*Journal of Scientific Research & Reports*
*3(3): 514-531, 2014; Article no. JSRR.2014.010*

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# Microbial and Physiochemical Quality of Effluent Water from a Brewery in Benin City, Midwestern Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author BI designed the study, wrote the protocol, and wrote the first draft of the manuscript. All authors managed the literature searches, analyses of the study performed the spectroscopy analysis and author FIO managed the experimental process and MA identified the species of plant. All authors read and approved the final manuscript.*

***Original Research Article***

***Received 23rd September 2013***
***Accepted 18th November 2013***
***Published 16th December 2013***

## ABSTRACT

**Aims:** The present study w as set up to investigate the physicochemical and microbial contents of waste water discharged into the Ikpoba Rivers as well as water samples obtained from the river at different points of collection with a view to determining impact on the water body.
**Study Design:** The design chosen for the study was complete randomization, considering the homogeneity of the experimental plots from which samples were collected.
**Place and Duration of Study:** Samples were collected during the late rainy season of August 2012 from both the Ikpoba River, Benin City, Nigeria and from effluent treatment plants in a brewery in Benin City.
**Methodology:** Samples were collected in three different locations; in the brewery, from the brewery effluent samples point, as well as in the Ikpoba River, where brewery effluent

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samples mixes with the river water. Non-effluent samples were also collected from the brewery, these included glycol, condensate, boiler feed, brew cold and cooling tower water (CTW). Samples of the brewery effluent discharged into the river were also collected from the brewery effluent samples channel. On the Ikpoba River, five different sampling point were identified; contact point (CP) of the discharged brewery effluent samples with the river water, 5m and 10m before contact point, as well as 5m and 10m after contact point, respectively.

**Results:** The pH of glycol was 7.8, compared to those of the condensate and boiler feed which were both 5.8 and 5.5 respectively. The pH of the brewery effluent samples was 5.8, however at the point of contact of brewery effluent samples with Ikpoba River, pH dropped to 4.8. The surface water temperature ranges of non-effluent samples materials was 29.6 – 29.9ºC, as compared to 29.2ºC which was the temperature of the brewery effluent samples before contact with Ikpoba River. The heavy metals detected in the non-effluent samples and brewery effluent samples samples were iron, magnesium, copper and zinc. Lead was only detected in the non-effluent samples while nickel and vanadium were not detected in both samples.

**Conclusion:** Results showed that the effluents samples from the industry altered the physical, chemical and biological nature of the receiving water body. However, comparison with WHO and FMENV standards showed no deviation from required benchmarks, and as such the samples were adjudged ecologically safe.

*Keywords: Brewery; effluent; microbial content; physicochemical; aste water.*

## 1. INTRODUCTION

Wastes are generated by various anthropogenic activities, and the improper management of the vast amount of these wastes has become one of the most critical problems of developing countries. More challenging is the unsafe disposal of these wastes into the ambient environment. Water bodies especially freshwater reservoirs are the most affected. This has often rendered these natural resources unsuitable for both primary and/or secondary usage [1]. Industrial brewery effluent samples are responsible for contamination of natural water bodies has emerged as a major challenge in developing and densely populated countries like Nigeria. Estuaries and inland water bodies, which are the major sources of drinking water in Nigeria, are often contaminated by the activities of the adjoining populations and industrial establishments [2]. River systems are the primary means for disposal of waste, especially the brewery effluent samples, from industries that are near them. These brewery effluent samples from industries have a great deal of influence on the pollution of the water body and can alter the physical, chemical and biological nature of the receiving water body [3]. Wastes entering these water bodies are both in solid and liquid forms. These are mostly derived from industrial, agricultural and domestic activities. Consequently water bodies have become highly polluted. The resultant effects of this on public health and the environment are usually great in magnitude [4]. The present study is driven by the fact that the river understudied is the source of livelihood for the local people who live along the river course.

