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Microbiological and Physicochemical Characteristics of Abattoir Wastewaters in Bayelsa and Rivers State

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Wastewater samples from abattoirs in Bayelsa and Rivers State were evaluated for their microbiological and physicochemical properties. The microbiological parameters were analyzed using standard microbiological techniques while the Physicochemical parameters were analyzed based on the APHA standard method. The mean range of the total heterotrophic bacterial, bacterial, total coliform, total *Vibrio,* total hydrocarbon utilizing, total heterotrophic fungal and hydrocarbon utilizing fungal counts of the wastewater were: ($1.9\times10^7$to 5.2×10^7, 2.1×10^5 to 4.0×10^5, 1.0×10^3 to 3.1×10^3, 1.0×10^5 to 2.2×10^5, 1.8×10^5 to c4.1×10^5 and 1.1×10^3 to 3.2×10^3)CFU/ml respectively. Kruskal-Wallis H test showed that there was no significant difference ($P\geq0.05$) in the counts for the bacterial and fungal counts. *Bacillus* sp (17.32%), *Escherichia coli (*16.33%), *Streptococcus* sp (15.34%)*, Staphylococcus* sp (14.35%), *Pseudomonas* sp (11.38%)*, Shigella* sp (10.39%) *Alcaligenes* sp (9.9%)*, Vibrio* sp (6.93%) and *Salmonella* sp (4.93%)*,* were the bacteria isolated*.* The percentage of occurrence of hydrocarbon utilizing bacteria isolates were: *Bacillus* sp (34.28%), *Pseudomonas* sp (26.71%), *Alcaligenes* sp (20%). *Escherichia coli* (14.28%), *Staphylococcus* sp (5.71%). Among the fungi isolated *Aspergillus niger, Fusarium* sp*, Geotrichum* sp were found in 18.75% each while *Penicillium* sp *recorded 23% occurrence, Aspergillus*

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fumigatus and *Candida* sp *were found in 6.25% occurrence each but Mucor* sp exhibited 8.33% of occurrence. The percentages of occurrence of hydrocarbon utilizing fungi were *Aspergillus* sp (22.22%), *Fusarium* sp (16.66%), *Penicillium* sp (33.33%), *Geotrichum* sp (16.66%), *Mucor* sp (5.55%) and *Candida* (5.55%). The mean of physicochemical parameters was: pH: 6.7-9.1, EC: 156-4981, Turbidity: 11.1-149NTU, TSS: 62-239, TDS: 104-3320, NO_3: 20.97-30.97, SO_4: 14.5-54.6, PO_4: 1.78-20.21, salinity: 41.25-2800, DO: 4.29-41.18, COD:3600-5476.0 and BOD^-:1600.0-7761.4. The high microbial load and physicochemical parameters in these wastewaters is alarming and could pose serious human health and environmental challenges if the disposal of untreated wastewater continues unabated.

Keywords: Abattoir; wastewaters; E. coli; Salmonella; Vibrio; BOD; nitrate; phosphate.

1. INTRODUCTION

The United Nations Environmental Programme has strengthened efforts across the globe to reduce the threat of pollution, however, human activities such as farming, are still prevalent in many regions of the world and animal farming for example, continues to have a negative influence on the ecosystem and biodiversity [1]. The environment is a very vital component for the existence of man and other biotic organisms. Environmental health comprised many aspects of human health including the quality of life determined by physical, biological, social and psychological factors [2]. The abattoir industry in Nigeria is a vital component of the livestock industry which provides domestic meat to over 150 million people as well as job opportunities for the workforce. Abattoir industries, on the other hand, are less established in developing nations like Nigeria in contrast to industrialized nations where these facilities used in treatment of abattoir wastes before discharge into the environment are abundant [3]. The activities carried out in abattoirs usually lead to the production of large wastes which are mostly not properly managed and in Africa, reports have shown that these wastes have a high potential of causing serious environmental problems to the environment where they are discharged [4]. It was also reported in a previous study that the abattoir operation which is very relevant to man due to the beneficial service in meat production since it provides meat for human consumption and other useful byproducts could also be a source of hazards to the public health due to the chunk of waste it generates [2]. It was also stated that, owing to the complex nature of slaughterhouse wastes, they may be extremely harmful to any area where they are released, particularly since there are residues of metals in animal organs, blood, kidneys, and liver, as well as a diverse microbial population in faeces [5].

The meat processing industry uses 29% of all freshwater utilized by the agriculture sector throughout the world. Water used in cleaning carcasses of slaughtered animals and washing of slaughter house floor has been referred to as abattoir wastewater [6]. Wastewaters from abattoir are sometimes discharged untreated into the environment or it could flow through constructed drainage channels into water bodies and this could lead to gross pollution of the receiving environment. Most wastewater released in developing countries are mostly untreated before being discharged into water bodies [6]. In a previous study, it was reported that most of the wastewater from the Sokoto abattoir are discharged into the surrounding soil, while the rest is directed into the River Rima via the abattoir drainages as a result of the buildup of certain nutrients and heavy metals, soil fertility may deteriorate, resulting in low production in the surrounding farmlands, as well as injuries and the extinction of aquatic life [2]. The risk of zoonotic infections among the consumers of such irrigated areas cannot be ruled out since both river and soil water is utilized for irrigation farming along the river banks [7]. The aim of this study is to evaluate the microbiological and physicochemical properties associated with abattoir wastewater in Bayelsa and Rivers State.

