Isolation and Identification of Microorganisms from Abattoir Effluents from Oyo, Oyo state, Nigeria

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ABSTRACT--- ***Effluent samples from three points (discharge point, upstream and downstream) were collected from Akunlemu abattoir in Oyo town, Nigeria. Physicochemical parameters and microbiological analyses of the samples were carried out. Total viable count was by pour plate technique and the most probable number (MPN) by the multiple tube fermentation technique. The temperatures of the samples were between 31.0-33.3^{0}C and pH range between 5.83-6.80. The total viable count for all the samples exceeded the limit of 1x10^2 cfu/ml which was between 8.3x10^5-10.2x10^5 cfu/ml, with sample C recording the highest value. All the samples exceeded the limits for Total dissolved solid (TDS), Total solid (TS), Total suspended solid (TSS), Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD) with sample B having the highest values – BOD 2765 mg/L, COD 5185 mg/L TSS 2981 mg/L, TDS 550 mg/L and TS 3528 mg/L. Microorganisms isolated included Proteus sp, Staphylococcus aureus, Escherichia coli, Klebsiella sp Pseudomonas sp, Aspergillus niger, A. flavus, Fusarium sp and Penicillium sp.***

Keywords--- Abattoir, effluent, physicochemical parameters, microorganisms

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1. INTRODUCTION

Wastewater discharged most of the times in developing countries is mostly not treated before being released into water bodies Water used in cleaning carcasses of slaughtered animals and washing of slaughter house floor is referred to as abattoir wastewater [1]. Pollution of the environment from effluents form abattoir can be direct or indirect from the different processes that are involved [2], while effluents from abattoirs have caused deoxygenation of rivers [3] and contamination of ground water [4]. Wastewater from abattoir contains blood, manure, hair, fat, feather and bone with BOD and TS levels as high as 8000 mg/L and 800 mg/L respectively [5]. Other studies on abattoir effluents have been carried out on contaminants in soil and aquatic environment in Nigeria [6][7][8].

. Water borne diseases such as diarrhea, typhoid and cholera which are prevalent can be contacted as a result of contamination with untreated wastewater [9]especially in developing countries. The release of untreated abattoir effluent into water bodies is a public health issue because some residents drink and make use of such water without treatment e.g boiling and this could lead to the outbreak of water borne diseases such as cholera, typhoid and diarrhea. Also, enteric pathogens and excessive nutrients into water bodies are introduced as a result of effluent from animal slaughtering and this also leads to contamination of ground water [10]. Studies have also been carried out by[11][12][13] [8]. Disposal of animal feces inappropriately into the receiving environment may cause oxygen depletion, and lead to excessive availability of nutrients into these receiving environment while increasing the accumulation of toxins in the biological systems[14].

This study was carried out to examine the level of contamination of the abattoir effluent before its discharge and after into the river.

2. MATERIALS AND METHODS

Sample collection

Sterile flasks were used for collection of samples before and after discharge into a river using the grab sampling method of [5]. The samples were then taken to the laboratory for analyses and these were done according to the procedures in the standard methods of examination of water and wastewater [15].

Measurement of physicochemical parameters

The temperatures were measured using a mercury thermometer and the pH using a Hanna pH meter PHS 25-H198107. Determination of TS, TSS, TDS. BOD and COD were carried out using the methods of [16].

Culture media preparation

Culture media used were Nutrient agar (NA), Potato dextrose agar (PDA) and Eosin methylene blue (EMB) agar (LAB M) and were all prepared according to the manufacturer's specification. Serial dilution of the effluents were carried out and 1 ml each of the diluents were aseptically introduced into different plates after which sterile prepared medium was introduced using the pour plate technique and incubated at the37^0C for 24 hrs. Biochemical tests were carried out on pure bacterial cultures using standard methods e.g oxidase, catalase, motility [17][18][19].

In estimating the fungi, PDA plates supplemented with streptomycin inoculated with the serial diluents of samples by pour plate technique and incubated at 30^0C for 72 hrs [20]. Macroscopic and microscopic examinations including staining for morphological characteristics were carried out on fungal isolates and identification was done based on the characteristics.

3. RESULTS

Three effluent samples were collected from Akinlemu abattoir in Oyo town for physicochemical and microbiological analyses. Table 1 shows the physicochemical of the samples. All the three samples were turbid and had offensive odors. The pH was between 5.83 and 6.80 and the highest was recorded for sample A, 6.80. The temperatures of the samples were between 31.0^0C and 33.3^0C with samples A and C having the same temperature of 31.0^0C. Sample B recorded the highest temperature of 33.3^0C. The BOD_5 range was between 75-2765 with Sample B (abattoir effluent at the point of discharge into the drainage) having the highest. Sample B had the highest values for BOD_5- 2765; COD- 5185; TS- 3528; TDS- 550 and TSS- 2981. The TS of all the samples were above the 2000mg/L limit with sample B (abattoir effluent at the point of entry into the drainage) having the highest value of 3,528 mg/L.

Table 2 shows the total viable count, the total colifom count and the most probable number (MPN) of the samples. The total viable counts of the samples were between 8.3-$10.2x10^5$ cfu/ml with sample C – mixture of the stream water and the abattoir effluent having the highest value of $10.2x10^5$ cfu/ml. The highest value for total coliform count- $5.8x10^5$ cfu/ml was recorded for sample B. The values recorded for the most viable number for all the samples were high- >240 MPN/100ml.

Table 3 shows the bacterial and fungal isolates from the effluent samples. Bacteria from sample A included *Staphylococcus aureus*, *Proteus* sp, *Escherichia coli* and *Pseudomonas* sp. From sample B, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* were isolated. All the samples had *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp present. For the fungal isolates, *Aspergillus niger* was present in samples A and C while *Aspergillus flavus* was isolated in Samples B. *Penicillium* was isolated from sample B and *Fusarium* was isolated from Sample B and Sample C.