High levels of pollutants in river water systems causes an increase in biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), toxic metals such as Cd, Cr, Ni and Pb and faecal coliform and hence make such water unsuitable for drinking, irrigation and aquatic life [5, 6, 7]. Tolba [8] reported that it is in these countries that the quality of water and often the quantity is lowest, sanitation and nutrition the worst and disease most prevalent. Effluent discharge practices in

Nigeria are yet too crude and society is in danger, especially in the industrialized part of the cities. For example, along a 10km course along the river is situated markets, at least 2 breweries that empty their effluents into the river, a cassava factory, as well as at least 2 abattoirs. Ezeronye and Amogu [9] opined that the Federal Environmental Protection Agency (FEPA) established to check these environmental abuses has had little or no impact on pollution control in our cities.

Industrial brewery effluent samples are a main source of direct and often continuous input of pollutants into aquatic ecosystems with long-term implications on ecosystem functioning including changes in food availability and an extreme threat to the self-regulating capacity of the biosphere. These industrial discharge or wastes include heavy metals, pesticides, polychlorinated biphenyls (PCBs), dioxins, poly-aromatic hydrocarbons (PAHs), petrochemicals, phenolic compounds and microorganisms [10]. Wastewater from Brewery Industry originates from liquors pressed from grains and yeast recovery and have the characteristic odour of fermented malt and slightly acidic [11]. Brewery effluent samples are high in carbohydrates; nitrogen and the cleaning and washing reagents have been proved water pollutants. The introduction of wastewater, high in organic matter and essential nutrients bring about changes in the microflora. Ekhaise and Anyansi [12] reported high counts of bacterial population in Ikpoba River in Benin City Nigeria receiving a brewery industrial brewery effluent samples. Similar results were reported by Kanu et al., [11] of the effect of brewery discharge into Eziama River, Aba, Nigeria.

In Nigeria, cities like Kaduna, Lagos and Aba depend very much on its rivers for the means of the livelihood, particularly those living near these rivers. However, the rush by African countries to industrialize has resulted in discharge of partially treated or raw wastes into the surrounding bodies of water since the development of treatment facilities cannot keep pace with the rate at which the wastes are generated by the industries [13]. Having earlier noted that the Ikpoba River was a source of drinking water for locals who live around, it is therefore the aim of the present study to investigate the physicochemical and microbial properties of waste water discharged into the Ikpoba Rivers as well as water samples obtained from the river at different points of collection.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Samples were collected at three different locations; in the brewery, from the effluent point, as well as in the Ikpoba River, where brewery effluents mixed with the river water. Non-effluent samples were also collected from the brewery; these included glycol, condensate, boiler feed, brew cold and cooling tower water (CTW). These were collected because they formed part of the brewing process and also find their way eventually into the brewery effluent samples channel after use.

Samples of the brewery effluent discharged into the river were also collected from the brewery effluent channel. On the Ikpoba River, five different sampling points were identified; contact point (CP) of the discharged brewery effluent with the river water, 5m and 10m before contact point, as well as 5m and 10m after contact point, respectively. Apart from samples collected at contact point, those collected at 5m and 10m before and after contact point, were collected uniformly at 1m from the river bank and on the same side of the river where the brewery effluent channel made contact with the Ikpoba River (see Figs. 1 and 2).

