

**ANALYSIS OF MICROBIOLOGICAL AND PHYSICOCHEMICAL
PARAMETERS OF WASTEWATER FROM TAMBUWAL ABATTOIR,
SOKOTO STATE, NIGERIA**

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Abstract

Pollution is threatening water quality worldwide. Abattoirs are examples of sources of polluted waste water that can affect biota. This study examined the microbiological and physicochemical parameters of wastewater from the Tambuwal Abattoir, Sokoto State in Nigeria. The key findings are: Microbiological analysis results include, 1. Bacterial isolates (12 bacterial isolates were identified, including Staphylococcus chromogene, Proteus Vulgaris, Enterobacter Aerogene, and others); 2. Fungal isolates (Fungal isolates were also identified, with varying macroscopic characteristics). 3. Coliform count: The coliform count was high, indicating potential contamination. Physicochemical Analysis; Temperature, Dissolved oxygen, Biochemical oxygen demand (BOD), and Chemical oxygen demand (COD) were lower, but pH was high, indicating potential pollution. Conclusively, the study highlights the need for quality analysis at abattoirs to safeguard public health and protect the interests of animal rearers.

Keywords: *Abattoir, waste water, health, pollution, public health, fungi, bacteria.*

Introduction

An Abattoir is any recognized place meant for the hygienic processing and conversion slaughtered animals to give meat for human consumption (Lawan *et al.*, 2010; Anele *et al.*, 2023). Equally, an abattoir (slaughter house) refers to a designated facility for killing animals to provide meat and related products for consumption. Therewith that, an abattoir is beneficial in many aspects, such as provision of meat for human consumption, as well as other by-products. However, during the activities of an abattoir, wastes are produced at various stages of operations. The waste could be in form liquid, solid or semi-solid, or wastewater (Bate *et al.*, 2023).

Significantly, abattoirs produced huge amounts of wastewater, that if allowed untreated may subject the environment into risks and serve as public health risks (Olanipekun *et al.*, 2021). Every abattoir has to have a good drainage system (Ndakaru & Ohwo, 2023). But, poor settings, in many parts of Nigeria, is not well provided, albeit, the drainage system in abattoirs is essential for draining the wastewater generated during the slaughter and cleaning processes. Unfortunately, many of the drainage systems serve as breeding grounds for pathogens and contaminants, threatening the public health and the environment (Anele *et al.*, 2023).

Water is an important substance that is required to provide adequate food supply to all biota (plants and animals), and it is essential for environmental health (Sarkingobir *et al.*, 2023). Due to the growth of human population, there is increase demand for water resources that are qualitative (Kilic, 2020). Poor water quality or water scarcity affects the entire worlds food supply, and regional supply are equally affected. It also affects biological biodiversity, health, standard of human life, and industrial services (Kilic, 2020). However, nowadays, there is increased in environmental problems that led to gross pollution of the environmental components, including water and consequently spurring diseases such as diarrhea, dysentery, typhoid, and others (Sumayya *et al.*, 2013). This spur the need to intensify monitoring of water in our environment. One of the source of waste or contamination to water in our environment include the abattoir (Sumayya *et al.*, 2013).

In an abattoir water can be utilized for cleaning of slaughtered goat, cattle, sheep, etc. Halls, equipment's, personnel, equally can be clean using water and in turn resulting the accumulation of waste water (Adamu & Dahiru, 2020). Livestock produce urine, and feces, that contain pathogens and chemicals that contaminate abattoir water, and seeping this kind of water into the soil, and water bodies could lead to pollution (Awari *et al.*, 2020). The act of discarding waste materials indiscriminately and into abattoir drainage system (or culverts) is very high among abattoir staff in Nigeria (Ijah *et al.*, 2022). Waste materials like hair, bones, skin, blood, flesh, fetuses, urine and feces are discarded sometimes improperly (Nuhu *et al.*, 2021). The waste at abattoir may contain high amount of pathogens such as bacteria and fungi, and chemicals that distort the microbiology and physicochemical balance of water (Ijah *et al.*, 2022).

