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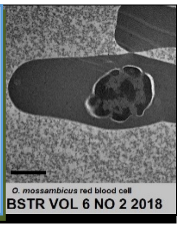

Microbiological and Physicochemical Analysis of Old Sokoto Abattoir Wastewater (Sewage) Contaminated with Blacksmith Activities

Dankaka S.M.[1*], Farouq A.A.[2] Bagega A.I.[1] and Abubakar U.[2]

[1]Department of Biochemistry, Bayero University, PMB 3011, Kana, Kano State, Nigeria.
[2]Department of Microbiology, Usmanu Danfodiyo University, PMB 2346, Sokoto, Sokoto State, Nigeria.

Corresponding author:
Saifullahi Mustapha Dankaka
Department of Biochemistry,
Bayero University Kano,
PMB 3011, Kana,
Kano State,
Nigeria.
Tel: 2348099174546
E-mail: saifudankaka@gmail.com

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ABSTRACT

The physicochemical and microbiological characteristics of wastewater samples from Old Sokoto Abattoir in Sokoto North Local Government Area, Sokoto state, Nigeria were studied over four weeks period. The physicochemical parameters of the wastewater samples studied namely pH, temperature, biochemical oxygen demand (BOD), dissolved oxygen (DfO), phosphate, nitrate, and chemical oxygen demand (COD) were within the standard limit for wastewater to be discharged into the environment. Heavy metals like nickel, copper, and lead were not detected (ND) except for iron with a concentration range from 0-0.64 mg/L. Wastewater samples for week 1 had a higher bacterial count of 270×10^5 CFU/mL than wastewater samples for the other weeks. The total viable count for all the samples exceeded the limit of 1×10^2 CFU/mL, which was between 4.1×10^5 – 27.0×10^5. All the wastewater samples were found to harbour coliform organisms in numbers greater than the required WHO standard (1×10^2 CFU/mL) for water. The MPN count of wastewater was ranged from 190-1100 MPN/100 mL. The bacterial species isolated were *E. coli* (33.3%), *E. aerogenes* (20%), *S. aureus* (6.66%), *K. pneumoniae* (6.66%), *Proteus vulgaris* (13.3%), *Citrobater freundii* (6.66%), *Bacillus licheniformis* (6.66%), and *Bacillus* spp. The fungal isolates were *Mucor* spp (12.5%), *Aspergillus niger* (37.5%), *Aspergillus terreus* (12.5%), *Sporothrix schenkii* (12.5%), *Rhodotorula* spp. (12.5%), and *Penicillium* spp. (12.5%). This study showed that the isolation and population of microorganisms from wastewater in Old Sokoto Abattoir is an indication that the wastewater was highly polluted with microbial load and can cause an environmental hazard if not treated.

INTRODUCTION

Efforts have been made towards limiting the perceived threat of pollution around the world specifically by the United Nations Organs including the United Nations Environmental Programme. There were many international conferences and protocols employed. Rio de Janeiro conference in 1992 was a major effort, collating prior environmental issues and bringing them to the fore [1]. The environment is a very vital and necessary 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 in the environment [2]. The past two decades have witnessed a raising concern over environmental deterioration from pollution and exhaustion of natural resources. Organic and inorganic substances have been discharged into the environment as a result of domestic, agricultural and industrial activities [3] though, in many parts of the world, human activities including animal production are still negatively influence on the environment and biodiversity [1].

Abattoir is a slaughter house originated from the French word "abattre" meaning 'to strike down'. It is a building where animals including cattle and goats and so on are killed, dressed and supplied for consumption and other industrial purposes [4]. An Abattoir is a premise approved and registered by the controlling authority for sanitary. Slaughtering and inspections of animals, processing and effective preservation and storage of meat products are done for human consumption [5]. An Abattoir or slaughter is a specialised environment where meat processing is usually carried out. It is a building where animals are killed for their meat [6]. Abattoir operation could be very helpful to man;

in that, it provides meat for human consumption and other useful byproducts, which can be very hazardous to the public health in respect to the waste it generates [7]. The Abattoir industry is a vital component of the livestock industry in Nigeria, providing domestic meat supplies to over 150 million people and job opportunities for the teaming population [3, 8].