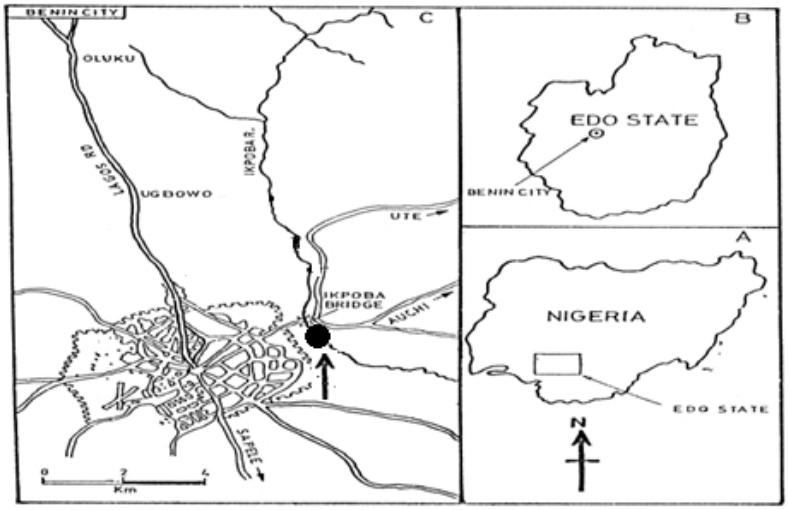


**Fig. 1. (a) Map of Nigeria showing geographic position of Edo state. (b) Map of Edo State showing the geographic position of Benin City, and (c) Schematic map showing location of Ikpoba River in Benin City. Sampling area is arrowed.**

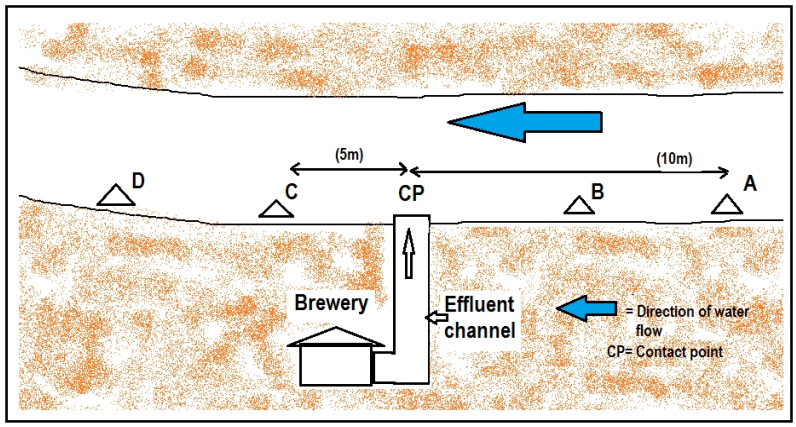


**Fig. 2. A diagrammatic representation of sampling points in the present study**

## 2.2 Physicochemical Parameters

The pH of each water sample was determined using the calorimetric method employing phenol red as indicator. In the laboratory, they were further confirmed using a Philip's pH meter.. The water temperatures were measured using mercury-glass thermometer with 0°C to 100°C calibration. The thermometer was left in water media for about 2 minutes before the reading was taken. Turbidity was carried out with the aid of a turbid meter. The turbid meter was standardized by putting the 90NTU cell in the sample compartment and adjusting the control knob to 90NTU (Nephelometric unit). This was later removed and the sample cell, containing 25ml of sample was put into the cell holder and the reading recorded. The electrical conductivity of the samples was determined using a HACH conductivity meter by

dipping the conductivity probe into a beaker containing the sample and taking the reading from the meter.

To determine total dissolved solid (TSS), the TSS meter as well as 0.01M and 0.00M KCl reagents were used. The meter was switched on and allowed to stabilize for 10 minutes. The TDS was then calibrated by pressing TDS and immersing the probe in the KCl solution. The probe was later rinsed and immersed in the sample solution. The TDS was read in mg/l.

The HACH Spectrophotometer, DR/2000 apparatus was used to determine total suspended solid in water. Measured 25ml of water was poured into a cuvette and read at zero at 810nm. Some 25ml of water sample was also poured into another cuvette and read again in the meter. The result is in mg/l of the non-filterable residue (suspended solid).

