Assessment of The Impact of EGBU Abattoir Effluent on the Microbiological Properties of Otamiri River

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ABSTRACT

A total of forty water samples collected between July and November, 2019 from five sampling points situated at distance of 0m, 10m, 50m, 100m downstream and 10m upstream (to reflect the ambient condition of the river prior to pollution with abattoir effluent) were used to ascertain the microbiological quality of the river. The mean bacterial counts ranged from 1.3 x 10^6 cfu/ml to 9.0 x 10^6 cfu/ml of total aerobic heterotrophic bacterial count, total fungal count ranged from 0.5 x 10^6 cfu/ml to 1.1 x 10^6 cfu/ml and the total coliform count ranged from 9 x 10^4 to 3.5 x 10^6 MPN/100ml. The results revealed that the effluent discharge point had the highest microbial load which was evident in the total aerobic heterotrophic bacterial count of 9.0 x 10^6cfu/ml, the total coliform count of 3.5 x 10^6 MPN/100ml and total fungal count of 1.1 x 10^6cfu/ml. The bacteria were mostly enteric organisms and their frequency of isolation included; *Salmonella* species (19.51%), *Enterococcus* species (14.63%), *Proteus* species (12.20%), *Klebsiella* species (7.32%), *Staphylococcus aureus* (9.76%), *Escherichia coli* (21.95%) and *Enterobacter* species (14.63%). While fungal isolates were; *Aspergillus flavus* (14.29%), *Mucor* species (28.57%), *Aspergillus niger* (33.33%) and *Penicillium* species (23.81%). Egbu abattoir effluents have negative microbiological impact on Otamiri River, exposing the health of those who directly use the water for various purposes to hazard.

Key words): Assessment, Otamiri River, Effluent, Isolates.

Introduction

The abattoir is known to provide domestic meat to the people as well as generate employment opportunities. (Ogbonnaya, 2008) reported that the abattoir industries in Nigeria are less developed and therefore do not have adequate facilities for the treatment of abattoir effluents before disposal. Hence, abattoir effluents are contaminated with microorganisms of diverse species (Nafarnda et al., 2012); (Adegunloye, n.d.); (Ogunnusi & Dahunsi, 2014) and can constitute potential health risks from waterborne pathogens (Nafarnda *et al.*, 2006).

The Otamiri River runs through several villages in Owerri and its environ and is readily accessible but is easily disposed to pollution as a result of various human activities. Due to the high population density and inadequate sanitation, the river is turned into dumping sites for refuse and wastes. The people living along a particular segment of the river simply demarcate a portion of the river that is relatively upstream with respect to their location and draw water from there for their domestic uses. Unfortunately, someone's upstream water source is another person's downstream waste disposal point and most rural dwellers are unaware of the gravity of the risk attached to the use of such polluted water. Hence, according to (Mittal, 2004) and (Omole & Longe, 2008), there are conflicts in water usage because of the difference in water qualities required for different users having diverse interests (commercial ventures, domestic purposes, religious purposes that involves direct skin contact with the water).

Contamination of river body by abattoir effluent which is the main of this study could constitute significant environmental and health hazards (Coker *et al.*, 2001; Nafarnda et al., 2006;

Osibanjo & Adie, 2007). (Adelegan, 2002), documented that the animal blood is released untreated into the stream while the consumable part of the consumable parts of the slaughtered animal are washed directly into the flowing water. In addition, wastes from abattoirs also contain undigested feed, flesh bits, fats and bones. These abattoir wastes are characterized with high level of organic matter (Coker et al., 2001; Nafarnda et al., 2006), which supports the growth of microorganisms. Some of the microbes isolated from surface water polluted by discharge of abattoir effluent have been shown to cause diseases such as acute gastroenteritis, salmonellosis and typhoid fever caused by enteropathogenic *Escherichia coli*, *Salmonella paratyphi* strains and *Salmonella typhi* respectively (Denis et al., 2005; Muoghalu & Omoch, 2000). Studies done in Canada (Mittal, 2004) and Nigeria (Nafarnda et al., 2012; Ogunnusi & Dahunsi, 2014; Osibanjo & Adie, 2007) revealed high microbial load of abattoir effluent.