Some of the wastewater from Sokoto Abattoir flows into the surrounding soil environment while the remaining is channeled through the Abattoir drainages into River Rima. The resultant repercussion could be the deterioration of soil fertility due to the accumulation of certain nutrients and heavy metals that may lead to low productivity in the surrounding farmlands in addition to injuries and destruction of aquatic lives. Since the water from both the river as well as the soil is used for irrigation farming along the river banks, the possibility of zoonotic diseases amongst the consumers from such irrigated fields cannot be ruled out [9].

Most wastewater released in developing countries are mostly untreated before being discharged into water bodies. Water used in cleaning carcasses of slaughtered animals and washing of slaughter house floor is referred to as Abattoir wastewater [10]. The influence of wastewater effluents in the quality of receiving water bodies is manifold and depends on the release volume, chemical and microbiological concentration/composition of the effluents [3]. The removal of sewage from inhabited areas is a vital consideration in the overall concept of life support system [11]. In the year 2000, the United Nations confirmed that 2.64 billion people had insufficient means to sanitation. However, in Africa and Asia, approximately half of the population had no access whatsoever to sanitation. In many developing countries like Nigeria, more than half of the population undergo hardship from the deficiency of harmless water supply and secure sanitation as available reports demonstrated gross site contamination of most major fresh water bodies across the nation resulted from the release of industrial effluents, sewage and agricultural waste among others [12].

A blacksmith is a metal smith who creates objects from wrought iron or steel by forging the metal using tools to hammer bend and cut. Blacksmith produces objects including cooking utensils and weapons. Aluminium is the most bountiful metallic element that makes up 8% of the earth crusts [13, 14]. Aluminium and its compound constitute about 8% of the earth surface, which exist naturally in silicates, cryolites and bauxite rocks. Aluminium has not been classified concerning carcinogenicity; nevertheless, 'aluminium production' has been classified as carcinogenic to mankind by the International Agency for Research on Cancer (IARC) [15]. Therefore, this study intends to determine the microbiological and physicochemical characteristics of wastewater (sewage) from Old Sokoto Abattoir which is contaminated by black smith activities.

MATERIALS AND METHODS

Study area

The Old Sokoto Abattoir is located at Kara market of Sokoto North local government area in Sokoto state, Nigeria located to the extreme North west Nigeria between longitudes $4^0$8'E $6^0$54'E and latitude 12^0N and $13^0$58'N. The Abattoir (old) is divided into different parts; the butchering section where the animals are rendered, the rinsing section where the parts of the animals are rinsed, butchering section where the emergency animal is rendered and dug pit where the intestines are emptied. The wastes from the Abattoir are collectively released into the soil and drainage without treatment, the destination of which is dry season farmlands.

Collection of samples

Wastewater samples were collected from the Abattoir for 4 weeks. Sterilisation of 1 L gallon using 70% ethanol and rinsed for 10 min was used to collect the sample in the morning during the peak activities between 9:00 am and 10:00 am. The wastewater samples were collected. The area is characterised by blacksmith activities to produce local pot by melting aluminium and iron obtained from beverages cans, which contaminate the wastewater. The aluminium enters the drain wastewater either in melted form or solid, which might cause a problem to the farmers when this water inhibits the growth of their farm products. Samples were collected at six days interval in the month of May and June and transported in an icebox to the microbiology laboratory in Usmanu Danfodiyo University Sokoto for analysis. A total of 4 wastewater samples were collected.

Preparation of media

The media used for this study include; nutrient agar (NA), potato dextrose agar (PDA) MacConkey broth (MB) and Eosin methylene blue (EMB). All of which were prepared and sterilised according to manufacturer's instruction.

Microbiological analysis

Microbiological analysis including enumeration of total bacterial counts, enumeration of the total fungal count, determination of the total coliform and faecal coliform counts were conducted according to standard method [3, 4].

Identification and characterisation of fungi

The fungi isolated were identified according to the method carried out by [16]. The results of the microscopy were compared with illustrations contained in colour atlas of mycology.

Identification and characterisation of bacterial isolates

Pure cultures of the heterotrophic bacterial isolates were identified by cultural, morphological and biochemical characteristics according to standard method [17-19].

Determination of physicochemical parameters.

Physicochemical parameters such as pH, temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), phosphate, and nitrate were carried out according to to standard method [16,10].

Procedure for determining the level of heavy metals (iron, nickel, copper and lead)

The concentration of the respective heavy metals (iron, nickel, copper and lead) present in the water and effluent sample was determined with the aid of an atomic absorption spectrophotometer using to standard method [16, 20].

