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Effect of Brewery Effluent on the Microbiological Quality of Ikpoba River and Surrounding Borehole Waters in Benin City, Nigeria

O. O. Akpomie[1], H. S. Buzugbe[2] and P. M. Eze[2*]

[1]Department of Microbiology, Delta State University, Abraka-Nigeria, Nigeria.
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OOA designed and supervised the study. Authors HSB and PME managed the laboratory analyses. Authors HSB and PME managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The microbiological quality of Ikpoba River, Benin City-Nigeria and the surrounding borehole waters was investigated to assess the extent of pollution from Guinness Nigeria Plc, Benin City.
Methodology: Microbiological parameters analyzed were total bacterial, fungal and coliform counts, and identification for indicator organisms.
Results: For river water samples (RW1-RW4), mean total viable aerobic bacterial, fungal and coliform counts ranged from $0.06x10^8$ to $35.0x10^8$ CFU/mL, $0.6x10^4$ to $2.5x10^4$ CFU/mL, and $0.006x10^6$ to $2.3x10^6$ CFU/mL respectively; and for borehole water samples (BH1-BH5), mean counts ranged from $1.3x10^4$ to $3.3x10^4$ CFU/mL for bacteria, 4.0x10 to 8.2x10 CFU/mL for fungi, and 0 to $6.1x10^2$ CFU/mL for coliforms. Only BH1 and BH5 showed the absence of coliforms.
Conclusion: From the results of this study, it was observed that the effluent discharge from Guinness Nigeria Plc may not totally be responsible for the microbial contamination of the Ikpoba River and the surrounding borehole waters, as other sources of contamination, especially from

**Corresponding author: Email: ezep2004@hotmail.com;*

human activities, were noticed. It is therefore important that governments and concerned institutions put measures in place to remove the sources of contamination in water bodies that supply locals with water for household use. Industrial effluent also, should be effectively treated before they are discharged into the water body and industries should strictly adhere to the standards and guidelines specified by various water and environment regulating bodies.

Keywords: River water; borehole water; microbiological quality; brewery effluent.

1. INTRODUCTION

Effluent discharges into the environment have been on the increase in Nigeria since 1960 due to active industrialization and urbanization and the accompanying increase in commercial activities [1]. Copious amount of wastes is produced due to human activities and the capacity of the environment to absorb these wastes is limited. On the other hand, waste or effluent management has remained under developed and very unsatisfactory leading to environmental pollution, depletion, global warming, deforestation, shoreline erosion or degradation of natural ecosystems [2]. Consequently, many potential pollutants have found their way into ground and surface waters causing harm to the environment, and also, to man, plants and animals.

Water pollution is a serious problem globally, involving the discharge of dissolved or suspended substances into ground water, streams, rivers and oceans [3]. Surface waters are usually exposed to microbial contamination from run-off inputs, soils, and any waste deliberately or inadvertently dumped into such waters. These can result to pollution, increase in microbial load, eutrophication, visible aesthetic nuisance and loss of recreational amenities [4].

A major source of pollution in developing countries is industrial activities and this has gradually increased the problem of waste disposal [5]. Increased industrial activities have led to pollutional stress on surface water both from industrial, agricultural and domestic sources [1]. However, the quantity of waste discharge from industries depends on the activities and usage of water. Breweries for example are known to consume water of about 4 to 8 cubic meters per cubic meter of beer produced [3]. Brewery plants have also been known to cause pollution by discharging effluent into receiving stream, ground water and soil [6].

Untreated wastes from processing factories located cities are discharged into inland water bodies resulting to stench, discoloration and a greasy oily nature of such water bodies [7]. These wastes pose a serious threat to associated environment, including human health risks and several researches have confirmed the negative effects of industrial effluent discharge on rivers [5,6,8,9,10]. There is therefore the need to control the pollution of surface and ground water since the health and wellbeing of the people have a directly dependent on the availability of good water.