The effluent from abattoir processes can be regarded as point source or nonpoint source of water pollution owing to the fact that waste from these processes can be directly dumped or indirectly through runoff actions into the same river. Point sources occur when pollutants are emitted directly into the water body. Surface waters being a normal habitat for aquatic animals could have consequential impact on man either directly or indirectly when polluted since less than 1% of the world's fresh water is readily accessible for human use (UNESCO, 2006). According to report by (WHO/UNICEF, 2005) and (UNESCO, 2006), large numbers of surface water bodies in developing countries are polluted. The people that make use of water from such water bodies are definitely adversely affected by the effects of the discharges from abattoirs and other pollution sources, thereby putting a large proportion of the users at environmental and public health risks (Nafarnda et al., 2012; Osibanjo & Adie, 2007). In addition, other pollution sources in Owerri that discharge their wastes into Otamiri river and have considerable pollution effect include; cassava processing industries, paint industries, rubber factories, sawmills, oil mills, motor servicing workshops, car wash services, sand dredging operations as well as the activities of cattle-rearing.

According to (Biradar et al., 2014), eutrophication of water bodies is rapidly in the increase due to growing rise in the quantity of sewage and anthropogenic stress. Therefore, monitoring the quality of surface water by microbiological factors is of significance since it permits the direct evaluation of the hygienic state of the water body as the coliforms particularly *Escherichia coli* if present in the water are indication of faecal pollution and consequential hazard of contracting diseases (Nogueira et al., 2003; Usha et al., 2008; Vandysh, 2004). Considering the various sources of pollution identified with Otamiri River, abattoir effluent is the most notable because of its high biological constituent. There is therefore the need for regulation which cannot be formulated without the scientific evaluation of the impact of the abattoir effluent on the river.

Hence, this study is based on the interest to have the primary information on microbial quality of Otamiri river, possible portability and gravity of pollution of the river. The data obtained will help in advising the policy makers on the need to make effective water policies as well as enhancing treatment processes to combat Otamiri pollution by abattoir effluent in other that the water will be portable. Therefore, this current study is aimed at assessing the impact of abattoir effluent on the quality of Otamiri River.

Materials and Method

Brief Description of Study Area

The Egbu (Somachi) abattoir is located near the popular Relief market in Owerri North, Eastern Nigeria as seen in figure 1. The abattoir has existed for several decades with an average daily kill of about 120 cattle. The Otamiri River runs through several villages including Egbu in Owerri North, covering an area of about of about 10,000 square kilometers with an average annual rainfall of 2250 – 2500mm and a mean temperature of 27^{O}C throughout the year.

