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Microbial quality assessment of an abattoir effluent discharged into Waterside Riverin Aba, Abia State, Nigeria

*Edward, K. C.[1],Nnochiri, O. A.[1], Owuamalam, P. O.[1], Onyekachi, O. V.[1], Akumah, C. N.[1]

[1](Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria)

Abstract:*The microbial quality of an abattoir effluent discharged into Waterside River in Abawas investigated. Samples were taken from the abattoir effluent, and from upstream and downstream of the receiving water body, and were examined for pathogenic microorganisms. The mean counts of the different isolates were obtained. The isolates include; Escherichia coli, Pseudomonas sp., Klebsiella sp., Salmonella sp., Shigella sp., Staphylococcus aureus, Enterobacter sp., Aspergillus sp., Rhizopus sp. and Penicillium sp. The ranges for microbial counts at room temperature and at 37°C were;9.8×10^4 to 2.2×10^7 cfu/ml(total aerobic plate count), 7.5×10^4 to 1.7×10^7 cfu/ml (total coliform count), $3.4\times10^3$to 7.0×10^4 cfu/ml (Salmonella count), $2.2\times10^3$to 5.6×10^4 cfu/ml(Shigella count), 2.0×10^4 to 5.4×10^5cfu/ml (Escherichia coli count),7.0×10^3 to 6.7×10^5cfu/ml (Staphylococcus aureus count) and 2.0×10^3 to 5.0×10^4 cfu/ml (total fungal count). In all the samples analyzed at both 37°C and at room temperature, the effluent had the highest count, while the upstream had the least count. The results show the negative impact of the abbatoir effluent wastedischarge on the receiving water body. The presence of the isolated pathogens is of public health importance and indicates poor abattoir hygiene. Thus, adoption of appropriate abattoir wastewater treatment measures are recommended.*

Keywords*–Microbial quality, abattoir effluent, Waterside River, Aba, Nigeria.*

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I. Introduction

An Abattoir is a special facility designed and licensed for receiving, holding, slaughtering and inspecting meat animals and meat products before release to the public [1]. During slaughter and meat processing, wastewater is generated, consisting of mainly intestinal contents, blood and water. Abattoir effluents most often enter natural bodies of water, like groundwater, streams, lakes, rivers and oceans, as a result of natural drainage patterns and sequences [2-3]. In Nigeria, abattoir wastes are sources of embarrassment that requires immediate remedy. When untreated wastewater is discharged into any receiving water body, it makes the water unfit for human use because there are potential pathogens that can exist in the waste [4].Several studies have revealed that abattoirs in developing countries have an unhygienic environment [5-6]. These studies have detected the presence of pathogens that are known causes of diarrheal diseases and a possible hazard to human health in the abattoirs' wastewater and receiving water bodies [7-8]. This pollution arises from activities in meat production as a result of failure in adhering to Good Manufacturing Practices (GMP) and Good Health Practices (GHP) [9]. Pathogens present in animal carcasses or shed in animal waste may include; Rotaviruses, Hepatitis E virus, *Salmonella* sp., *Escherichia coli*, *Yersinia enterocolitica*,*and Campylobacter*sp. [10]. The consequence of infection by pathogens originating from animal wastes can range from temporary morbidity to mortality, especially in high risk individuals. Due to the difficulties in quantifying pathogens, indicator fecal coliforms have been monitored for more than 100 years [11]. In order to reduce pollution rate, effluent discharge guidelines have been put together by several agencies that are involved in environmental health. Therefore, this study is aimed at assessing the microbial load of the abattoir effluent, and the microbial effect of the effluent on the nearby river (Waterside); and to compare the growth rate of the isolates at 37℃ and at room temperature.

II. Materials and methods

2.1 Sample collection

A total of nine (9) wastewater samples were aseptically collected at the abattoir, from a point where it is thoroughly mixed and close to the discharging point (outlet), and also from the nearby river (Waterside) during the morning hours, between 8am and 9am, at the time when slaughtering was at its peak. Water from Waterside Riverwas collected from two (2) different points namely; upstream (before mixing with the abattoir effluent) and downstream (after mixing with the abattoir effluent). The samples were collected using sterile water sampling bottles, stored in an ice-box, and were transported to the laboratory, where they were processed within 2-3 hours of sampling.