Some studies have reported quality assessment of abattoir water and relations in different parts of the country. Awari *et al.*, (2020) show the presence of *Aspergillus fumigatus spp.*, *Fusarium spp.*, *Candida spp.*, *Trichoderma spp.*, fungi species in abattoir soil in Port Harcourt in Nigeria and indicating contamination with microbes. Ijah *et al.*, (2022) analyzed abattoir water in Minna, Nigeria for quality, and result indicates that *Bacillus*, *Escherichia*, *Aspergillus*, *Pseudomonas*, *Staphylococcus*, *Penicillium* were identified. And the physicochemical results show higher levels of parameters, except pH. In a study that assess several abattoirs have revealed that, most of the abattoirs in Kaduna have no proper waste management plan or actions (Nuhu *et al.*, 2021). In another study in Sokoto, Gandu area, Sumayya *et al.*, (2013) performed a study assessing abattoir water, and the results indicate that the water was not fit for human consumption because the physio values were below WHO guidelines and standard. Therefore, a

comprehensive analysis of the physicochemical and microbiological characteristics of abattoir drainage systems is crucial to ensure food safety and environmental sustainability. This study is aimed at analyzing the microbiological and physicochemical analysis of the wastewater at starting point from Tambuwal Town's Abattoir, Sokoto, Nigeria.

2. MATERIALS AND METHODS

Materials and methods for the research on the physicochemical and microbiological analysis of waste water at starting point of an abattoir will typically include the following steps:

2.1 Sample Collection

Wastewater samples were obtained from (starting point) at the Tambuwal town's Abattoir; therewith, the samples were properly identified and as well labeled and shuttled aseptically to the laboratory for analysis.

2.2 Preparation of media

The media that was applied in the conduct of this work were, MacConkey broth (MB), nutrient agar (NA), and Eosin methylene blue (EMB). The preparation and sterilization were ensured based on the guidelines and instruction in APHA (2020).

2.3 Physicochemical Analysis

Physicochemical parameters such like temperature, PH, dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), phosphate, and nitrate were carried according to standard method (APHA, 2020).

2.4 Microbiological Analysis

Microbiological analysis including enumeration of total bacterial counts, enumeration of the total fungal count, and determination of the total coliform counts were done according to standard methods in Ogbomida *et al.*, (2016).

2.4.1 Isolation and identification of the Bacterial isolates

Bacteria had to be isolated from the wastewater sample using nutrient agar medium. The sample had to be serially diluted using sterile distilled water, and as well the dilutions had to be inoculated in the nutrient Agar plates, and incubated at 37°C for 24 hours. Pure cultures of the bacterial isolates were identified through cultural, morphological and biochemical characteristics according to standard method (Oyeleke & Manga, 2008).

2.5 Identification of the bacterial isolates

2.5.1 Coliform count

A sample of the wastewater had collected in a sterilized container. Then diluted to obtain a suitable colony count. Nutrient agar plates were prepared with a commercially available coliform agar media or using the following: 10g peptone, 5g yeast extract, 10g sodium chloride, 15g agar, and 1000mL distilled water. The media will be autoclaved to sterilize it. The prepared agar plates were inoculated with the diluted wastewater sample using a sterile loop or pipette. Then, sample was spread evenly over the surface of the agar (using aseptic techniques). The inoculated agar plates were had to be incubated at 35°C for 24-48 hours (a temperature ideal for the growth of coliform bacteria). Upon the incubation, the number of coliform colonies present on the agar plates were counted. A magnifying glass was utilized to properly count the colonies. Calculation of the coliform count had to be done through multiplying the number of colonies counted by the dilution factor used. This gives the number of coliform bacteria found in the

original sample. The results of the coliform count were interpreted based on regulatory guidelines or standards for water quality (APHA, 2020).

2.5.2 Gram Staining

Initially (firstly), a single (pure) colonies using a sterile wire loop was taken from an agar plate of still growing bacteria (often 18–24 hours old) and heat-fixing the cells (which kills them) onto a microscope slide. In turn, the cells had to be stained using a basic dye, crystal violet for sixty (60) seconds, which stains all bacterial cells blue. Secondly, there was addition of iodine potassium (Lugol's iodine) iodide solution, that was left for 1 minute. The solution gets into the cells and a water-insoluble complex was made with the crystal violet dye. Thirdly, cells are treated with alcohol or acetone solvent for thirty (30) seconds, therein the iodine crystal violet complex is soluble. Due to solvent treatment, only Gram-positive cells remain stained. After the staining, cells are treated with a counter stain (a red acidic dye like Safranin) for 1-2minutes, so as to make Gram-negative (decolorized) cells visible. Counter stained gram-negative cells exist as red, while gram-positive cells exist as blue. Examination of slide microscopically using an x100 objective is ensured (APHA, 2020).