Statistical analysis

The data obtained from the study were subjected to statistical Analysis of Variance (ANOVA) at 5% level of significance.

RESULTS AND DISCUSSION

The results of this study are presented in **Tables 1** to **4**. **Table 1** shows the physicochemical analyses of the wastewater samples. The temperatures of the samples were between 29°C and 31°C with week 1 and week four samples having the same temperature of 30°C, respectively. Week 3 wastewater samples recorded the highest temperature of 31°C. The pH was 6.9 to 7.4 where the highest was recorded for week 2 sample 7.4, with week 1 and week three samples having the same pH of 6.9. The DO of the samples was between 4.2 mg/L to 8.5 mg/L with week four samples having the highest DO of 8.5 mg/L. The BOD of the samples was 10.3 mg/L to 14.1 mg/L. Meanwhile, the COD of the samples was 0.8 mg/L to1.5 mg/L. The phosphate and nitrate of the samples were between 0.29 to 0.31 mg/L and 15.6 to 20.4 mg/L, respectively.

Table 2 depicts the analyses of some heavy metals such as nickel (Ni), lead (Pb), copper (Cu), and iron (Fe). **Table 3** shows the range of viable count of bacteria and from $41x10^5$ to $270x10^5$ CFU/mL. and the total viable count of fungi ranging from $2.0x10^5$ to $3.5x10^5$ CFU/mL with week two samples having the highest count of $3.5x10^5$ CFU/mL. **Table 4** illustrates the fungal isolates in Old Sokoto Abattoir wastewater, while **Table 5** shows bacterial isolates in old Sokoto Abattoir sewage.

Table 1. The physicochemical parameters of Old Sokoto Abattoir wastewater.

Parameter	Week 1	Week 2	Week 3	Week 4	Total	Mean	Range
Temperature (°C)	30	29	31	30	120	30	29-31
PH	6.9	7.4	6.9	7.2	28.4	7.1	6.9-7.4
DO (mg/L)	4.2	4.8	5.5	8.5	23	5.72	4.2-8.5
BOD (mg/L)	14.1	12.9	13.7	10.3	51	12.75	10.3-14.1
COD (mg/L)	1.3	1	0.8	1.5	1.19	0.2975	0.8-1.5
Nitrate (mg/L)	17.2	15.6	20.4	17.6	70.8	17.7	15.6-20.4
Phosphate (mg/l)	0.29	0.3	0.29	0.31	4.6	1.15	0.29-0.31

Table 2. Heavy metal analysis of Old Sokoto Abattoir wastewater

Metals (mg/L)	Cu	Ni	Pb	Fe
Week 1	ND	ND	ND	0.1804
Week 2	ND	ND	ND	ND
Week 3	ND	ND	ND	0.6396
Week 4	ND	ND	ND	0.5674
Total	-	-	-	1.3847
Mean	-	-	-	0.3462
Range	-	-	-	0-0.6396

Table 3. Total viable count of bacteria and fungi in Old Sokoto Abattoir wastewater

No of Weeks	Bacteria (CFU/mL)	Fungi (CFU/mL)
Week 1	$27.0x10^6$	$2.5x10^5$
Week 2	$5.0x10^6$	$3.5x10^5$
Week 3	$4.1x10^6$	$3.0x10^5$
Week 4	$7.0x10^6$	$2.0x10^5$
Total	$4.31x10^7$	$11.0x10^5$
Mean	$1.08x10^7$	$2.75x10^5$
Range	$4.1x10^6$ - $27.0x10^6$	$2.0\text{-}3.5x10^5$

Table 4. Fungal isolates from Old Sokoto Abattoir wastewater

Isolate code	Organism
WK1H1	Mucor spp.
WK1H2	*Aspergillus niger*
WK2H1	*Aspergillus niger*
WK2H2	*Sporothrix schenckii*
WK3H1	*Aspergillus terreus*
WK3H2	*Aspergillus niger*
WK4H1	*Rhodotorula* spp.
WK4H2	*Penicillium* spp.