2. MATERIALS AND METHODS

2.1 Sample Collection

Wastewater samples were collected using the method of Adesemoye [1]. Sterile 1.5 litre bottles were used to aseptically collect the abattoir wastewater (i.e. wastewater produced after washing the carcasses of slaughtered animals and roasted cowhide). The control sample for this experiment was collected from the freshwater source of the abattoir before use for animal dressing. The wastewater samples were

Table 1. Map coordinates abattoir locations

Location	Northing (N)	Easting (E)
Igbogene	5^0 2' 17.8188"	6^0 24'14.958"
Tombia	4^0 57' 17.8092"	6^0 20'53.2428"
Opolo	4^0 56'52.764"	6^0 20'3.984"
Swale	4^0 53'42.9576"	6^0 16'39.7164"
Rumuokoro	4^0 52' 11.64"	7^0 01' 026"

collected at three different points as the wastewater was running off the drains after animal dressing. About 500ml of sample water was collected from each point and were pooled together to constitute a composite sample. The samples were collected from abattoirs in Yenagoa Local Government Area of Bayelsa State and from abattoirs in Obio-Akpor Local Government Area of Rivers State. The samples on collection were aseptically transported in ice-packed coolers to the Microbiology laboratory of the Rivers State University and were analyzed immediately.

2.2 Isolation, Enumeration and Characterization of Microorganisms

2.2.1 Total Heterotrophic Bacteria (THB)

This was determined with the nutrient agar using the pour plate technique as described by Prescott *et al.* (2011). Ten millilitres (10ml) of wastewater sample were weighed into 250ml conical flask containing 90ml sterile normal saline to give an initial 10^{-1} dilution. With the aid of sterile pipette, 1ml was taken from this dilution into the test tube containing 9ml of sterile normal saline to achieve 10^{-2} dilution. This sequence was repeated until dilution 10^{-6} was obtained. 1ml aliquot of 10^{-6} dilution was transferred aseptically unto sterile petri dishes and 10ml of molten sterile agar cooled to about 45^{o}C was added aseptically, swirled and allowed to solidify. Samples were plated in duplicates. Plates were incubated in an inverted position at 35 ± 2^{o}C for 24 hours after which viable colonies were counted.

2.2.2 Total Coliform Bacteria (TCC)

This was determined with MacConkey Agar using the spread plate technique as described by Prescott [8]. Ten millilitres(10ml) of wastewater sample were weighed into 250ml conical flask containing 90ml sterile normal saline to give an initial 10^{-1} dilution. One ml was taken from this dilution into the test tube containing 9ml of sterile normal saline to achieve 10^{-2} dilution. This sequence was repeated until dilution 10^{-6} was obtained. Aliquot (0.1ml) of 10^{-4} dilution was transferred aseptically unto the surface of pre-dried sterile petri dishes. Plates were spread using sterile bent glass rod, and were incubated in an inverted position at 35 ± 2^{o}C for 24 hours after which viable colonies were counted.

2.2.3 Total *Vibrio* Count (TVC)

This was determined with Thiosulphate Citrate Bile Salt (TCBS) Agar using the spread plate technique as described by Prescott [8]. Ten millilitres (10ml) of sample was transferred into 250ml conical flask containing 90ml sterile normal saline to give an initial 10^{-1} dilution. With the aid of sterile 1ml pipette, 1ml was withdrawn from the initial dilution into a test tube containing 9ml of sterile normal saline to achieve 10^{-2} dilution. This sequence was repeated until dilution 10^{-6} was obtained. Aliquot (0.1ml) of 10^{-4} dilution was transferred aseptically unto the surface of pre-dried sterile petri dishes. Plates were spread using sterile bent glass rod, and were incubated in an inverted position at 35 ± 2^{o}C for 24 hours after which viable colonies were counted.

2.2.4 Total Hydrocarbon Utilizing Bacteria (THUB)

The Vapour Phase Transfer method of Mills and Colwell [9] was adopted to determine the population of hydrocarbon utilizing bacteria. Aliquots (0.1ml) of the serially diluted samples were inoculated on mineral salt agar media using the spread plate technique as described by Odokuma [10]. Sterile filter paper discs soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar was placed aseptically to the cover of the inoculated agar plates. The plates were incubated for 5 days at 35 ± 2^{o}C after which viable colonies were counted. After the incubation period, mean of the colonies were recorded.

2.2.5 Total Heterotrophic Fungi Count (THF)

This was determined using the Potato Dextrose Agar (PDA**)** onto which 1% of lactic acid was added to suppress bacterial growth [11]. The spread plate technique as described by Prescott *et al.* (2011) was adopted. An aliquot (0.1ml) of the appropriate serially diluted samples were inoculated in duplicates onto sterile pre-dried PDA plates and then spread evenly with a sterile glass spreader. The plates were incubated at 25^{o}C for about 5 days after which the colonies were counted and the mean of the count recorded accordingly [12].

2.2.6 Total Hydrocarbon Utilizing Fungi (THUF)

The Vapour Phase Transfer method of Mills and Colwell (1978) was adopted to determine the population of hydrocarbon utilizing fungi. Aliquots (0.1ml) of the serially diluted samples were inoculated on mineral salt agar media added with 1% lactic acid to suppress bacterial growth [11] using the spread plate technique as described by Odokuma [10]. Sterile filter paper discs soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar was placed aseptically to the cover of the inoculated agar plates. The plates were incubated at 25^{o}C [12] for about 5 days. After the incubation period, mean of the colonies were recorded.

2.3 Purification of Isolates

After the incubation periods, morphologically distinct and discreet colonies were streaked on respective agar plates to obtain pure cultures. Pure isolates of total heterotrophic bacteria and total hydrocarbon utilizing bacteria were obtained by picking (with sterile wire loop) distinct culturally and morphologically different colonies from the various media plates and streaked on pre-dried NA plates while pure cultures of fungal isolates were streaked on pre-dried PDA plates. Plates for bacteria were incubated at 35 ± 2 ^{o}C for 24 hours while the fungal plates were incubated at 25^{o}C for about 5 days.

