperature polymerizes a thousand bases per minute. If there is no limitation due to limiting substrates or reagent at each extension step, the amount of DNA target is doubled leading to the exponential amplification of the specific DNA fragment.

Denaturing Gradient Gel Electrophoresis
The PCR products were gel-extracted using Zymo Research, Zymoclean^{tm} Gel DNA Recovery kit (Stephen *et al.* 1997).

To check if the PCR generated the anticipated DNA fragments called the amplicons, an agarose gel electrophoresis was employed for size separation of the PCR products. The sizes of the PCR products were determined with a DNA ladder-a molecular weight marker which contains DNA fragments of known size, run on the gel alongside the PCR products.

Reagents/Materials
Agarose gel was prepared by dissolving 1.0gram of the powder in 100ml of TBE buffer with 10µl ethidium bromide and heated in microwave for 3 minutes at 100°C), loading dye, DNA lather, Gel tank, Elecrophoresis machine and Transilluminator equipment.

Procedure
TBE buffer (200 ml) was first poured into the gel tank containing the gel mass then 5µl of DNA ladder mixed with the loading dye was added into the first well in the gel followed by the negative control (5µl nuclease-free water) and then 5µl of the amlpicon (PCR product) each mixed with the loading dye and added into each of the sample-well of the agarose gel.

After the addition of materials, the gel tank was connected to the electrophoresis machine and run for 45 minutes. During electrophoresis, the amplicons in the gel matrix placed in the gel tank moved from negative pole to positive under the influence of the electrolytes supplied by the TBE buffer. After gel electrophoresis, the gel was placed in phototransilluminator equipment for documentation of the separated products which appear inform of bands. The height of the bands depends on the base pair of the target DNA on the ladder.

Sequencing and Blast
The purified PCR products were sequenced in the forward and reverse directions on the ABI PRISM^{tm} 350xl Genetic Analyser. Purified sequencing products (Zymo Research, ZR-96 DNA sequencing clean-up kit ^{tm}) were analyzed using CLC main workbench 7 followed by a BLAST search (NCBL).

Determination of Bacterial Plate Count of Abattoir and Receiving Water Body Effluents
The bacterial plate count of each effluent sample was determined using multiple-tube most probable number (MPN) fermentation method of Sharma (2007). The presumptive, confirmed and completed tests were carried out as described in the Standard Methods for the Examination of water and wastewater, American Public Health Association. The numbers of positive findings were enumerated and statistical tables (MPN tables) were used to determine bacteria counts.

One (1) ml of each sample was serially diluted up to 10^{-5} folds using distilled water as diluent and incubated at 35°C for 24 hours.
After incubation, the colony forming unit of the samples was calculated using the formula:
Number of colonies × reciprocal of dilution.
Coliforms were grown in E M B Agar (36 g in 1000 mL distilled water) and then enumerated by counting the bacteria colony using colony counter.
Coliform forming unit/ml= Number of colonies × Reciprocal of dilution

Table 1: **Physicochemical Analysis of Abattoir Effluents and Receiving Water Bodies**

Effluent Samples	*Total dissolved solids mg/l*	*Total Hardness Mg/l Caco3*	*Total alkalinity Ppm*	*Turbidity NTU*	*pH*	*Nitrate-Nitrogen Mg/l*	*Conductivity*	*BOD Mg/l*
Abattoir waste water	250	195	10.4	5.0	5.8	12.50	23.50	280.30
Akwata stream	183.40	192	10.8	4.8	6.2	8.0	24.0	178.13
Coal camp water body	145.20	185	10.2	5.2	5.9	6.5	25.2	167.0
Zik Avenue water body	138.40	188	10.7	4.8	5.7	4.2	24.8	158.10
Uwani water body	132.30	173	10.4	4.6	6.0	3.4	25.4	150.20

Table 2: Average Bacterial Count in Abattoir and Receiving Water Bodies Upstream and Down Stream

Effluent sample	*Total bacterial count (TC)*	*Faecal count (FC)*	*Faecal Streptococcus*	*Faecal E. coli*
Abattoir	4.8×10^6 to 5.8×10^5	8.2×10^4 to 3.2×10^4	5.2×10^4 to 2.0×10^4	1.2×10^4 to 2.0×10^3
Receiving water body	6.2×10^3 to 1.5×10^3	2.6×10^3 to 2.2×10^2	1.5×10^3 to 1.2×10^2	4.5×10^3 to 6.0×10^2