2.2 Sample dilution, isolation, enumeration and identification of microorganisms

2.2.1 Media used and their preparation

Nutrient Agar (Titan Biotech Ltd. Rajasthan, India), MacConkey Agar (Titan Biotech Ltd. Rajasthan, India), Blood Agar, Eosin Methylene Blue Agar (Titan Biotech Ltd. Rajasthan, India), Sabouraud Dextrose Agar (Titan Biotech Ltd. Rajasthan, India), *Salmonella-Shigella* Agar (Titan Biotech Ltd. Rajasthan, India), Mannitol Salt Agar (Titan Biotech Ltd. Rajasthan, India), Simmon's Citrate Agar (Titan Biotech Ltd. Rajasthan, India), Peptone Water (Titan Biotech Ltd. Rajasthan, India), and Methyl Red-Voges Proskauer (MR-VP) Broth (Titan Biotech Ltd. Rajasthan, India) were used. The media were prepared according to the manufacturers' instructions, and were brought to boiling before sterilization (except for *Salmonella-Shigella* Agar which was only boiled at 100℃ with frequent agitation) at 121℃ for 15 minutes at 15psi.

2.2.2 Serial dilution

One (1) ml of each water sample was aseptically measured into 9mls of sterile normal saline, and was properly mixed by aspiration, producing a dilution of 10^{-1}. Successive decimal dilutions were then obtained.

2.2.3 Isolation and enumeration

The pour plate method was used. From each of the sample dilutions, 1ml aliquot was aseptically taken using a sterile pipette and was inoculated into sterile Petri dishes; after which, prepared culture media were aseptically poured into the Petri dishes, which were swirled gently to mix the sample and the media. The plates were inverted and incubated at 37^{o}C for 18-24 hours and also at room temperature; Sabouraud Dextrose Agar plates were incubated for 72-120 hours. The developed colonies were counted using a colony counter, and the results were recorded; plates containing 30-300 colonies were selected. Average of duplicate plates were counted and recorded as the number of colony forming units (cfu/ml) of each the wastewater samples. The plate counts per ml were recorded using:1/V x N x D

Where V = Volume of inoculums;N = Number of colonies counted;D = Dilution factor used.

Pure cultures of the isolates were obtained by aseptically streaking representative colonies with different morphological features, which appeared on the cultured plates onto freshly prepared Nutrient Agar plates. The Nutrient Agar plates were incubated at 37^{o}C for 24 hours, and discrete colonies which developed were transferred into McCartney bottles containing agar slants, and were stored in the refrigerator at 4^{o}C, and served as a stock culture for subsequent characterization tests.

2.2.4 Identification and characterization of the isolates

Bacterial isolates were identified based on standard microbiological, cultural, morphological and biochemical characteristics as described by [12] and [13], while the fungal isolates were identified by comparing the macroscopic and microscopic characteristics with those described by the Public Health Agency of Canada [14].

2.3 Statistical analysis

The total coliform count of the abattoir effluent and those of the upstream and downstream of the receiving water body were compared using a One Way Analysis of variance. The counts were first transformed using Logarithm to base 10 values(Log_{10})before the statistical treatments were done. A Least Significant Difference (LSD) Test was further performed to compare significant differences between the mean values as decribed by [15].

Table 1: Microbial counts of the isolates

	Upstream		Raw effluent		Downstream	
	At 37^{o}C	At room temp.	At 37^{o}C	At room temp.	At 37^{o}C	At roomtemp.
TAPC (cfu/ml)	1.30×10^{5}	9.8×10^{4}	2.2×10^{7}	1.7×10^{7}	1.5×10^{7}	1.3×10^{7}
TCC (cfu/ml)	9.1×10^{4}	7.5×10^{4}	1.7×10^{7}	1.3×10^{7}	1.2×10^{7}	9.1×10^{6}
SaC (cfu/ml)	4.8×10^{3}	3.4×10^{3}	7.0×10^{4}	5.9×10^{4}	5.8×10^{4}	4.2×10^{4}
ShC (cfu/ml)	3.5×10^{3}	2.2×10^{3}	5.6×10^{4}	4.3×10^{4}	4.7×10^{4}	3.8×10^{4}
EcC (cfu/ml)	2.9×10^{4}	2.0×10^{4}	5.4×10^{5}	4.2×10^{5}	3.9×10^{5}	3.5×10^{5}
StC (cfu/ml)	8.4×10^{3}	7.0×10^{3}	6.7×10^{5}	5.7×10^{5}	5.8×10^{5}	4.6×10^{5}
TFC (cfu/ml)	-	2.0×10^{3}	-	5.0×10^{4}	-	3.0×10^{4}

Key: TAPC = Total aerobic plate count; TCC = Total Coliform count; SaC = *Salmonella* count; ShC = *Shigella* count; EcC = *E. coli* count; StC = *S. aureus* count; TFC = Total fungal count

III. Discussion

This study was conducted to determine the microbial quality of an abattoir effluent discharged into Waterside River in Aba, Abia State. The study tried to assess the possible health hazard of the effluent being discharged into the receiving river, and also the growth rate of the organisms at 37^0C and at room temperature. The bacterial isolates include; *Escherichia coli, Staphylococcus aureus, Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., *Shigella* sp., and *Pseudomonas* sp., while the fungal isolates include;*Aspergillus*sp., *Penicillium* sp., and *Rhizopus* sp.