2.6 Biochemical Tests

2.6.1 Indole test

The Indole test is utilized in measuring the ability of an organism to break an amino acid tryptophan to release indole. Tryptophan is broken by tryptophanase enzyme to take three possible end products, including the indole. Indole production is determined by Kovac's or Ehrlich's reagent (which is composing of 4 (p)-dimethylamine benzaldehyde, that reacts with indole to yield a red colored compound). Each isolate's speck of was inoculated into 5ml of sterile peptone water (enriched with 1% tryptophan) in a test tube and, incubated at 37°C for 24-48hours to the culture; then, 0.5ml of Kovac's reagent was put and gently shaken. Noticing a positive indole test ensured if a red color was observed in the reagent layer (on top of the agar deep) within seconds of addition. Persistent yellow colour is an indication of a culture that is indole negative, or be slightly cloud (Oyeleke & Manga. 2008).

2.6.2 Methyl Red and Voges-Proskauer Test (M.R.V.P)

This test is used in differentiating bacteria which ferment glucose with the production of acetyl methyl carbon oil (acetone). The media contains peptone salt and glucose. Colonies from the stock culture will be inoculated into methyl red medium and incubated at 37°C for 48hrs. Two drops of methyl red solution will be added and shaken. The presence of a red color indicated positive for methyl red while a yellow colour indicated negative for methyl red (Cheesborough, 2000).

2.6.3 Citrate test

This is utilized in identifying the organisms that are utilizing sodium citrate for provision of sole carbon, a well and inorganic ammonium salts as the exclusive nitrogen source. This test is applied to differentiate *Enterobacteriaceae* and other gram-negative rods. Koser citrate was prepared by weighing 1.5g of ammonium phosphate, 2.5g of Sodium citrate, 1g of potassium dehydrogenate phosphate, 0.2g of Magnesium sulphate, and as well 0.1g of bromothymol blue. Then, dissolved in 1L of distilled water, and subjected to homogenization and put in test tubes, then corked with cotton wool. Inoculation of speck of each isolate is done into Koser's citrate medium, as well the incubation was performed at 37°C for 72hours. Noticing positive citrate was signified by presence of blue color, while the initial green color identifies negative result (Oyeleke & Manga, 2008).

2.6.4 Catalase test

The shaking of a container of hydrogen peroxide solution was ensured to release the dissolved oxygen. In turn, one drop of the solution was added on a clean glass slide and loopful 24hours old inoculums were

added on the drop of H₂O₂. The noticing pressure of gas bubbles signifies a positive test; but absence of bubbles signifies a negative reaction (Oyeleke & Manga., 2008).

2.6.5 Urease test

The media called Christensen urea agar was prepared through weighing 1g of glucose, 20g of plain agar, 0.1g of phenol red, 1g of peptone, 1.2g of disodium hydrogen orthophosphate, and 5g of sodium chloride, and equally the materials were dissolved in 100ml distilled water that is heated to ensure complete dissolution. The pH used was 6.8 using electrode pH meter, in order achieve yellow color. Then, dispensing into universal bottles and sterilized by autoclaving at 121°C for 15minutes. 5ml of 40% sterile preparation was aseptically taken into the universal bottle, allowed to ensure solidification in slanting position. Every isolate's speck had to be added in Christensen's urea agar, incubated at 37°C for 24hours. Liberation of red color signifies urease positive, but initial yellow color signifies negative test (Oyeleke & Manga, 2008).

2.6.6 Triple Sugar Iron (TSI)

This medium entails three sugars (lactose, glucose, and sucrose). Certain organisms ferment the entire three sugars to give an acid, in turn which changes the color of the indicator from red to yellow. Upon attack, the sugar and protein release ammonia. Then, the media allow the detection of H₂S, which is identified by the presence of a black color, along the stabbed line. Motility had to be detected be to the presence of growth along the area been stabbed by the straight wire loop. The release of gas was detected by the presence of gas bubbles (or crack on the agar) in the test tube or entire disruption of the medium. The picking of colonies from the sub-cultured plate was ensured using a sterile straight wire loop, and stabbed on the butt; streaked on the surface of the slope. The incubation was done at 37°C for 48hrs (Cheesbrough, 2000).

2.7 Identification of fungi

The fungi were macroscopic identified by checking out to the color, appearances; while the microscopic examination was done by carving a portion of the culture, fixing it on a clean glass slide, and addition of drop of lacto phenol cotton blue using a sterile inoculating needle and covered with a clean cover slip. The preparation was seen using a microscope using x10 and x40 objectives. Identification had to be based on colonial and cellular morphology of the fungi as they appear in the mycology atlas (Cheesbrough, 2000).