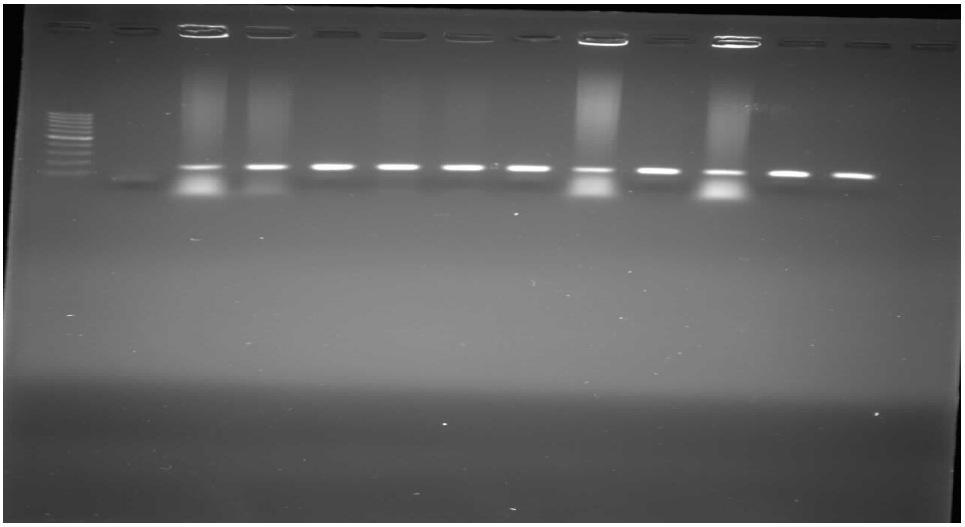

Fig. 1 Gel image of *Escherichia coli* (ZH 063) with amplicon size of 147 bp

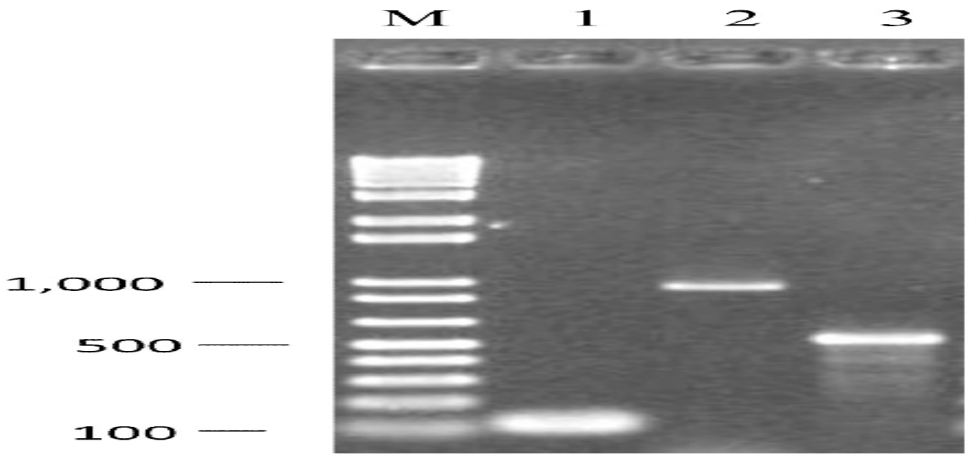

Fig. 2 Gel image of *Streptococcus faecalis* with amplicon size of 1000bp

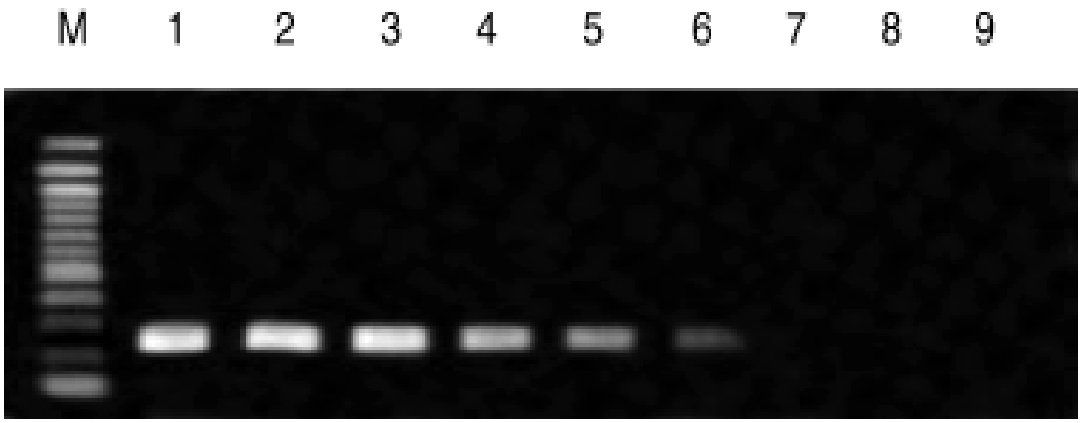

Fig. 3 Gel image of *Geobacillus stearothermophilus (NBRC 12250)* with the amplicon size of 225 bp