2.3.1 Identification of bacterial isolates

Pure bacteria isolates were identified by the method described by Cheesebrough [13]. Pure bacterial isolates were subjected to Biochemical tests which include oxidase test, catalase test, indole test, methyl red test, Voges Proskauer test, starch hydrolysis test, urease test, citrate test, sugar fermentation test and Triple Sugar Iron agar test. Procedure for the respective biochemical tests were carried out according to standards (Cheesbrough, 2005). Bacterial isolates were identified with reference to the Bergey's Manual of Determinative Bacteriology [14].

2.3.2 Identification of fungal isolates

Pure mould isolates were identified using their morphological features followed by microscopic examination of their wet mounts prepared with lactophenol-cotton blue and reference made to a [15] fungal identification atlas by Barnett and Hunter). Yeast isolates were also identified using their morphological characteristics, followed by microscopic examination of their wet mount prepared with normal saline, reference was also made to a fungal identification atlas by Barnett and Hunter [15]. The yeast isolates were further identified using Gram-staining, Sugar fermentation, oxidation and fermentation tests.

2.4 Physicochemical Parameters

The physicochemical parameters of soil samples were analyzed based on the APHA-standard methods for the examination of water and wastewater [16].

2.4.1 Determination of Ph

The Hanna HI pH meter (Model 8424, USA) was used. The meter was switched on and then standardized with two buffer solutions of higher (10) and lower (4) pH by dipping the electrode in the buffer solutions. The electrode of the pH meter was rinsed thoroughly with distilled water and was finally dipped into the water sample and the pH value recorded.

2.4.2 Determination of salinity

Salinity was taken from the sample above. The salinity probe was calibrated in a '0' (zero) salinity water. The probe was then placed in the wastewater sample to read the salinity values.

2.4.3 Determination of electrical conductivity

A calibrated (0.01M KCl) conductivity meter was inserted into the wastewater samples and the values were read and recorded.

2.4.4 Determination of turbidity

The turbidity of the wastewater samples was measured using Schimadzu UV-160A double beam UV recording Spectrophotometer at the wavelength of 400nm. The machine is a 20-parameter programmed and calibrated spectrophotometer. The program number four (4) for turbidity was entered and the meter was automatically ready for turbidity measurement at 400nm. Following standardization with distilled water, the measurement for the samples was made and the concentrations read off from the in-built calibration curve which is displayed on the screen.

2.4.5 Determination of total suspended solids

This parameter was determined by entering program number twenty (20) for total suspended solids on the Schimadzu UV-160A recording spectrophotometer at 400nm . After standardization with distilled water (blank), the samples were then introduced and the values read off as displayed on the screen.

2.4.6 Determination of total dissolved solids

The gravimetric method as described by APHA [16] was used. The evaporating dish was heated first at 200℃ in the oven for 1 hour. It was subsequently cooled to room temperature, weighed and stored in a desiccator. Fifty milliliters of the samples were filtered using a Whatman No 1 filter paper and transferred to the pre-weighted dish and then evaporated to dryness in the oven and dried for 1 hour at 105^0C. The dish was subsequently cooled to room temperature in a dessicator and weighed. The level of Total Dissolved Solids (TDS) was calculated as shown below;

$$TDS/L \quad = \frac{(A-B)\ x\ 100}{Sample\ volume}$$

Where;

A = Weight of sample + dish in mg
B = Weight of dish in mg

2.5 Biological Oxygen Demand (BOD_5)

Biochemical Oxygen Demand (BOD_5) was determined after incubation of wastewater samples for five days at 20℃. BOD tests are generally carried out by measuring the amount of dissolved oxygen present in the sample before and after incubation in the dark for 5 days at 20°C.

The Azide Modification method as described by APHA (1995) was used.Phosphate buffer solution, Magnessium sulphate solution, Calcium chloride solution and Ferric chloride solution were prepared as the dilution water. The water sample was diluted by 2%. Into the 125ml BOD bottles, 100ml of the 2% diluted samples were placed using a 50ml long tipped pipette. Extra 25ml of the diluted sample was added to the bottle to bring it to brim. The stoppers were inserted living no air bubble in the bottle. An initial determination of DO was taken before dilution from one of the duplicate bottles. The samples were then incubated including a blank for 5 days in the dark at 20℃ on the 5^{th} day, the DO was measured in the incubated samples and the blank.

$$BOD\ (mg/L) \quad = \frac{DO0\ -\ DOd}{\%dilution}$$

Where;

D_0 = DO of the sample immediately after saturation with air (initial DO)
D_d = DO of the sample after 5 days incubation (final DO)

2.5.1 Determination of chemical oxygen demand

The Dichromate Open Reflux method of Chemical Oxygen Demand (COD) determination (was adopted. 0.4g mercuric sulphate ($HgSO_4$) was weighed into a refluxing flask and 20ml 0f sample was added. Then, 10ml of $K_2Cr_2O_7$ were slowly added using a pipette and gently swirling. After which 30ml $HgSO_4$ in H_2SO_4 solution (Silver sulphate in conc H_2SO_4) was added. The refluxing condenser was then connected. The mixture was then refluxed for 2 hours until the colour of the mixture changed from orange to greenish yellow. The system was cooled and the condenser washed into a conical flask, the content was left to cool to room temperature. With the aid of a 0.05M Ferrous Ammonium Sulphate (FAS) solution, the excess dichromate solution was titrated using ferroin as the indicator. A blank titration was done.

The Chemical Oxygen Demand (COD) was calculated as shown below;

$$mg\ COD\ = \frac{A - B\ x\ M\ x\ 8000}{vol\ of\ sample(ml)}$$

Where:

A = Volume of FAS used for the blank
B = Volume of FAS used for sample
M = Molarity of FAS
8000 milliequivalents weight of oxygen x 1000 ml/l

2.5.2 Determination of alkalinity

One hundred milliliters of the sample were transferred to a 250ml Erlenmeyer flask. Two drops of phenolphthalein indicator were added and titrated with 0.02N HCl and monitored for colour change. Otherwise, 2 drops of methyl orange indicator were added and titrated till colour changes to yellow from pink at pH 4.3 with 0.02N HCl. The volume of the titrant used was recorded. The total alkalinity was then calculated.

$$Alkalinity\ mg\ (CaCO3/L) = \frac{vol\ of\ titrant\ \times\ C\ \times\ 50{,}000}{vol\ of\ sample}$$

Where:

C = Concentration of titrant

2.5.3 Determination of total hardness

One hundred milliliters of the sample was placed in a 250ml conical flask. 5ml of Ammonia/ Ammonium chloride buffer was added followed by 5 drops Eriochrome black T indicator. The burette was filled with a 0.01M EDTA solution and was titrated. A change from the wine red colour to a marine blue colour at the end point was observed.