Ikpoba River is a fourth order stream located in Benin City, Edo State in South Western Nigeria. The river serves as a source of water for domestic purposes (including drinking and cooking). Also, fishing and other human activities (such as bathing, swimming, washing, etc.) take place in the river. According to Ekhaise and Anyansi [9], the Ikpoba River receives a variety of wastes ranging from industrial, agricultural, domestic and natural sources and these wastes introduce foreign microorganisms, organic and inorganic matter, in addition to indigenous micro-flora. The Oregbeni community flanks the river on one side behind Guinness Nigeria Plc and Bendel Breweries Ltd. The products of the brewery operations include large volumes of wastewater, conveyed over a distance of 2.5km by an underground tunnel and discharged into the receiving river [9].

This study is aimed at determining the effects of Guinness Nigeria Plc effluent discharge on the microbiological qualities of Ikpoba River and the surrounding borehole waters, which is the major source of drinking water for people in that locality.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The samples were obtained and the study conducted in the month of April, 2014. A total of nine sampling points for both river and borehole waters were selected for this study (Fig. 1). Four sampling points for river water: RW1 (from the effluent drainage), RW2 (point of effluent discharge into the river), RW3 (upstream, 20

meters away from the point of effluent discharge) and RW4 (downstream, 20 meters away from the point of effluent discharge into the river). Five sampling points for borehole water: BH1 (borehole water supply located 200 meters away from river), BH2 (borehole water supply located 350 meters away from river), BH3, BH4 and BH5 (borehole water supply located at different points about 1000 meters away from river). Samples for microbiological analyses were collected in sterile 1 Liter glass bottles, and sample collection was done using the WHO water sampling method [11]. The river samples were collected by holding the bottle near its base in the hand and plunging it, neck downwards, below the surface. The bottles were then turned until the neck points slightly upwards and the mouth being directed towards the current. Borehole water samples were collected directed from the taps. The tap was cleaned and opened fully and the water was allowed to run for about two minutes to permit clearing the service lines and then the sample collection bottles were filled without splashing.

Two (2) samples per site were collected for both river and borehole waters and the mean values of the parameters analyzed were calculated.

2.2 Microbiological Analyses

2.2.1 Total viable aerobic (heterotrophic) plate count (for bacterial and fungal counting)

The total viable aerobic plate count was carried out using the pour plate method as described by the European Pharmacopeia [12]. One (1)mL of each water sample was aseptically pipetted into a sterile 9 cm Petri plate and 20mL of sterilized molten agar medium (that has been kept below 45ºC) was added to the plate. This was then carefully swirled to properly mix the medium with the inoculum. For bacterial counting, Tryptone Soya Agar (Oxoid, UK), also called Soybean Casein Digest Agar, was used and for fungal enumeration, Sabouraud Dextrose Agar (Oxoid, UK) was used. After the solidification of the agar, plates were incubated in an inverted position at 35-37ºC (for bacterial counting) and 25-27ºC (for fungal counting) for 2-3 days. Plates were observed daily for presence of countable colonies.

2.2.2 The total coliform count

The total coliform count was performed by the pour plate method using Chromocult Coliform Agar (Merck, Germany). The Chromocult Coliform Agar is a selective and differential chromogenic culture medium that enables the detection, differentiation and enumeration of *Escherichia coli* and coliforms. It gives reddish or pinkish coloration to coliforms, except for *E. coli* which may appear as dark blue or violet colonies. One (1) mL of each water sample was transferred aseptically to a sterile 9 cm Petri plate and 20 mL of the sterilized molten agar medium (that has been kept below 45ºC) was added to each plate. This was then properly mixed then carefully swirled to properly mix the medium with the inoculum. The medium was allowed to solidify and was then incubated at 35-37ºC in an inverted position for 24-48 hours. After incubation, the plates were examined for the presence of countable colonies of coliforms.

2.2.3 Isolation and identification of microorganisms

The isolation and identification of fungal isolates were carried out based on their cultural, morphological and microscopic characteristics as described by Bunchan and Gibbon [13].

For bacterial isolation and identification, various cultural, staining, and biochemical testing procedures were carried out as described by Cheesbrough [14] and Barnett and Hunter [15].

3. RESULTS AND DISCUSSION

3.1 Results

The mean values of microbial counts are summarized in Tables 1 and 2.