Table 5. Bacterial isolates in Old Sokoto Abattoir wastewater

Sample Isolate Code	Organism Isolated
N.A WK1H1	*Klebsiella pneumoniae*
N.A WK2H1	*Citrobacter freundii*
N.A WK2H2	*Staphylococcus aureus*
N.A WK3H1	*Bacillus* spp.
N.A WK3H2	*Bacillus licheniformis*
N.A WK4H1	*Proteus vulgaris*
N.A WK4H2	*Proteus vulgaris*
EMBWK1H1	*Escherichia coli*
EMBWK1H2	*Enterobacter aerogenes*
EMBWK2H1	*Escherichia coli*
EMBWK2H2	*Escherichia coli*
EMBWK3H1	*Enterobacter aerogenes*
EMBWK3H2	*Escherichia coli*
EMBWK4H1	*Escherichia coli*
EMBWK4H2	*Enterobacter aerogenes*

Key:
WK=Weeks
N.A= Nutrient Agar
EMB= Eosin Methylene Blue Agar
H= Point of Sample Collection
N.AWKH= Isolate on Nutrient Agar in Weeks
EMBWKH= Isolate on Eosin Methylene Blue Agar in Weeks

DISCUSSION

From the results of this study, the temperatures of wastewater ranging from 29°C to 31°C and were similar to the temperatures of wastewater reported by [4] of 29.5°C to 37.5°C in Adere Abattoir, and [10] reported temperatures of 31°C to 33.3°C in Abattoir effluent in Oyo, Oyo state, Nigeria. The temperature was within the limit for wastewater discharge <40°C.

The pH of the wastewater was near neutral (6.9-7.4), which plays a role in determining both the qualitative and quantitative abundance of microorganisms in the wastewater. This is in line with the limit for wastewater discharge of 6-9. Besides, this result is in agreement with that obtained by [22] of 6.92-8.18. The initial neutral pH that characterised the onset of this study has contradicted the observation by [1] who recorded an acidic pH of wastewater from Abattoir wastewater in Lagos, Nigeria. The anaerobic degradation of organic compounds releases ammonia, which reacts with carbon dioxide produced during the anaerobic process, resulting in ammonia bicarbonate, which may contribute to the increase in the pH values [3]. The highest DO of the samples was 8.5 mg/L with 14.1 mg/L BOD and 1.5 mg/L COD. There was a significant difference between the parameters at ($p<0.05$).

This is in line with the limit from the FEPA for the discharge of wastewater from the Abattoir into the water body. The presence of nitrate and phosphorus can be attributed to the feed of the cows. The nitrate of the wastewater was ranged from 17.2 mg/L - 20.4 mg/L, which is higher than the standard limit of NESREA (10 mg/L). The high concentration of nitrate can be attributed to high concentration of organic matter content of the wastewater samples resulted from the decomposition of protein and nitrogenous compound, which when broken down give rise to simpler substances including ammonia. This is parallel with the finding of [3] who reported nitrate from Eyean Abattoir wastewater ranging from 1.25 mg/L- 28.43 mg/L.

Heavy metals are natural constituents of the earth's crust. The term heavy metal is collectively applied to a group of metals and metals-like elements with a density greater than 5 g/cm^3 and atomic number above 20. Living organisms require trace amount of some heavy metals including cobalt, copper, iron and zinc. Excess levels of essential metals, however, can be detrimental to living organisms, since they cannot be degraded or destroyed. Therefore, they tend to accumulate in the soil and sediments. Leaching of these heavy metals into the ground water is also a major cause of concern especially due to the recalcitrant nature of the metals [8].

Iron (Fe) was the only heavy metal detected in the wastewater and ranged from 0 - 0.6396. The permissible limit of the Fe is not available in NESREA permissible limit. Iron (Fe) is an essential micronutrient for plants whose deficiency presents a major worldwide agriculture problem. Moreover, iron is not easily available in neutral to alkaline soils, rendering plant's iron deficient despite its abundance [22]. Even though Fe is hardly presented in living matter, it is still an essential element that is critical for the life of plants [22]. In some cases, however, Fe application might cause nutritional disorder due to the antagonistic effect of Fe with other cationic micronutrients particularly manganese [23].

The mean for the total bacterial count ($1.08x10^7$) and fungal count ($2.75x10^5$) was higher for the wastewater. Going by WHO standard, any water contaminated to this extent or level is neither good for domestic use nor is it supposed to be released into the environment directly without treatment. There was a significance difference at ($p<0.05$) of the bacterial population from wastewater samples in week one and other samples from the other weeks. The abundance of microorganisms might be due to the abundance of nutrients in the wastewater in which the microbes thrive [8].