1ml EDTA ≈ 1mg $CaCo_3$

2.5.4 Determination of phosphate

Available phosphate was determined using the method of Bray and Kurtz [17] Fifteen millilitres (15ml) of 1M solution of Ammonium fluoride and 25ml of 0.5M HCl were added into a 500ml volumetric flask. 460ml of distilled water was added to make up the mark. Two millilitres (2ml) aliquot of the wastewater were transferred into 20ml test tube, 5ml distilled water added followed by 2ml of ammonia solution. The contents were mixed well. Finally, 1ml of stannous chloride was added and mixed again. The colorimeter was set at 660^{o}nm and absorbance values were taken. The amount of phosphate in the sample was determined from the standard curve prepared with phosphate standard solutions.

2.5.5 Determination of Nitrate

Nitrate level was determined using spectrophotometric method. Schimadzu UV-160A recording Spectrophotometer (Plate 3.4 above) was used. In the Spectrophotometer, the stored program number fourteen (14) was entered at the wavelength 425nm. The zeroing vial was then filled with 25ml of the sample (blank). A second vial was filled with 25ml of the sample and the contents of one nitriVER v reagent powder pillow was added. The vial was swirled to mix and a one minute reaction period was allowed. The zeroing vial then used to zero the instrument, the prepared sample vial was placed in the cell holder and the result in mg/l NO3 was displayed.

2.5.6 Determination of sulphate

Turbidimetric method as described by APHA [16] was used. One hundred milliliter of the sample was measured into a 250ml Erlenmeyer flask followed by addition of 20ml buffer solution and then stirred, while stirring a spoonful of $BaCl_2$ crystals was added. This was stirred for 60 seconds at a constant speed. After stirring, the solution was poured into the absorbance cell of photometer and turbidity measured at 420nm. The SO_4^- was estimated by comparing turbidity reading with a standard working sulphate concentration from 0.01mg/ml – 0.1mg/ml treated as sample above, with turbidity measured at 420nm using distilled water as blank.

Calculation:

SO_4^{2-} = Absorbance of sample x Gradient of the standard graph.

Ten millilitres (10ml) of sample was pipetted into a 25ml volumetric flask and made up to volume with distilled water. Iml of a gelatin-$BaCl_2$ reagent (0.6g gelatin in 200ml hot distilled water + 2g $BaCl_2$) was added, making the volume up to mark and mixed. The content is left to stand for 30minutes (the test solution formed a whitish precipitate) and was measured at 420nm using a HACH DR 890 colorimeter.

2.5.7 Determination of chloride

The Silver – Nitrate Titration method of APHA (2012) was used. Twenty-five milliliter of the

sample was measured into 100ml conical flask. Two drops of potassium dichromate were added and then titrated with silver nitrate until the appearance of a brick red colour which is the end point. The titre volume was recorded.

$$Amount\ of\ Chloride\ (Mg/l) = \frac{Titre\ (ml)\ \times\ 100}{Vol.of\ sample\ (ml)}$$

2.6 Statistical Analysis

The mean and standard deviations of the microbial counts were computed. The log counts were used in plotting the graph. Also, the frequencies of microorganisms across the abattoir sites were determined. The Analysis of variance (ANOVA) was used in checking for significant difference in the microbial counts across the abattoir samples while Kruskal Walis H test was used in separating the means. All analysis was done using SPSS (version 27).

3. RESULT

Results of the mean values of total microbial (bacterial and fungal) counts obtained from the abattoir wastewaters are presented in Fig. 1. The Figure shows the Total heterotrophic bacterial counts, total hydrocarbon utilizing bacterial count, total fungal count, total hydrocarbon utilizing fungal count, total coliform counts and total *Vibrio* counts.

The highest and lowest mean total heterotrophic bacterial count of 5.2×10^7CFU/ml and 1.9×10^7CFU/ml was obtained in abattoir wastewater samples from Opolo and Tombia abattoir samples, respectively. The highest and lowest mean total hydrocarbon utilizing bacterial count of $2.2\times10^5$and 1.0×10^3CFU/ml was obtained in wastewater samples from Tombia and Swale abattoir samples respectively. Mean Total coliform count ranged from 2.1×10^5 to 4.0×10^5CFU/ml. The highest and lowest coliform count was recorded in samples from Opolo Abattoir and Rumuokoro abattoirs respectively. Mean *Vibrio* count ranged from 1.0×10^3to3.1×10^3 CFU/ml. *Vibrio* sp were not detected in the control.

Kruskal-Wallis H test showed that there was no statistically significant difference in the microbial counts across the sampled locations p >0. 05.

The mean counts of the total heterotrophic and hydrocarbon utilizing fungi from abattoir wastewater samples ranged from 1.8×10^5 to 4.1×10^5CFU/ml and 1.0×10^3 to 3.2×10^3 CFU/ml, respectively. The highest fungal load of the wastewater sample was recorded in samples collected from Tombia abattoir while the samples from Opolo had lowest fungal load. The total hetrotrophic fungal load in the control sample was similar to those recorded Igbogene and higher than those recorded for Opolo and Swale, respectively. Kruskal-Wallis H test showed that there was no statistically significant difference in the total fungal counts and hydrocarbon utilizing fungal counts of the samples (P>0.05).