Data for mean values of the total viable aerobic plate counts are presented in Tables 1 and 2. The total viable aerobic bacterial count of the river samples was high. Borehole water samples also recorded bacterial counts, but far lower than the values recorded for the river samples. Also, among all borehole water samples, BH4 recorded the highest coliform population in the total coliform count, but BH1 and BH5 recorded no coliform growth. All samples from both the river and borehole recorded fungal growths. Among the river samples, RW1 and RW2 recorded higher fungal counts, while compared to other borehole samples, BH3 and BH4 recorded higher fungal numbers.

Tables 3 and 4 show the presence of certain microorganisms in the water samples. A total of twelve organisms were identified, which include eight bacterial isolates and four fungal isolates.

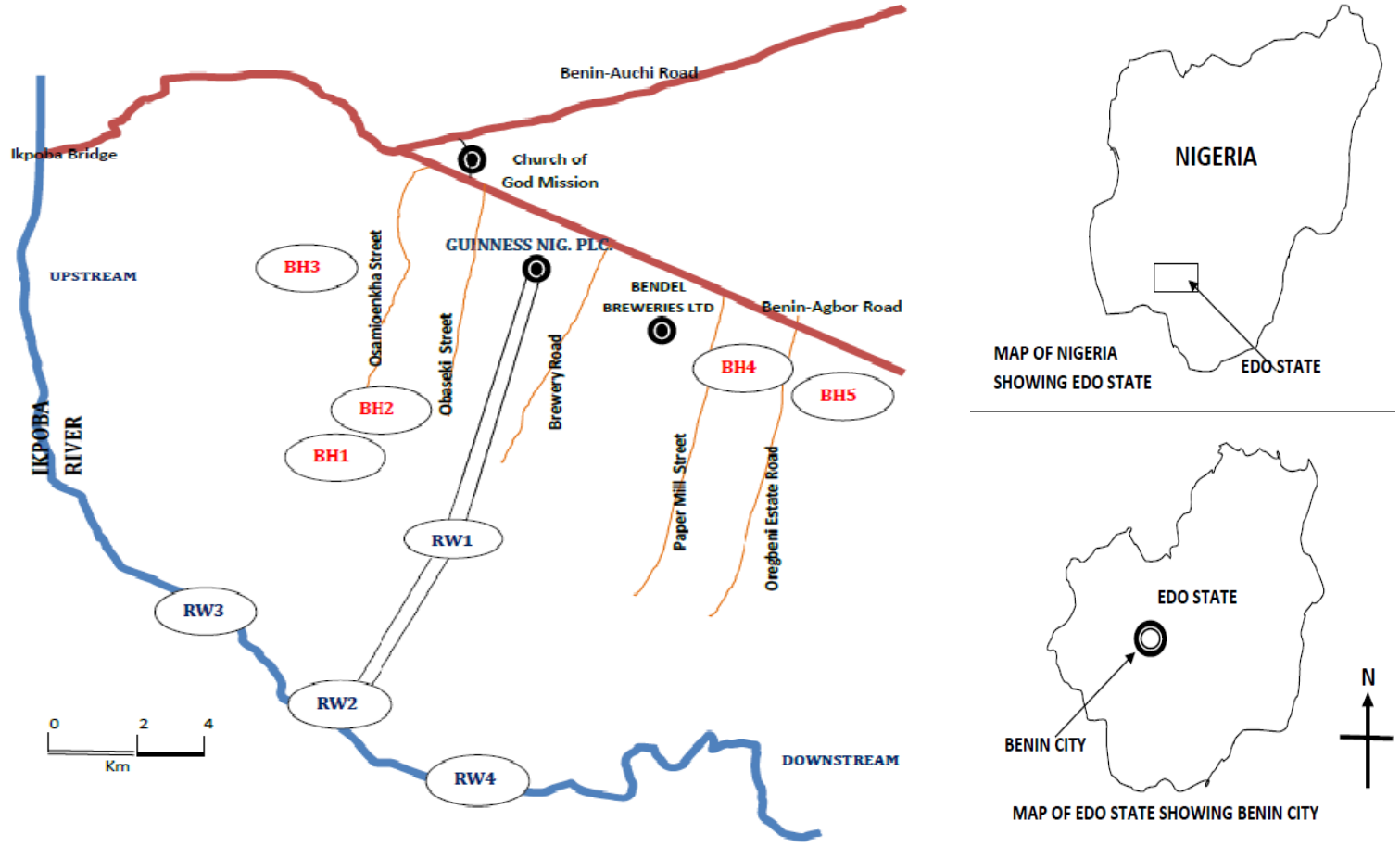

Fig. 1. Map of Ikpoba River showing sample collection points

Table 1. Total bacterial, coliform and fungal counts for river water samples

Parameters	River water samples			
	RW1	RW2	RW3	RW4
Total viable aerobic bacterial count (CFU/mL)	$35.0x10^8$	$9.0x10^8$	$0.07x10^8$	$0.06x10^8$
Total Coliform count (CFU/mL)	$1.7x10^6$	$2.3x10^6$	$0.006x10^6$	$0.006x10^6$
Total Viable Aerobic Fungal count (CFU/mL)	$2.5x10^4$	$1.7x10^4$	$0.6x10^4$	$0.7x10^4$

Table 2. Total bacterial, coliform and fungal counts for borehole water samples

Parameters	Borehole water samples				
	BH1	BH2	BH3	BH4	BH5
Total viable aerobic bacterial count (CFU/mL)	$3.3x10^4$	$2.8x10^4$	$2.7x10^4$	$2.7x10^4$	$1.3x10^4$
Total Coliform count (CFU/mL)	0	$4.6x10^2$	$2.3x10^2$	$6.1x10^2$	0
Total viable aerobic fungal count (CFU/mL)	4.0x10	4.0x10	8.0x10	8.2x10	7.2x10

Table 3. Bacterial isolates identified in water samples

Bacterial isolates	RW1	RW2	RW3	RW4	BH1	BH2	BH3	BH4	BH5
Escherichia coli	-	+	+	-	-	+	+	+	-
Pseudomonas aeruginosa	+	-	-	+	+	+	-	+	-