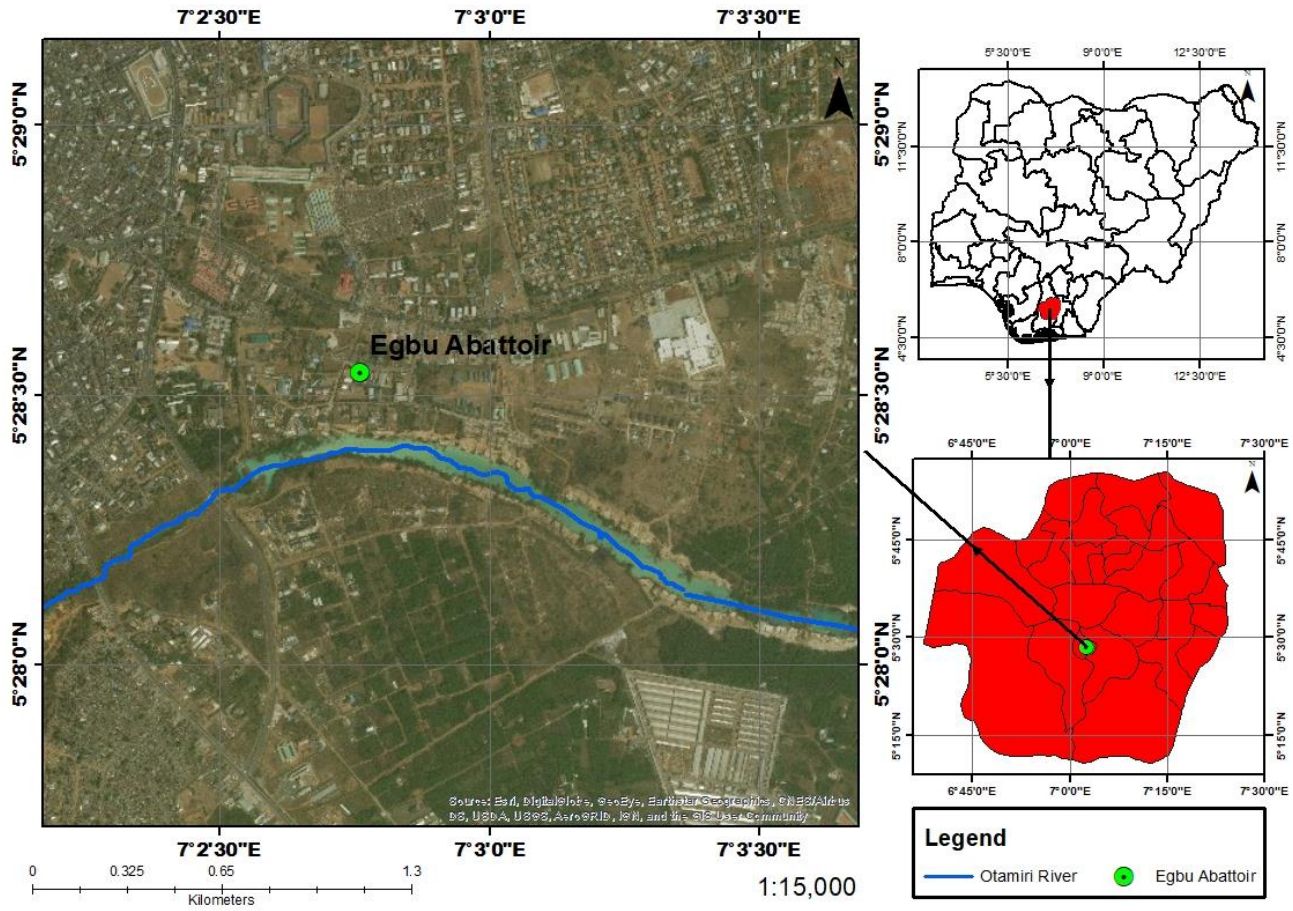

Figure 1: A Map Showing the Study Area

Sample Collection

A total of forty (40) water samples were collected in the morning (08.00 to10.00 am) at the peak hours of effluent discharge into the river and evening (04.00 to 05.00 pm) between July and November, 2019. The grab sampling method was employed using wide mouthed 500 ml sterilized Pyrex glass bottles with tight screw dust proof stoppers. The bottles were filled leaving a top space of about 2.5 cm (Nafarnda et al., 2012). Samples were obtained from four points spread over 100m downstream of the effluent discharge point and an ambient water sample was also collected 10m upstream to reflect on the water quality prior to pollution by the abattoir effluent giving a total of five sampling points namely; sampling point at effluent discharge point, sampling point located 10m downstream from effluent discharge point, sampling point located 50m downstream from effluent discharge point, sampling point located 100m downstream from effluent discharge point and sampling point located 10m upstream from effluent discharge point. A meter measuring tape was used to determine the distances. The water samples were transported without delay in icepacks to the Microbiology laboratory of Federal Polytechnic Nekede, Owerri for immediate analysis.

Microbiological Analysis

All the media used (Nutrient agar, MacConkey agar, Sabouraud dextrose agar, lactose broth and Eosin methylene blue agar) were prepared according to manufacturers' instructions. Microbial counts of water samples were determined using the spread plate inoculation technique. An aliquot (0.1ml) of ten-fold serial dilution (10^1-10^4) of each sample was inoculated on various media using a sterile spreader. Inoculation of MacConkey agar and Nutrient agar were for enumeration of total aerobic heterotrophic bacterial (TAHB) count whereas, Sabouraud dextrose agar (SDA) was for total fungi (TF) count. Plates for

bacterial isolation were incubated at 37^{O}C for 24 hours while, SDA plates were incubated at 25^{O}C for 5 days. After incubation, distinct colonies that developed were counted and expressed as colony-forming units per milliliter (cfu/ml). The mean averages of the total counts were calculated and recorded.