The result of bacteria isolated from the abattoir wastewater is presented in Fig. 2.

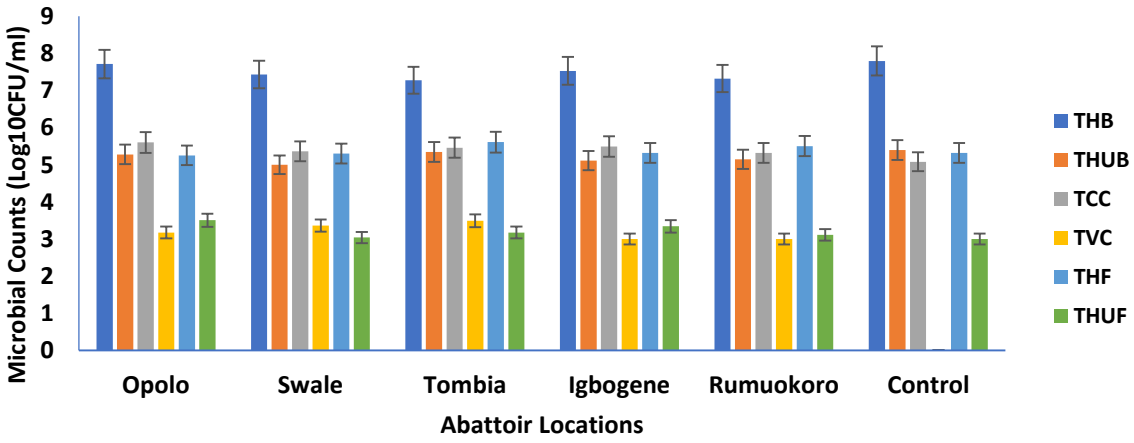

Fig. 1. Microbial load of the different Abattoir Wastewater samples

Key: THB = Total heterotrophic bacteria; THUB = total hydrocarbon utilizing bacteria; TF = total fungi; THUF = total hydrocarbon utilizing fungi; TCC = total coliform counts, TVC = total Vibrio counts

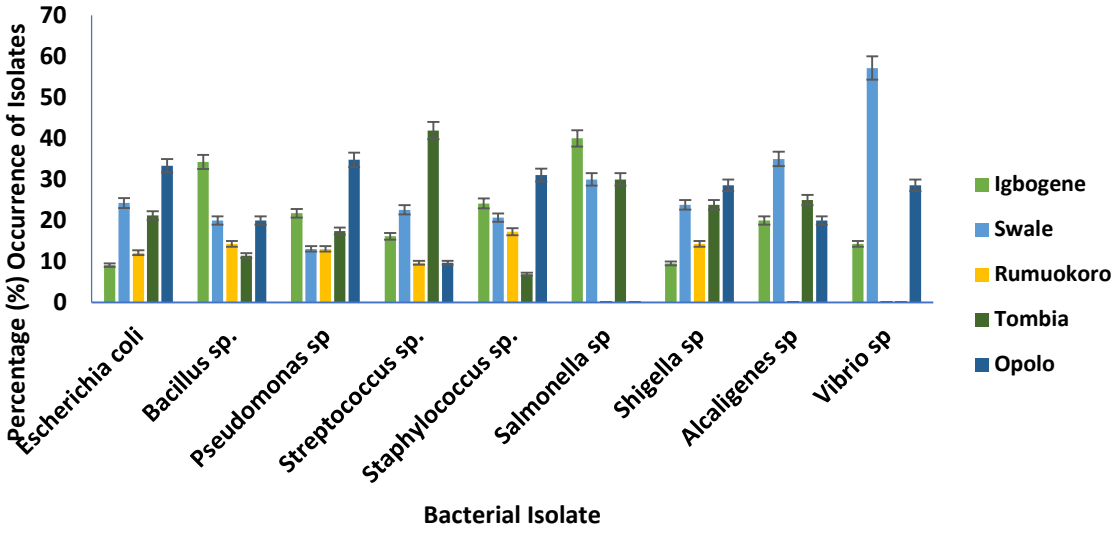

Fig. 2. Frequency of Occurrence of Bacteria Isolated From The Abattoir Wastewater

Bacteria isolated include *Alcaligenes* sp, *Bacillus* sp, *Escherichia coli*, *Pseudomonas* sp, *Staphylococcus*sp, *Streptococcus* sp, *Salmonella* sp, *Shigella* sp, *and Vibrio* sp. Organisms like *Bacillus* sp, *Pseudomonas* sp, *Streptococcus* sp and *Shigella* sp were identified from all the wastewater samples. *Salmonella* sp and *Vibrio* sp were characterized in samples from three abattoir locations while *Alcaligenes* sp were isolated from all the samples except from the Rumuokoro wastewater sample. The percentage of occurrence of the total heterotrophic bacteria characterized from abattoir wastewater are *Bacillus* sp (17.32%), *Escherichia coli* (16.33%), *Streptococcus* sp (15.34%), *Staphylococcus* sp. (14.35%), *Pseudomonas* sp (11.38%)., *Shigella* sp (10.39%) *Alcaligenes* sp (9.9%). *Vibrio* sp (6.93%) and *Salmonella* sp (4.93%). The percentage of occurrence of hydrocarbon utilizing bacteria isolates obtained were *Bacillus* sp (34.28%), *Pseudomonas* sp (26.71%), *Alcaligenes* sp (20%), *Escherichia coli* (14.28%) and *Staphylococcus* sp (5.71%).