3. RESULTS AND DISCUSSION

3.1 Results

The results of the microbiological and physicochemical analysis of the wastewater at starting point of Tambuwal Abattoir are represented in Tables 1 to 5. Table 1 shows the Gram reactions and the morphological observations of the bacteria isolated from the wastewater sample. Table 2 shows the macroscopic characteristics of the fungi isolated from the wastewater sample. Table 3 shows the biochemical characterization of the bacterial isolates. Table 4 shows the physicochemical analysis of the wastewater sample. Table 5 shows the coliform count of the bacterial isolates. The identified bacterial isolates include *Staphylococcus chromogene*, *Proteus Vulgaris*, *Enterobacter Aerogene*, *Proteus Vulgaris*, *Staphylococcus Aureus*, *Staphylococcus Hominis*, *Morganella Morganii*, *Serratia marcescens*, *Staphylococcus Aureus*, *Proteus Vulgaris*, *Serratia Mercescens* and *Staphylococcus Aureus*. *Staphylococcus Aureus*, *Proteus Vulgaris* and *Serratia Mercescens* were identified more than once from different samples.

Table 1: Gram reaction and the morphological observations of the bacterial isolates

S/N	Isolates	Gram Reaction	Morphology
1	K1A	+	Cocci
2	K1B	+	Cocci
3	K2A	+	Cocci
4	K2B	-	Rod
5	K1Aemb	+	Cocci
6	K1Bemb	+	Cocci
7	K2Aemb	+	Rod
8	K2Bemb	-	Rod
9	K3A	+	Cocci
10	K3B	+	Cocci
11	K4A	+	Cocci
12	K4B	+	Cocci

Table 2: Macroscopic characteristics of fungi isolated from the wastewater samples

S/N	ISOLATES	COLOUR	TEXTURE	REVERSE
1	K1A	White	Powdered	Milkish
2	K1B	White	Powdered	Milkish
3	K2A	White	Powdered	Milkish
4	K2B	Black	Powdered	Milkish
5	K3A	Dark green	Powdered	Grayish
6	K3B	Blackish brown	Powdered	Black
7	K4A	Dark green	Powdered	Grayish
8	K4B	Dark brown	Powdered	Milkish

Table 3: Biochemical characterization of the bacterial isolates

S/N	ISO	GR	SH	CAT	L	GL	SUC	CIT	MOT	IND	UR	MR	VP	H ₂ S	GP	ST	CO
1	K1A	+	Cocci	+	-	+	+	-	+	-	+	+	-	-	+	-	-
2	K1B	-	Rod	+	-	+	-	-	+	+	+	+	-	+	+	+	-
3	K2A	-	Rod	+	+	+	+	+	+	-	-	-	+	+	+	+	-
4	K2B	-	Rod	+	-	+	+	-	+	+	+	+	-	+	+	-	-
5	K1Aemb	+	Cocci	+	+	+	+	-	+	-	+	-	+	-	+	-	+
6	K1Bemb	+	Cocci	+	+	+	+	-	+	-	+	-	+	+	-	+	-
7	K2Aemb	-	Rod	+	-	+	-	-	+	+	+	+	-	-	-	-	-
8	K2Bemb	-	Rod	+	-	+	+	+	+	+	-	-	+	-	+	-	-
9	K3A	+	Cocci	+	+	+	+	-	+	-	+	-	+	-	+	-	+
10	K3B	-	Rod	+	-	+	+	-	+	+	+	+	-	+	+	-	-
11	K4A	-	Rod	+	-	+	-	+	+	+	-	-	+	+	+	-	-
12	K4B	+	Cocci	+	+	+	+	-	+	-	+	-	+	-	+	-	+

KEY:

ISO: Isolates **GR:** Gram Reaction **SH:** Shapes **CAT:** Catalase **L:** Lactose
GL: Glucose **SUC:** Sucrose **CIT:** Citrate **MOT:** Motility **IND:** Indole **UR:** Urease
MR: Methyl Red **VP:** Voges Proskauer **H₂S:** Hydrogen Sulphide **GP:** Gas Production
ST: Starch **CO:** Coagulase

IDENTIFIED ORGANISMS:

Staphylococcus chromogene, *Proteus Vulgaris*, *Enterobacter Aerogene*, *Proteus Vulgaris*, *Staphylococcus Aureus*, *Staphylococcus Hominis*, *Morganella Morganii*, *Serratia marcescens*, *Staphylococcus Aureus*, *Proteus Vulgaris*, *Serratia Mercescens* and *Staphylococcus Aureus*.