Bacterial and fungal colonies were picked at random from plates containing the highest countable dilution and purified by sub-culturing into appropriate media and incubated for biochemical analysis (Rompre et al., 2002)

Enumeration of faecal coliform count of the water samples was carried out by a one step-tube most probable number (MPN) technique using lactose broth growth medium with tubes incubated at 44^{O}C for 24 hours. Aliquots (10ml, 1ml and 0.1ml) of each water sample were added to single or double strength medium as appropriate. Tubes showing growth and gas production after 24 hours were recorded positive. The positive tubes were further cultured on Eosin methylene blue (EMB) agar plates for confirmed coliform test (Rompre et al., 2002). The numbers of positive results were enumerated and statistical MPN tables used to determine bacteria counts in MPN/100ml.

Identification of Isolates

The bacterial isolates were screened and identified based on their morphological, physiological and biochemical characteristics as described by (Biradar et al., 2014; Rompre et al., 2002).

Results

Table 1 shows mean values of the total microbial load of Otamiri River at various distances from the abattoir effluent discharge point. Total aerobic heterotrophic bacterial count (TAHBC) ranged from 1.3 x 10^6 cfu/ml to 9.0 x 10^6 cfu/ml while total fungal count (TFC) ranged from 0.5 x 10^6 cfu/ml to 1.1 x 10^6 cfu/ml.

Table 1: Microbial Loads of the Otamiri River Sampling Points

Sampling Points	NA TAHBC (cfu/ml)	MCA TAHBC (cfu/ml)	SDA TFC (cfu/ml)
OTSP 1	9.0 x 10^6	7.0 x 10^6	1.1 x 10^6
OTSP 2	6.5 x 10^6	5.5 x 10^6	0.9 x 10^6
OTSP 3	5.1 x 10^6	4.3 x 10^6	0.9 x 10^6
OTSP 4	3.1 x 10^6	2.0 x 10^6	0.5 x 10^6
OTSP 5	1.7 x 10^6	1.3 x 10^6	0.7 x 10^6

Keys: cfu/ml= Colony forming unit per milliliter, TAHBC = Total aerobic heterotrophic bacterial count, TFC= Total fungal count, OTSP 1 = Otamiri river samples at effluent discharge point, OTSP 2 = Otamiri river samples at sampling point 10m downstream from effluent discharge point, OTSP 3 = Otamiri river samples at sampling point 50m downstream from effluent discharge point, OTSP 4 = Otamiri river samples at sampling point 100m downstream from effluent discharge point, OTSP 5 = Otamiri river samples at sampling point 10m upstream from effluent discharge point.

The coliforms were morphologically confirmed on Eosin methylene-blue agar as *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella* species as presented in table 2.

Table 2: Confirmed test for Coliforms

Morphological Characteristics coliform on EMB	Possible Coliform
Raised, blue-black colonies with metallic sheen	*Escherichia coli*
Raised, pinkish, dark-centered large colonies	*Enterobacter aerogenes*
Irregular, raised, purple colonies with dark-purple edge	*Klebsiella* species

Key: EMB = Eosin Methylene-blue agar

The morphological and biochemical characteristics of the bacterial isolates as shown in Table 3 above, unraveled the bacteria contaminants as *Salmonella* species, *Streptococcus* species, *Proteus* species, *Klebsiella* species, *Staphylococcus aureus*, *Escherichia coli* and *Enterobacter* species.

Table 3: Morphological and Biochemical Characteristics of the Bacterial Isolates

Cultural Characteristics	Gram reaction	OX	CA	COA	CIT	IT	KIA					Possible Bacteria
							S	B	H_2S	G	MT	
Creamy, circular, opaque colonies	- rod	-	-	-	-	-	R	Y	+	-	+	*Salmonella* species
Milkish, irregular flat, non-mucoid colonies	+ cocci	-	-	-	-	-	R	Y	-	-	-	*Streptococcus* species
Shiny swarming mucoid colonies	- rod	-	+	-	+	+	R	Y	-	-	+	*Proteus* species
Shiny, raised, circular, large mucoid colonies	- rod	-	-	-	+	-	Y	Y	+	-	+	*Klebsiella* species
Dull-orange, raised, non-mucoid colonies	+ cocci	-	+	+	-	-	No reaction				-	*Staphylococcus aureus*
Pinkish, circular, raised colonies on MCA	- rod	-	+	-	-	+	Y	Y	-	+	+	*Escherichia coli*
Dull pinkish, irregular, large colonies flat	- rod	-	-	-	+	-	Y	Y	-	+	+	*Enterobacter* species

Key: + = Positive, - = Negative, OX = Oxidase test, CA = Catalase test, COA = Coagulase test, CIT = Citrate test, IT = Indole test, KIA = Kigler iron agar, MT = Motility test, S = Color of slope, B = Color of butt, H_2S = Hydrogen sulphide production (blackening), G = Gas production, R = Red coloration (alkaline production), Y = Yellow coloration (acid production).