**2.2.1 Chemical oxygen demand**

Some 25ml of water sample was pipette into a conical flask, 10ml of 0.00833 $K_2CrO_7$ solution was then added. A pinch of $HgSO_4$ and 10ml of $Ag_2SO_4$ ¯ $H_2SO_4$ solution were added and a few beads. A reflux greaseless condenser was fit and heated gently to boiling and then, boiled for exactly 10 minutes. It was left to cool while the condenser was rinse with 50ml of water. The flask was cooled under running tap. Two drops of ferroin indicator was added and titrated with 0.025M $Fe(NH_2)_2$ $(SO_4)_26H_2O$ until the colour changed from blue-green to red-brown. A blank determination was done on a 25ml of water. The difference in value between the two titres gave the titre of the sample.

**2.2.2 Total hydrocarbons**

Some 50ml of the water sample was measured into 150ml separating funnel. Measured 25ml of hexane was added and then shaken for 2 minutes manually. The stopper was removed and allowed to settle for 20 minutes. The water layer was drained off and the hexane layer collected and read at 460nm. The hexane was used as the blank. Measured 1.18ml of forcados was pipette and then made up to 1 litre with n – Hexane. From this, working standards of 0, 10, 20, 40, 60, 80, and 100ppm were prepared.

THC was therefore calculated as:

THC (mg/l) = Instrument Reading x Slope Reciprocal x 0.5mg/l

**2.2.3 Sodium, potassium calcium and magnesium determination**

Exactly 2.5ml of concentrated $HNO_3$ (Analar grade) was added to 25ml of water sample in a clean Teflon beaker. The mixture was heated on a hot plate to concentrate the sample to about 10ml. Heating of the sample continued with periodical addition of 1ml portion of concentrated $HNO_3$ until a clear solution was obtained. The clear solution was then allowed to cool after which it was transferred into 25ml standard flask and made up to the mark with distilled water. Blank samples were prepared for background correction.

**2.2.4 Determination of heavy metals**

Heavy metals were analyzed using the Atomic Absorption Spectrophotometer (AAS). Each metal has a hollowed cathode lamp for its determination. The water sample is sprayed through a nebulizer into an air-acetylene flame resonance line in element, which was

generated in a hollow cathode lamp and was simultaneously passed through the flame. The absorbance of radiant energy by the element of interest was related to its concentration in the water sample by Beer–Lambert law.

## 2.3 Microbiological Analysis

Potato dextrose agar (PDA) and nutrient agar (NA) were used during the course of this study. The medium used was sterilized by autoclaving at 15psi (121ºC) for 15minutes. Chloramphenicol and fulscin at 0.02gm per 200ml of medium was introduced at pouring to inhibit the growth of bacteria and fungi. Inoculation and transfer of culture were carried out on sterile inoculating bench CRC model HSB 60*180, after wiping with methylated spirit. Some 9mls of distilled water was pipetted into six McCanthy bottles prepared in duplicate and labelled $10^1$ to $10^6$ for serial dilution preparation and was labelled A10, B5, C5, D10, contact point and brewery effluent samples. The bottles were sterilized in an autoclave at 121ºC for 15minutes. McCanthy bottles also labelled A10, B5, C5, D10, contact point and brewery effluent samples containing the samples represented the stock solution.

From the stock bottles, with a sterile pipette 1ml each was transferred from the stock bottle into the bottle labeled $10^1$ to $10^6$ for serial dilution preparation for each of the samples collected. Some 0.1ml aliquots from the $10^6$ tubes were aseptically inoculated onto already prepared plates of nutrient agar and potato dextrose agar using the spread plate method of inoculation. The nutrient agar (for bacteria) was incubated at 37ºC for 24 hours while the potato dextrose agar plates (for fungi) were incubated at ambient laboratory temperature (28 ± 2ºC) for 72 hours. After the incubation period, plates with distinct colonies were counted and recorded as cfu/g. The bacterial isolates were identified and characterized using cultural, morphological and standard biochemical tests as described by Cheesebrough [14]. The fungal isolates were identified according to the methods described by Oyeleke and Okusanmi [15] based on their colour of aerial hyphae and substrate mycelium, arrangement of hyphae, and conidial arrangement.