Result presented in Fig. 3 represents the distribution of characterized fungi isolates in abattoir wastewater samples from the examined locations. Fungal species identified include *Aspergillus niger, Aspergillus fumigatus, Penicillium* sp, *Fusarium* sp, *Geotrichum*sp, *Mucor* sp and the yeast *Candida* sp. *A. niger* was found in all abattoir wastewater samples with the highest count in the sample from Tombia abattoir. *A. niger*, *Fusarium* sp and *Penicillium* spwas found in all samples but sample from Igbogene had the highest counts while the least count was in samples Rumuokoro and Opolo. *Penicillium* sp was more frequent in Tombia wastewater. *Geotrichum* sp was isolated from all the wastewater samples save Igbogene sample. *Mucor sp* was isolated from samples obtained from Igbogene, Tombia and Swale wastewater samples. The yeast, *Candida* sp was isolated from Igbogene, and Swale samples. *Mucor* sp was isolated from Igbogene, Tombia and Swale abattoir wastewater samples.

In summary, *Aspergillus* sp were the most frequently isolated with a percentage occurrence of 18.75% and 6.25% for *Aspergillus niger* and *Aspergillus fumigatus,* respectively. *Penicillium* sp had 23% occurrence. While others are: *Geotrichum* sp (18.75%), *Fusarium* sp (18.75%) and *Mucor* sp (8.33%). The percentages of occurrence of fungi with hydrocarbon utilizing potentials are *Penicillium* sp (33.33%), *Aspergillus* sp (22.22%), *Fusarium* sp (16.66%), *Geotrichum* sp (16.66%), *Mucor* sp (5.55%) and *Candida* occurred at 5.55%.

The result of the mean values and range (in parenthesis) of physicochemical properties of the abattoir wastewater samples examined are presented in Table 1. The highest mean pH value was 7.19 obtained from Swale while the least pH value was 6.83 obtainable from Opolo wastewater. The highest mean electrical conductivity was 4981(μScm^{-3}) as obtained in Tombia abattoir while the least value recorded was 156(μScm^{-3}) obtained in Swale samples.

The turbidity test of the soil samples indicated the highest value in Tombia (149NTU) and the least was in Rumuokoro (16.9NTU). Total Dissolved Solids (TDS) values ranged between 3320-104mg/kg in Tombia and Swale respectively. TSS values recorded were between 62and 237mg/l. The salinity range of the abattoir wastewater samples were 41.25mg/l (Swale wastewater)and2800 (Tombia wastewater). The DO values for abattoir wastewater ranged from 4.29mg/l (Igbogene)and41.18mg/l (Rumuokoro).

DO is a measure of the degree of pollution by organic matter, the destructive of organic substances as well as the self-purification. COD values obtained ranged from 3600–5110.7 mg/l. The highest COD value was recorded in Swale (5470mg/l) and lowest in Igbogene with 3600mg/l value. The BOD values ranged from 1605.0–7761.4mg/l. The highest BOD_5 was recorded in Swale and least in Opolo (7761.4 and 1600.0mg/l, respectively).

Statistical comparison (P≤0.05) of the physicochemical properties of wastewater with the control samples indicated a significant difference in all the parameters examined.

Total suspended solid valued ranged from 23 to 62mg/l while Total dissolved solids ranged from 104–1011. The concentration of nitrate ranged from 22.07 to 30.97mg/l; 10.4–54.6 mg/l sulphate and 1.78–20.11 mg/l phosphate and the mean nitrate value is below the WHO limit of 250 mg/l. The mean phosphate is higher than the WHO limit of 5mg/l for the discharged of wastewater into rivers.

4. DISCUSSION

The abundance of *Bacillus* in the wastewater may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment [18]. More so, the presence of *E. coli*, *Salmonella, Shigella* and *Vibrio* spin the abattoir wastewater may be attributable to the discharge of the content of animal bowels onto the water. These findings are in conformity with that of Adesemoye [1] as well as Ogbonna and Igbenijie [19] who also recorded these mentioned organisms in their study. This study also confirmed the presence of hydrocarbon utilizing bacteria belonging to the genera*Alcaligenes*, *Bacillus*, *Escherichia*, *Micrococcus*, *Proteus*, *Pseudomonas*, and *Staphylococcus* in these environmental samples. Drilling wastes have been reported to be degraded by bacterial isolates identified as species of *Staphylococcus*, *Serratia*,*Acinetobacter*, *Alcaligenes*, *Clostridium*, *Enterobacter*, *Nocardia*, *Bacillus*, *Actinomyces*, *Micrococcus* and *Pseudomonas* sp *by* Benka-Coker and Olumagin [20]. This present study also reported the characterization of six genera of fungi from contaminated abattoir wastewater. They include members of the genera *Aspergillus, Fusarium, Geotrichum*, *Mucor*, *Candida* sp and *Penicillium* sp. The finding is similar to the microorganisms reported by Abolagba and Igbinevbo [21]. Anthropogenic activities such as