The frequency of occurrence of the above isolated bacteria in the water samples was as shown in table 4 below: *Salmonella* species 8 (19.51%), *Streptococcus* species 6 (14.63%), *Proteus* species 5 (12.20%), *Klebsiella* species 3 (7.32%), *Staphylococcus aureus* 4 (9.76%), *Escherichia coli* 9 (21.95%) and *Enterobacter* species 6 (14.63%).

Table 4: Frequency (%) of the Bacterial Species from the Otamiri River Samples

Bacterial Species	Frequency	Percentage Occurrence (%)
Salmonella species	8	19.51
Streptococcus species	6	14.63
Proteus species	5	12.20
Klebsiella species	3	7.32
Staphylococcus aureus	4	9.76
Escherichia coli	9	21.95
Enterobacter species	6	14.63
Total	**41**	**100.0**

The fungal isolates were as presented in table 5 and include; *Aspergillus flavus*, *Mucor* species, *Aspergillus niger* and *Penicillium* species. The frequency at which the isolated fungi occurred was as shown in table 6 below viz; *Aspergillus flavus* 3 (14.29%), *Mucor* species 6 (28.57%), *Aspergillus niger* 7 (33.33%) and *Penicillium* species 5 (23.81%). The most frequent occurred fungus was *Aspergillus niger* 7 (33.33%) while *Aspergillus flavus* had the least occurrence 3 (14.29%).

Table 5: Identification and characterization of fugal isolates

Cultural characteristics on SDA	Microscopy	Possible fungi
Whitish, cottony broom-like colonies with greenish center	Septate hyphae with conidia bearing sterigmata	*Aspergillus flavus*
Whitish, cottony broom-like colonies with grey center	Non-branched hyphae	*Mucor* species
Whitish, cottony broom-like colonies with yellowish-green center	Septate hyphae with conidia bearing sterigmata	*Aspergillus niger*
Whitish, cottony broom-like colonies with bluish-green center	Septate hyphae with spores	*Penicillium* species

Key: SDA = Sabouraud Dextrose agar

Table 6: Frequency (%) of the fungal Species from the Otamiri River Samples

Fungal species	Frequency	Percentage Occurrence (%)
Aspergillus flavus	3	14.29
Mucor species	6	28.57
Aspergillus niger	7	33.33
Penicillium species	5	23.81
Total	**21**	**100**

Discussion

According to (Nafarnda et al., 2012) as well as (Ogunnusi & Dahunsi, 2014), abattoir effluent habours a variety of microorganisms which when discharged into water bodies pollute the water. The results of the bacterial and fungal load at the various sampling points as shown in table 1 were evident to this fact. The mean total aerobic heterotrophic bacteria count (TAHBC) ranged between 1.3 x 10^6 cfu/ml and 9.0 x 10^6 cfu/ml while total fungal count (TFC) ranged between 0.5 x 10^6cfu/ml and 1.1 x 10^6cfu/ml. The highest counts for mean aerobic heterotrophic bacteria count (TAHBC) and total fungal count (TFC) were observed and recorded at the effluent discharge (ED) point. In that order, the higher counts were recorded at downstream points signifying higher pollution at those points nearer to the effluent discharge point and reduction in the microbial load was observed as the distances from the effluent discharge point decreases. The estimation of faecal coliform count (FCC) at different points using the MPN method also indicated that the effluent discharge (ED) sampling point had the highest mean faecal coliform MPN count of 3.5 x 10^6 MPN/100ml. The effluent discharge sampling point and the downstream points also revealed the higher total fungal count (TFC) in decreasing order towards downstream further distances. In contrast, the upstream sampling point showed the lowest counts for all the microbial parameters employed as unraveled in table 1 and table 2. The total viable count for all the samples exceeded the limit of 1x10^2 cfu/ml which agrees with the reports of (Ogunnusi & Dahunsi, 2014) and (Nafarnda et al., 2012) signifying that the abattoir effluent is highly contaminated and ultimately polluted the Otamiri river that receives the effluent. However, the bacterial counts obtained in this study are higher than that recorded by (Nafarnda et al., 2012) for receiving water body. This may be attributed to the increased activities of the villagers dwelling close to Otamiri river who use of the water body for dumping refuse, other domestic activities as well as the activities of herdsmen and their cattle. Other pollution sources that discharge their wastes into Otamiri river include; cassava processing industries, paint industries, rubber factories, sawmills, oil mills, motor servicing workshops, car wash services and sand dredging operations must have considerable pollution effect. These point pollution and non-point pollution sources collaborate with the reports of Efok, (2008) and U.S Environmental Protection Agency (2010). There was no significant difference ($P < 0.05$) between the mean bacterial counts of abattoir effluent discharge point and receiving water bodies 100 m downstream which is an indication of contamination of receiving water bodies with abattoir wastewater and similar findings has been reported in other places (Nafarnda et al., 2012; Ogunnusi & Dahunsi, 2014)