A similar finding was reported by [5] which were $4.9x10^7$ to $7.3x10^7$ CFU/mL of bacteria and mean fungal count of $11.7x10^4$ CFU/mL from wastewater in Sokoto Abattoir. Adesemoye et al. [1] reported total bacterial count from Agege Abattoir soil in Lagos as $3.32x10^7$ CFU/g and fungal count of $1.60x10^5$ CFU/g, while [24] reported a total bacterial population of $2.08x10^3$ CFU/mL and total fungal population of $8.0x10^2$ CFU/mL from wastewater collection sites in Port Harcourt city, Nigeria. Total coliform count using MPN/100 mL water was 3,490 CFU/mL, which is above the WHO ($1x10^2$ CFU/mL) for drinking water. The high coliform count is an indication for the likelihood of the presence of pathogenic organisms that mostly cause water-borne diseases. The total viable count exceeded the limit of $1x10^2$ CFU/mL.

Bacteria isolated from the Abattoir wastewater were identified as *E. coli* 33.3%, *E. aerogenes* 20%, *S. aureus* 6.66%, *K. pneumoniae* 6.66%, *Proteus vulgaris* 13.3%, *Citrobacter freundii* 6.66%, *Bacillus licheniformis* 6.66%, *Shigella sonnei* 6.66%, and Bacillus spp. Fungal isolates were identified as *Mucor* spp 12.5%, *Aspergillus niger* 37.5%, *Aspergillus terreus* 12.5%, *Sporothrix schenkii* 12.5%, *Rhodotorula* spp. 12.5%, and *Penicillium* spp. 12.5%. Similar pathogenic microorganisms have been isolated from abattoir wastewater in different parts of the country. Isolated *S. aureus*, *E. coli*, *Proteus* spp, *Klebsiella* spp, *Penicillium* spp and *Aspergillus niger* has been identified from Abattoir effluents in Oyo, Oyo state, Nigeria [10]. The presence of *E. coli*, *Klebsiella pneumonia*, *S. aureus*, *E. aerogenes*, *A. niger*, *Penicillium* spp from Ijegbu-Igbo Abattoir effluent [8]. Moreover, the presence of *Bacillus s*pp, *A.* niger, *Penicillium* spp and *Mucor* spp from Abattoir wastewater in Lagos, Nigeria [1]. Ogbomida et al. [3] recorded isolated *Escherichia* spp., *Klebsiella* spp., *Enterobacter* spp., and *Staphylococcus* spp. from Abattoir wastewater. In addition, *E. coli*, *Klebsiella* spp., *Bacillus* spp and *Enterobacter* spp. from water used in Abattoir in Ado-Ekiti, Southwest Nigeria have isolated [4]. Meanwhile, *E. coli*, *K. pneumonia*, *Penicillium* spp., and *A. niger* were isolated from Sokoto Abattoir wastewater in Sokoto state, Nigeria [5]. Apart from that, Kacprzak et al. [25] isolated *Sporothrix schenkii* and *Rhodotorula* spp in inhabiting sewage sludge and untreated and treated wastewater, respectively.

Although *Aspergillus terreus* have been isolated from the wastewater samples in Old Sokoto Abattoir wastewater, *Sporothrix schenkii*, *Rhodotorula* spp., *Aspergillus terreus* and *Citrobacter freundii* were isolated from Old Sokoto Abattoir wastewater. Most of the research conducted did not report the presence of *Sporothrix schenkii*, *Rhodotorula* spp., *Aspergillus terreus* and *Citrobacter freundii* in Abattoir wastewater, which made this study as one of those reporting their presence in the Abattoir wastewater.

The wastewater from the Abattoir is discharged into open drainages untreated and the leachates from the series of decomposition of these wastes can introduce enteric pathogens into the Rima River, thus serving as a vehicle for gastrointestinal infections. Besides, it may introduce excess nutrients into surface water and percolates into the underlying aquifers to contaminate hand-dug wells. The high level of organic matter in the wastewater encourages rapid proliferation of O_2 consuming microorganisms to deplete the water of its dissolved oxygen leading to septic condition or anoxia, which is lethal to aquatic fauna [5].

Although Abattoir operation could be very useful to man in that it provides job opportunities and serves as a source of revenue generation to the government as well as meat for human consumption and other beneficial by-products, it can be very hazardous to the public health in respect of the waste it generated. The high level of contamination of Old Sokoto Abattoir as found in this study illustrates the danger associated with the release of untreated wastewater to the river and the surrounding environment, thus need to be treated. The isolation of these microorganisms is an illustration that the wastewater could be very hazardous if being released into the Rima River and surrounding environment if left untreated.

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