Proteus sp.	+	+	+	+	-	-	-	-	-
Serratia marcesens	+	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	+	+	+	+	-	-	-	+	-
Staphylococcus aureus	-	-	-	-	-	+	+	-	-
Streptococcus faecalis	-	-	+	+	-	-	-	-	-
Bacillus sp.	+	+	+	-	+	+	-	+	+

- : present; +: absent

Table 4. Fungal isolates identified in water samples

Fungal isolates	RW1	RW2	RW3	RW4	BH1	BH2	BH3	BH4	BH5
Mucor sp.	-	+	+	+	-	-	-	-	+
Rhizopus sp.	+	+	+	+	-	-	+	-	-
Aspergillus sp.	-	+	+	-	+	-	+	-	+
Fusarium sp.	+	+	-	+	+	-	-	-	-

- : present; +: absent

3.2 Discussion

The Ikpoba River in Benin City receives a variety of wastes ranging from industrial, agricultural and domestic to natural waste [9]. The result of this research shows the effect of Guinness Nig. Plc on the microbiological quality of Ikpoba River and the borehole waters around the river.

Microbial (bacterial and fungal) counts observed in all river samples were far higher than those recorded for the borehole water samples. The river water samples (RW1-RW4) recorded mean total viable aerobic bacterial, total viable aerobic fungal and total coliform counts that ranged from $0.06x10^8$ to $35.0x10^8$ CFU/mL, $0.6x10^4$ to $2.5x10^4$ CFU/mL, and $0.006x10^6$ to $2.3x10^6$ CFU/mL respectively (Table 1); and the borehole water samples (BH1-BH5) recorded mean counts that ranged from $1.3x10^4$ to $3.3x10^4$ CFU/mL for bacteria, 4.0x10 to 8.2 x10 CFU/mL for fungi, and 0 to $6.1x10^2$ CFU/mL for coliforms (Table 2). Only the borehole water samples BH1 and BH5 showed the absence of coliforms.The relatively high bacteria counts obtained in RW1 ($35.0x10^4$ CFU/mL) could be attributed to the high organic matter content of the brewery effluent and the ambient temperature of the effluent that may tend to favour the growth of mesophilic bacteria. Sayler et al. [16] reported that high microbial population in an aquatic system is a reflection of the input of microorganisms from extraneous