Table 4: Physicochemical analysis of the wastewater sample

S/N	PARAMETERS	K1A	K1B	K2A	K2B	K3A	K3B	K4A	K4B
1	Temperature (°C)	30	32	30	32	30	31	31	32
2	DO (mg/L)	9.2	5.3	7.0	7.5	9.2	6.2	6.5	7.2
3	PH	7.00	8.10	7.14	7.10	7.0	8.10	7.16	7.15
4	BOD (mg/L)	24.9	23.8	25.7	26.8	20.3	23.2	22.5	22.8
5	COD (mg/L)	4.8	4.0	3.3	3.5	3.8	4.1	4.4	4.25

KEY:**BOD**= Biochemical Oxygen Demand**COD**= Chemical Oxygen Demand**DO**=Dissolved Oxygen**Table 5: Coliform count of the bacterial isolates**

S/N	Isolates	3 out of 10ml test tube	3 out of 1ml test tube	3 out of 0.1ml test tube	Total No. of coliform
1	K1A	3	3	2	210
2	K1B	3	2	1	1,100
3	K2A	3	2	2	1,110
4	K2B	3	2	2	1,110
5	K3A	3	3	2	1,110
6	K3B	3	3	1	1,110
7	K4A	3	2	2	210
8	K4B	3	3	1	1,110

3.2 Discussion

According to the bacteriological analysis done in this work, the bacteria isolates are entirely different from abattoir analysis result in Dutse Jigawa Nigeria performed by Bate *et al.*, (2023). It was also in conflict with the result submitted in a study of Ijebu-igbo abattoir effluent. Anele *et al.*, (2023) also reported different bacteria species from abattoirs in Rivers State, Nigeria. The difference in bacteria species may be due to differences in the assessed environments, since environment determines the difference in animal rearing methods, types of animals reared, feeding methods, hygiene methods, and other related discrepancies (Anele *et al.*, 2023); albeit, there was the presence of *Staphylococcus* species in the assessment of abattoir water from Egbu, Imo state, Nigeria (Chinakwe *et al.*, 2022). The implications of determining the varied bacteria microbes in this work is, the water when drained into river or public drainage system and find it ways into drinking water may elicit public health problem of diarrhea, stomach ache, typhoid fever, gastroenteritis, and related public health concerns (Neboh *et al.*, 2013; Ndakaru & Ohwo, 2023).

It is important to monitor the water and pollution possibilities at abattoir to safeguard the environment and public health. In this study, the temperature, pH, and total dissolved oxygen findings are higher than results from Anwai River Nigeria. This might be due to the differences in pollution or contamination of the two different waters examined. It might also be a sign of more pollution in the examined abattoir water, when compared with the Anwai River Nigeria water examined by Ndakaru & Ohwo (2023). However, the BOD, pH, COD, Temperature, are respectively below the standard values of 50, 6-9, 80, and <40 set by Federal environmental protection Agency (FEPA) as reported in Chinakwe *et al.*, (2022).

On the other hand, the determination of physiological values of the water indicates that, the water may also have the potential to pollute the receiving water (rivers and drainages), and similarly increase the levels of organic constituents in soil, which may disturb soil microbes and consequently agriculture. In

the same vein, the presence of the bacteriological species as contaminants in the abattoir waste water indicates the tendency of ill health in the animals been slaughtered, animals are of zoonotic potential, may transmit them to the public, and the bacteria may weaken the health of animals, may have more susceptibility to other diseases (infectious agents), and consequently reducing the quality of meat or animal products been disposed to the public. Humans must obtain animals foods to have a well-balanced diet and by healthy, productive, actively and play. There is need to intensify animal health interventions, and treat wastewater before discharging to the environment (Abubakar & Tukur, 2014).

4. CONCLUSION

It is very important to conduct quality analysis at abattoirs to safeguard public health and protect the losses to the rearers as well. This study conducted an assessment of abattoir water in Tambuwal, Sokoto, Nigeria and the results show the levels of physicochemical parameters is high and as well indicated the presence of various bacteria species. This could pose loses to the rearers or sellers and may incite public health problems or environmental pollution as well. It is important to state the need for more interventions that aimed at providing quality control in abattoirs.

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