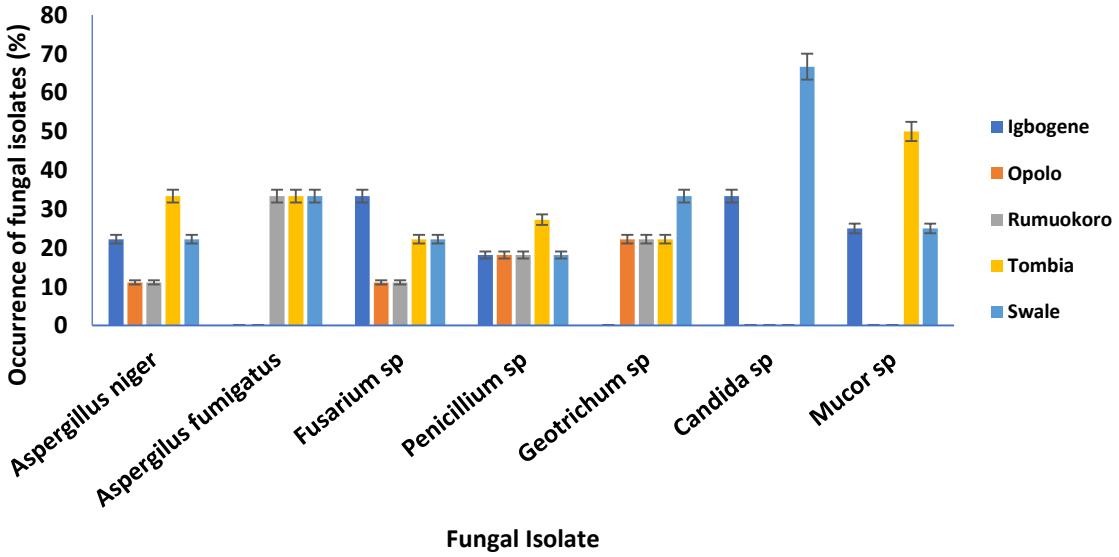

Fig. 3. Frequency of occurrence of Fungi isolated from the Abattoir wastewater

Table 1. Mean values and Range (in Parenthesis) of Physicochemical Properties of Abattoir Wastewater Samples

Parameter	Abattoir Location						WHO/FEPA Limit
	Igbogene	Opolo	Rumuokoro	Tombia	Swale	Control	
pH	6.83 (6.33 – 7.33)	6.87 (6.68 – 7.08)	6.94 (6.73 – 7.28)	6.98 (6.82 – 7.24)	7.19 (7.07 – 7.31)	6.73 (6.65 – 6.80)	6.5 – 8.5
Electrical Conductivity (μScm^{-3})	1517 (1336 -1767)	3440 (3380 -3508)	374 (360 - 394)	4981 (4972 - 4995)	156 (142 - 168)	74 (70-77)	1250
Turbidity NTU	21.9 (21.7 – 22.3)	28.4 (27.7 – 29.4)	16.9 (16.5 – 17.4)	149 (140 - 157)	11.1 (11.0 – 11.3)	0.598 (0.57 -0.642)	15
TSS (mg/l)	116 (110 - 124)	222 (203 - 235)	239 (218 - 287)	237 (242 - 287)	62 (62 - 68)	1 (-)	20
TDS (mg/l)	1011 (933 - 1000)	2293 (2200 – 2379	249 (245 - 252)	3320 (3290 - 3370)	104 (98 - 108)	49 (46 - 53)	200
Alkalinity (mg/l)	40 (39 - 41)	52 (49 - 53)	35 (32 - 39)	56 (50 - 60)	14.2 (14.0 – 14.6)	10 (8 - 13)	30-500
Total Hardness	600 (600 - 563)	950 (940 - 956)	50 (45 - 57)	1200 (1050 - 1300)	14.2 (13.8 – 14.2)	6 (5.0 – 7.5)	1500
Salinity (mg/l)	568 (568 - 580)	165 (157 - 170)	152 (144 - 157)	2800 (2798 - 2882)	41.25 (40.7 – 41.6)	26.3 (25.8 - 27)	
NO_3 (mg/l)	22.97 (20.8 -24.61)	30.97 (29.8 -32.01)	26.97 (26.2 – 27.6)	25.77 (24.77 -26.62)	20.97 (20.61 -21.21)	9.9 (9.5 – 10.2)	50
SO_4 (mg/l)	46	54.6	14.5	10.40	25.6	25.2	500
PO_4 (mg/l)	4.7 (4.6 – 4.9)	3.54 (3.4 – 3.62)	20.11 (19.23-21.1)	1.78 (1.1 – 2.34)	8.00 (7.5 – 8.7)	2.05 (1.95 – 2.1)	0.4
DO (mg/l)	4.29 (4.15 – 4.5)	5.25 (5.12 – 5.4)	41.18 (40.5 – 42.1)	4.92 (4.5 – 5.5)	6.05 (5.8 – 6.25)	6.58 (6.35 – 6.8)	4
COD (mg/l)	3600	4732.4	3790.5	5110.7	5476.0	1125.5	1000
BOD_5 (mg/l)	2203.0 (2103 -2295)	1600.0 (1581 -1620)	1605.0 (1600 -1610)	2587.0 (2555 - 2615)	7761.4 (7622.5-788.4)	512.4 (509.8 - 521)	20

roasting of cowhide meat for human consumption further generates diverse pollutants (such as PAHs and others complex organic pollutants) dispersed as particulate matter into the air and also deposited into the abattoir wastewater. Consequently, the assay for hydrocarbon degraders in the abattoir effluents. The ability of fungi to degrade hydrocarbons is of immense importance in bioremediation procedures. Out of the obtained six genera of fungi four demonstrated hydrocarbon utilizing potentials namely, *Aspergillus, Fusarium, Geotrichum*, and *Penicillium*. Some of these organisms have earlier been reported as hydrocarbon degraders by April [22]. Hydrocarbon-utilizers isolated from the abattoir effluent by Goddey and Rasheed [23] included *Candida* sp*., Rhodotorula* sp*., Fusarium* sp*., Penicillium chrysogenus and Aspergillus niger.* Amaku and Obire [24] demonstrated the ability of *Aspergillus*, *Penicillium*, *Mucor, Rhizopus* and *candida* species isolated from Forcados effluent to biodegrade hydrocarbons.

The occurrence of biomarkers of faecal contamination in wastewaters that are being discharged into the environment without any form of treatment poses serious risks to human health, environmental challenges and impact aquatic flora and fauna in cases where the effluents are channeled into adjoining rivers. The need for waste treatment before they are dumped on soil or discharged into the environment is very paramount because the thus polluted rivers are yet resourceful for other human activities.