Table 2, table 3 and table 4 confirmed the bacteria isolates identified by morphological and biochemical tests from the water samples as well as their decreasing order of frequency to be;

Escherichia coli (21.95%), *Salmonella* species (19.51%), *Streptococcus* species and *Enterobacter* species (14.63%) each, *Proteus* species (12.20%), *Staphylococcus aureus* (9.76%) and *Klebsiella* species (7.32%). Some of these bacteria isolates from the water samples have been incriminated to cause diseases, for example, acute enteritis caused by enteropathogenic *Escherichia coli* (Byamukama et al., 2005) as well as Salmonellosis and typhoid fever caused by *Salmonella paratyphi* strains and *Salmonella typhi* respectively (Muoghalu & Omoch, 2000). The fungal isolates were; *Aspergillus niger* (33.33%), *Mucor* species (28.57%), *Penicillium* species (23.81%) and *Aspergillus flavus* (14.29%). The most frequent occurred fungus was *Aspergillus niger* while *Aspergillus flavus* had the least occurrence (Table 5 and table 6). Apart from *Aspergillus flavus* which synthesizes harmful mycotoxin referred to as aflatoxin that cause aspergillosis; most of the isolated fungi from this study were mainly soil saprophytes that are not usually considered pathogenic. However, some saprophytic fungi may become opportunistic pathogens when exposed to persons with compromised immune system (Jawetz & Adelberg's, 2010). The effluent discharge sampling point exhibited the highest diversity of microbial species from the water samples followed by samples at sampling point 10m downstream from effluent discharge point, samples at sampling point 50m downstream from effluent discharge point and samples at sampling point 100m downstream from effluent discharge point. Otamiri river samples at sampling point 10m upstream from effluent discharge point had few species of microbial isolates. This suggests greater contamination of the Otamiri river body to be from the abattoir effluents and can as well contaminate underground water sources used for drinking.

The high coliform count and especially the presence of faecal indication bacteria (*Escherichia coli*) indicate faecal contamination thereby limits the use of the water body and making the water unsafe for consumption. People making use of the water without adequate treatment are prone to various diseases that are contracted through direct or indirect ingestion of pathogenic organisms that can cause various degrees of gastroenteritis and intoxication. Some of the of medically important microorganism from the water can also penetrate into open skin cuts, hair follicles, ears or nose that could manifest in various diseases including; boils, skin sores, respiratory infections, otitis media etc.

Conclusion

The relatively high microbial loads revealed at the effluent discharge point and downstream points reflect the large amount of inadequate treated abattoir waste water discharged into Otamiri water body, thereby polluting the river. Hence, this study has shown that the discharge of Egbu abattoir effluent into Otamiri river negatively impacts on the microbiological quality of the river, thereby exposing domestic, recreational and commercial users to avoidable health hazard.

Therefore, regulations and laws on effluent discharge from abattoir would promote healthy practices that will enhance the safety of the use of surface water bodies.

Recommendations

- The abattoir effluent disposal should be strictly monitored by the appropriate agencies so as to control environmental pollution and reduce health hazards.
- The government's intervention is needed in putting up effluent treatment facilities and introduction of better technologies to reduce quantity of wastes emanating from meat processing.
- Awareness should be created as well as education of the teeming population on the ill effects of polluted water on health.
- More research should be geared towards discovering better efficient methods of assessment and biological treatment of abattoir waste in order to reduce its pollution capacity and volume of waste produced from abattoir.

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