4. DISCUSSION

Physicochemical and biological parameters of abattoir effluents obtained were compared with the limits from the Federal Environmental Protection agency (FEPA) for discharge of effluents from the meat industries into water bodies. Bacteria and coliform counts were high in the effluents which pose an environmental risk to the receiving rivers. The study revealed that abattoir activities have direct and indirect effects on the water body, environment and health of the people. The offensive odors of all samples in the study were similar to those reported by [1]. They stated that the free running abattoir effluent produces a stench and foul odor which pervades the locality and constitutes an environmental nuisance. The results obtained for temperature of samples which fell between 31.0^0C and 33.3^0C were similar to that recorded by [21 32.0^0C-34.0^0C [1], The temperature range was within limit of <40 for effluent discharge. The pH of 5.83 for sample (B) was the only one that was not within the limit for effluent discharge of 6-9. This result was in line with that obtained by [22]. pH of abattoir obtained by [23] was 5.7-6.7: 6.92-8.18 [1].The high COD value of abattoir waste water and down stream water is an indication of high organic matter in the abattoir waste, supported by [5]. The high BOD level observed in this study is similar to that observed by [24]. The BOD and TSS of abattoir effluent are high as a result of the blood content and particulates respectively from the slaughter processes.

High bacterial count in the wastewater was due to the rich protein content of the whole blood which serves as a medium for growth of microorganism [25]. In another study, the TVC was between $4.9x10^7$ cfu/ml-$7.3x10^7$ cfu/ml [25]. [26] from abattoir in Agege Lagos reported mean bacterial count of $3.32x10^7$ cfu/ml. Report by[27] on study carried out had total bacterial population of $2.08x10^3$ cfu/ml from waterwater collection sites in Port Harcourt. Total coliform count $8.3x10^5$-$10.2x10^5$ cfu/ml, this is above the WHO 100 cfu for drinking water. High coilform count indicative of likelihood of occurrence of water borne diseases. The total viable count exceeded the limit of $1x10^2$ cfu/ml. This shows that the samples have been polluted due to discharge of untreated wastes from the abattoir [28]. Bacteria isolated include from this study were *Escherichia coli* and *Klebsiella* sp, fungi- *Aspergillus flavus, A niger*. [1] isolated *E. coli* with other microorganisms from Bodija abattoir wastewater in Ibadan. [29]reported isolation of *A. niger, Mucor* sp, *Penicillium* sp from two abattoirs in Lagos state. While effluent from the abattoir is not treated and is being washed into the river, this could lead to the introduction and proliferation of enteric pathogenic resulting to gastrointestinal infections [25].

Microorganisms deplete the dissolved oxygen in water and do proliferate as a result of the organic matter content that is high thus resulting in septic condition or anoxia which is lethal to aquatic fauna[30]

TSS increase as TS value increased with values from this study being TS 2003-3528, TSS 1773-2981. [4] reported on some abattoirs in Ibadan, Nigeria with high suspended solids and COD levels. High High TS value could be as a result of lack of sedimentation facility to separate the solid from the liquid waste before discharge. Limit for effluent discharge is 400 cfu/ml [31].

Organisms isolated included *Staphylococcus aureus, Escherichia coli, Klebsiella* sp, *Proteus* sp, *Pseudomonas* sp, *Fusarium* sp, *Aspergillus* sp and *Penicillium* sp which were also isolated by [29][1].

6. CONCLUSION

The results obtained from this study showed that the effluent from the abattoir exceeded the limit for effluent discharged into water bodies set by the FEPA thereby causing water contamination. The isolation of these microorganisms is an indication that the effluent could be hazardous if discharged into the water body without treatment.

Table 1: The physicochemical parameters of effluent samples

Samples	Color	Odor	Temp (^{O}C)	pH	BOD_5 (mg/L)	COD (mg/L)	TSS (mg/L)	TDS (mg/L)	TS (mg/L)
A	Slightly cloudy	Offensive	31.0	6.80	75	130	1773	232	2003
B	Brownish	Offensive	33.3	5.83	2765	5185	2981	550	3528
C	Brownish	Offensive	31.0	6.40	974	1755	2693	273	2970
Standard limit			<40	6-9	50	80	30	2000	2000

Key
A- stream water before mixing with the abattoir effluent
B- abattoir effluent at the point of entry into the drainage
C- mixture of the stream water and the abattoir wastewater

Table 2: Total coliform counts, Total viable counts and Most Probable Number (MPN) of coliform/100ml of water samples

Sample	Total coliform count (cfu/ml)x10^5	TVC (cfu/ml)x10^5	MPN/100ml
A	4.7	8.3	>240
B	5.8	9.2	>240
C	4.5	10.2	>240

Key
A- stream water before mixing with the abattoir effluent
B- abattoir effluent at the point of entry into the drainage
C- mixture of the stream water and the abattoir wastewater

Table 3: Bacterial and Fungal isolates from effluent samples

Sample	Bacteria	Fungi
A	*Staphylococcus aureus* *Proteus* sp *Escherichia coli* *Pseudomonas* sp	*Aspergillus niger*
B	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas* sp *Klebsiella* sp	*Penicillium* sp *Aspergillus flavus* *Fusarium*
C	*Escherichia coli* *Pseudomonas* sp *Staphylococcus aureus*	*Aspergillus niger* *Fusarium*

Key
A- stream water before mixing with the abattoir effluent
B- abattoir effluent at the point of entry into the drainage
C- mixture of the stream water and the abattoir wastewater

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