Results and Discussion

This study was carried out to determine the bacteriological characteristics of Ogbete Enugu abattoir effluents discharged into water bodies and their possible health hazards on receiving water bodies in Enugu, Nigeria. The result of physicochemical analysis showed high level of BOD and nitrate-nitrogen which indicates high concentration of organic matter. Presence of high organic matter in water bodies is capable promoting eutrophication resulting in high microbial activities and algal bloom growth which consequently makes the water unfit for domestic purposes. The results of the studies based on molecular analysis showed that the principal bacterial isolates from the abattoir effluents and the receiving water bodies were *Streptococcus faecalis, Geobacillus stearothermophillus* and *Echerichia coli*. The total bacterial count (TC), fecal bacterial count (FC), *Fecal streptococcus*, and *Esherichia. coli* in the abattoir effluents and the receiving water bodies upstream and downstream were determined using multiple tube most probable number (MPN) fermentation method of Sharma, (2007). The bacterial concentrations (MPN/100 mL) of abattoir effluents ranged from 4.8×10^6 to 5.8×10^5/100 mL, Fecal bacterial count FC ranged from 8.2×10^4 to 3.2×10^4/100 mL, *Fecal streptococcus* ranged from 5.2×10^4 to 2.0×10^4/100 mL, and 1.2×10^4 to 2.0×10^3/100 mL of *E. coli* while the receiving water bodies ranged from 6.2×10^3 to 1.4×10^3/100 mL of TC, 2.6×10^3 to 2.2×10^2/100 mL of FC, 1.2×10^3 to 1.2×10^2/100 mL of *F. streptococcus*, and 2.0×10^2 to 4.0×10^1/100 mL of *E. coli* 100 m upstream, and 6.6×10^5 to 6.0×10^5/100 mL of TC, 6.2×10^4 to 1.8×10^4/100 mL of FC, 1.8×10^4 to 6.0×10^3/100 mL of *F. streptococcus*, and 4.8×10^3 to 6.6×10^2/100 mL of *E. coli* 100 m downstream after mixing with the abattoir wastewater. Bacterial counts of 4.8×10^2 to 1.2×10^2/100 mL of TC, 4.0×10^2 to 2.6×10/100 mL of FC, 1.8×10^2 to

2.1 × 10/100 mL of *F. streptococcus*, and 4.8 × 10 to 1.2 × 10/100 mL of *E. coli* were observed in the water sources servicing the abattoir. Total bacterial count TC in the abattoir wastewater discharged exceeded the recommended limit for the discharge of effluents into water bodies and lands in Nigeria (FEPA. 1991). There was no significant difference ($P < 0.05$) between the mean bacterial counts of abattoir wastewater and receiving water bodies 100 meters downstream; is an indication of contamination of receiving water bodies with abattoir wastewater, similar findings has been reported in other places (Sobsey *et al.* (2002). The receiving water bodies are oten used for drinking, bathing, washing, watering of animals, watering of crops, and other domestic purpose downstream.

Fecal coliforms live in the digestive tract of warm-blooded animals; their counts are often used as a surrogate measurement for gastro-enteric pathogens, since the presence of fecal coliform bacteria is an indication of contamination by human or animal wastes. *E. coli* is the most prevalent member of the fecal coliform group; livestock harbour the bacteria and release it in their feces. And so the presence of *E. coli* in water is considered a specific indicator of fecal contamination and the presence of enteric pathogens is used as the general indicator organism that signals whether there has been fecal contamination or not. The high levels of total coliforms and *E. coli* in the abattoir wastewater and receiving water bodies are therefore an indication of the contamination of water sources with faecal material and possibly pathogenic organisms from abattoir wastewater discharged untreated; similar findings have early been reported Cadmus et al. (1999). The discharge of untreated abattoir wastewater could result in out breaks of *E. coli* infection as observed by Nelson, (1997); Cieslak et al. (1993). Bacterial pathogens such as Salmonella Barros et al. (2007), Campylobacter, and *Listeria monocytogenes* Pepperel (2003) have been isolated from abattoir wastewater. The microbial concentrations observed upstream in this study could be as a result of indiscriminate disposal of domestic wastes into water bodies by human beings in these areas.

Conclusion

This investigation discovered that untreated abattoir wastewater discharged into water bodies in Enugu, Nigeria contains bacterial counts above the recommended level for discharge into water bodies in Nigeria. Receiving water bodies were contaminated with bacteria pathogens that could impact on public health, especially that streams and rivers still serves as major sources of water supply in developing countries like Nigeria. The importance of adopting appropriate abattoir wastewater treatment measures to prevent the chances of contaminating water bodies and ground water in Nigeria is therefore recommended. Determination of specific pathogenic microorganisms in abattoir wastewater and their health impacts is recommended.

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