From the results obtained, the total aerobic plate count ranged from 9.8×10^4 cfu/ml to 2.2×10^7 cfu/ml, with the effluent giving the highest mean count, while the total coliform count ranged from 7.5×10^4 cfu/ml to 1.7×10^7 cfu/ml, with the effluent also giving the highest mean count. When compared with the Federal Environmental Protection Agency (FEPA)standard for discharge of industrial effluent into surface waters (4.0×10^2cfu/ml or 400MPN/100ml), the total coliform count exceeded the limits of discharge [16], and this is similar to the findings made by [17]. Fecal coliforms live in the digestive tract of warm blooded animals and their counts are often used as a surrogate measurement for gastroenteric pathogens. Presence of fecal coliforms in water is an evidence that human or animal waste has been, or, is present in the water. This is a cause of concern as many diseases can be spread through the fecal-oral route. Faecal coliforms in abattoir wastewater have earlier been reported by several researchers [18-20].

The *Salmonella* count ranged from 3.4×10^3 cfu/ml to 7.0×10^4 cfu/ml. Presence of *Salmonella* in the water poses a threat to humans who utilize the water for drinking or cooking, as it is the leading cause of enteric fever, gastroenteritis and other malignancies associated with the organism [21].

The *Shigella* count ranged from 2.2×10^3cfu/ml to 5.6×10^4 cfu/ml. *Shigella* when ingested can survive gastric acidity, and causes illness by infecting the colonic mucosa and multiplying in colonic epithelial cells [22-23]. Severe inflammatory bacillary dysentery or Shigellosis soon occurs with several manifestations, such as severe abdominal cramps, nausea, vomiting and fever[24]. *Shigella* has also been isolated from several other abattoir effluents [25-26].

Regarding the *Escherichia coli* count, the counts ranged from 2.0×10^4 cfu/ml to 5.4×10^5cfu/ml. *Escherichia coli* is the major indicator organism for fecal contamination. Most strains are harmless, but however, some strains such as *E. coli* O157:H7 can pose a serious threat to humans, causing severe stomach cramps, diarrhea and vomiting. Serious complications of *E. coli* O157:H7 infection can include kidney failure. The discharge of untreated abattoir wastewater could result in an outbreak of *E. coli* infection, as observed in several studies [27-29].

Further, the *Staphylococcus aureus* count ranged from 7.0×10^3 cfu/ml to 6.7×10^5 cfu/ml. *S. aureus* is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans. It is one of the most common causes of skin and soft tissue infections[30].

The fungal count ranged from 2.0×10^3 cfu/ml to 5.0×10^4 cfu/ml. The presence of the fungal isolates in this study poses a serious threat to the individuals who use the nearby river for several domestic purposes. *Aspergillus* species are the leading cause of Aspergillosis, which is an illness that has the ability of affecting the central nervous system in immune-compromised hosts [31]. *Rhizopus* species cause the group of infections referred to as Zygomycosis. This type of infection is also fatal to humans. *Penicillium* species are potential producers of mycotoxins which are injurious to health. *Penicillium* species also cause allergenic and asthmatic reactions in susceptible individuals.

The One Way Analysis of Variance showed that there was a significant difference ($P<0.05$; $P<0.01$) between the total coliform counts of the abattoir wastewater and the upstream and downstream of the receiving water body. When a least significant difference (LSD) test was further performed, it was discovered that the difference between the mean counts occurred between the abattoir effluent and downstream of the receiving water body; an indication of contamination of the receiving water body with abattoir wastewater. This was similar to the findings reported in other places [1; 4; 8; 32].

The microbial concentration observed in the upstream sample in this study could be as a result of usage of the water for bathing by the abattoir workers and also indiscriminate disposal of waste into water bodies by the inhabitants of the area. From the differences observed in the mean counts of the samples at 37^oC and at room temperature, one could infer that the organisms thrive better at 37^oC than at room temperature. This could be because, the isolates were more of pathogenic organisms, which have an optimal growth temperature of 37^oC.

IV. Conclusion

This study observed that untreated abattoir wastewater discharged into the Waterside River in Aba, Abia State, contained bacterial counts above the recommended level for discharge into water bodies in Nigeria. The water-body was contaminated with bacterial pathogens that could impact negatively on public health,owing

to the fact that the river still serves as major source of water supply for the surrounding area. Therefore, the study recommends the siting of abattoirs away from residential areas and nearby streams and rivers, and the implementation of **a**ppropriate abattoir wastewater treatment measures to prevent the contamination of water bodies and underground water in Nigeria.

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