The pH is a prevailing environmental factor which affect the metabolic activities of microbes by influencing the functioning of enzymes, hormones and proteins. pH is also a major factor in all chemical reactions associated with formation of, alteration and dissolution of chemical [25]. The pH of the wastewater samples examined in this study waswithin the WHO permissible limit of 6.5 – 8.5. The electrical conductivity is the measure of a material's ability to allow the transport of an electric charge. It is measured by the presence of total concentration of ions, temperature of the system, e.t.c. The conductivity levels in the samples of Igbogene, Opolo and Tombia were above the European Economic Community (EEC) maximum limits while conductivity of Rumuokoro and Swale were within the EEC maximum of 1250 μScm^{-3}. The low values reported in the Rumuokoro and Swale abattoirs indicates low organic matter deposition and decomposition in the wastewater. The obtained high values might be as a result of decomposition and mineralization of organic matter within the abattoir environment.The electrical conductivity values were observed to be very high in abattoirs that make use of confined containers to wash the meat and cowhide being processed (viz., Opolo, Igbogene and Tombia abattoirs) and least in Swale and Rumuokoro abattoir where animal products are washed in running stream. It was observed that the handlers don't change the water until the close of work for the day. This gives room for all ions, organic and inorganic particle including other suspended solids to build up in the wastewater container as the operation for the day progresses.

Consequently, turbidity was also highest in Tombia (which recorded highest value of conductivity). Turbidity level values ranged from values which were above the WHO permissible limit in four out of five abattoirs assessed. High turbidity obstructs the penetration of light and consequently the water body loses its capacity to support the growth of diverse aquatic organisms. Total Suspended Solids (TSS) is the filterable particles in water while Total DissolvedSolids (TDS) is the dry-weight of particles trapped by the filter. TSS includes a wide variety of material, such as silt, decaying plant and animal matter, zooplankton, algae and bacteria, industrial wastes, and sewage. High concentrations of suspendedsolids can cause many problems for stream health and aquatic life. TSS and TDS values recorded in wastewater samples from all the abattoirs were higher than the WHO limits. These TSS and TDS values are above the WHO permissible limits. These observed high concentration values were as a result of organic particles scaled from the surface of the roasted cowhide, blood, gut content and other particulate materials from abattoir floor and tables being discharged into water. U.S. Environmental Protection Agency (EPA) advises against consuming water containing more than 200mg/l of TDS, although many health specialists believe that ideal drinkingwater should be less than 50mg/l of TDS or lower. Alkalinity refers to the acid neutralizing capability of a water body. It is a measure of the buffering capacity of a system which indicates that the water with high alkalinity is considered to be well buffered against acidic input. The recorded alkalinity values recorded in Swale wastewater was the only value lower than the WHO permissible limit of 30-500mg $CaCO_3$/l while other abattoirs recorded values within the acceptable limit.

Nutrients including nitrate, sulphate and phosphate were assessed in abattoir wastewater. These chemical substances are required for microbial growth and proliferation. Although nitrate and sulphate values were higher than phosphate values yet the values are below WHO permissible limits. Nevertheless, the highest values of these nutrients were recorded in Igbogene and Opolo. These abattoirs make use of confined containers to wash abattoir products (there is no flowing water body). Consequently, the accumulation of these nutrients. Swale abattoirs having the privilege of a flowing water body in which abattoir products are washed recorded lower values of these nutrients. Excess concentration of nitrate can give rise to methaemoglobinemia, a situation whereby hemoglobin is oxidized to methemoglobin, the heme iron becomes (Fe^{3+}) and is incapable of binding oxygen. Methemoglobinemia is suspected in any cyanotic patient with no evidence of heart and lung disease. Cyanosisis due to decreased oxygen saturation. Phosphates had the lowest recorded values (among nutrients assessed) and the values are higher than the WHO limit of 0.4mg/l for domestic and industrial waters in all the abattoirs. 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. Dissolved oxygen (DO) is a measure of the degree of pollution by organic matter, the destructive of organic substances as well as the self-purification. Dissolved oxygen level with values below 2.0mg/l is indicative of environmental stress [26]. The values recorded in this study were above the FEPA permissible limit in all the abattoir stations. Biochemical Oxygen Demand (BOD) is the amount of dissolved oxygen needed (i.e., demanded) by aerobic biological organisms to break down organic materials such as sulphides, ferrous ions, and nitrogen, present in a given water sample at certain temperature over a specific time duration. BOD_5 indicates the amount of organic matter degradable by microbial metabolism on the assumption that the water has no bactericidal or bacteriostatic effects (APHA, 2012) [27,28]. Therefore, the more organic material presents in the abattoir wastewater, the higher the BOD. BOD_5 and COD values recorded in wastewater from all the abattoirs were higher than the WHO permissible limit. This can be attributed to the fact that abattoir activities generate huge organic and inorganic wastes. Also, highest values were observed in Swale abattoir which is the largest abattoir (which biggest slaughtering capacity). The physic-chemical parameters obtained during this study showed that the abattoir activities have negative impact on the water and the immediate environment, thus abattoir activities constitute a major source of pollution. This observation has been recorded by other researchers who worked on the influence/impact of abattoir activities on the environment [1,25].

The United States Environmental Protection Agency (US EPA) has categorized abattoir wastewaters as one of the most environmentally harmful industrial wastewaters because improper disposal is one of the causes of river deoxygenation and soil and groundwater contamination [24].

5. CONCLUSION

The drive to increase food production to satisfy the increasing demand for meat and its products has led to the need for increased production by adopting industrial and anthropogenic techniques which has resulted in the release of millions of tonnes of toxic and recalcitrant pollutants into the environment. Although abattoir activities are unavoidably beneficial to mankind in that meat and other products are a major supply of protein to the Nigerian populace, nevertheless the high level of pollutants and untreated wastes being generated in the course of animal dressing procedures are very alarming and disturbing. The high microbial load (especially of microbes of faecal origin in untreated wastewater) in these wastewaters is alarming and could pose serious environmental/human health risks if indiscriminate disposal of untreated wastewater is allowed to continue. Thus, the poor standards of these abattoir facilities currently in use in the Niger Delta Region should be improved upon or rebuilt and equipped with modern facilities and equipment. Also, regular checks should be carried out on the effluents discharged as well as the activities carried out in these abattoirs.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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