sources and availability of growth supporting organic matter. In addition to the effluent discharged into the river, incidence of human activities such as defecation, washing, bathing and discharge of sewage into the river may also be a contributing factor in the increase in microbial load of the river.

The WHO [17] stated that the greatest risk to public health from microbes in water is associated with consumption of drinking-water that is contaminated with human and animal excreta (although other sources and routes of exposure may also be significant). The presence of coliforms, *E. coli* and fecal streptococci in the water samples indicated fecal pollution of the water. The presence of coliforms in the borehole water samples may either be attributed to the proximity of the borehole to septic tanks or pit latrines, which is in negation to recommendations for constructing boreholes at least 30 meters away from septic tanks or pit latrines [18], or to faulty piping systems which may allow leakage of these agents into the borehole water. The high coliform counts recorded for river samples may be as a result of human activities such as defecation, urination, washing and even, discharge of sewage and other domestic wastes into the river. The heavy presence of these organisms in the river samples and in some of the borehole water samples (BH2, BH3 and BH4) indicates the unsanitary condition of the water and their unfitness for drinking. Unfortunately, these water sources (river and borehole) serve as the major source of drinking water for people of this locality. Other bacteria such as *Proteus species, K. pneumoniae* and *S. aureus* were also isolated from the river water samples and this is similar to the results of a previous study on the Ikpoba River [10].

Although the borehole water samples recorded microbial counts far lower than those recorded for the river water samples, they all failed to meet microbiological standards. Apart from the indicator bacteria (Coliforms and *E. coli*), other bacteria encountered, which include *Bacillus species, S. aureus, P. aeruginosa, Proteus species, S. marcesens and K. pneumoniae* (Table 3), may have contaminated the water from various sources. Some fungal contaminants were also isolated from the water samples. These include species of *Aspergillus, Mucor, Rhizopus* and *Fusarium* (Table 4).

It can be seen from the results of this study that the effluent discharge from Guinness Nigeria Plc may not totally be responsible for the high microbial contamination of the Ikpoba River, as other sources of contamination, especially from human activities, were also observed. Also, the high microbial levels of the brewery effluent and river water did not show any significant influence on the borehole waters around the river. This could be attributed to the fact that the river is not too deep to have contaminated the aquifer and also, that the sand, being a very good filter, was able to carry out a proper filtration of some of the contaminants. It is however assumed that the microbial counts recorded for the borehole water samples may have resulted from contamination of the tanks and pipes, or from other sources.

4. CONCLUSION

The results of this study reveal the unsanitary state of water consumed in this part of the country as most of the samples, including the borehole waters (which are the major source of drinking water for people in Nigeria), contain bacterial indicators of fecal pollution which were found to be above recommended levels for drinking water.

There is the need for state governments and concerned institutions to provide pipe-borne water as a source of drinking water for people in this region and throughout the country; put measures in place to remove the sources of contamination in water bodies that supply locals with water for household use; and also educate the people of the region on the relationship between water sanitation hygiene and healthy living.

It is recommended that users of borehole waters should be educated on the dangers associated with constructing septic tanks or pit latrines close to boreholes or vice versa and the use of good piping systems that will prevent seepage of harmful agents into the water and also the benefits of filtering and boiling water before drinking. These will go a long way in checking the likely outbreak of epidemics of water-related diseases.

It is also important that industrial effluent be effectively treated before they are discharged into the water body so as to reduce its negative effects on both the aquatic habitat and the quality and safety of the water for domestic purposes. Industries should adopt efficient preventive measures and also strictly adhere to the standards and guidelines mapped out by various

water and environment regulatory bodies as these will greatly reduce the pollution of water bodies with harmful agents.

CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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