**Plate 1. The brewery effluent samples channel**

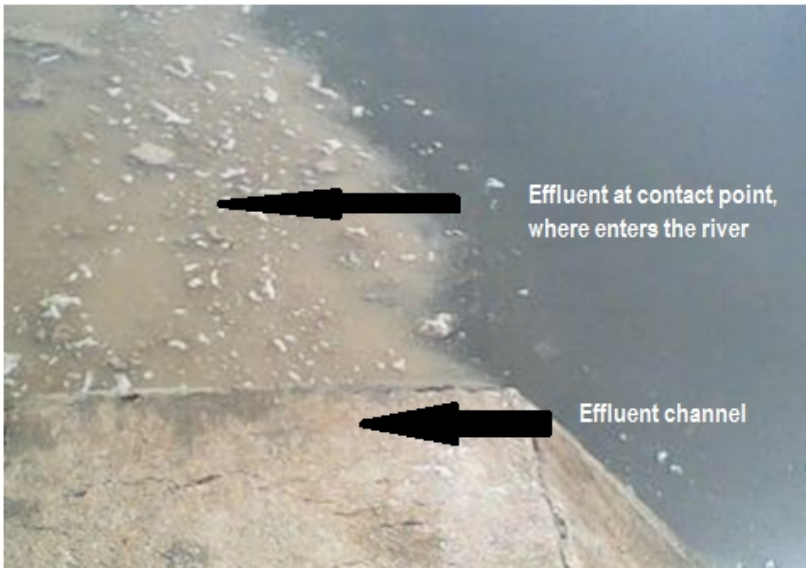


**Plate 2. Brewery effluent samples (arrowed) being discharged into the Ikpoba River**

## 2.4 Computation of Hazard Quotient (HQ)

HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern. The hazards Quotient is expressed by the following equation:

$$HQ = \frac{\text{Measured concentration}}{\text{Toxicity reference value or selected screening benchmark.}}$$

When HQ > 1: Harmful effects are likely due to contaminant in question
When HQ = 1: Contaminant alone is not likely to cause ecological risk
When HQ < 1: Harmful effects are not likely

Benchmarks are available at Efroymson et al. [16].

## 3. RESULTS

Results of the present study showed comparative assessment of physical chemical parameters and microbial composition of brewery effluent discharge from a brewery in Edo state, Midwestern Nigeria. The study also compared physicochemical property of brewery effluent discharge with those of liquid materials used in brewery process, that eventually end up as part of the brewery effluent discharge at some point in the brewery process. An assessment was also made of water samples in the Ikpoba River Benin City, where the brewery effluent samples was discharged.

The pH of glycol was 7.8, compared to those of the condensate and boiler feed which were both 5.8 and 5.5 respectively (Table 1). The pH of the brewery effluent samples was 5.8; however at the point of contact of brewery effluent samples with Ikpoba River, pH dropped to 4.8. At 5m before contact point, pH was 5.6, and at 5m after contact point pH was 5.3.

**Table 1. Physiochemical parameters of brewery effluent discharge from a brewery in Edo State, Midwestern Nigeria**

| | pH | Temp. °C | EC µs/cm | Sal g/l | Turb. NTU | Col Pt.Co | TDS Mg/l | TSS | COD | $HCO_3$ | THC | Na | K | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Non-effluent samples** | | | | | | | | | | | | | | |
| Glycol | 7.8 | 29.9 | 600 | 0.27 | 70.8 | 84.0 | 300 | 401.2 | 117.6 | 56.8 | 4.09 | 33.6 | 10.8 | 1.64 | 1.65 |
| Condensate | 5.8 | 29.9 | 30 | 0.02 | 1.6 | 3.2 | 15 | 4.0 | 28.5 | 22.4 | 1.30 | 6.1 | 2.1 | 0.98 | 0.31 |
| Boiler feed | 5.5 | 29.6 | 70 | 0.03 | 1.0 | 1.3 | 35 | 3.4 | 20.0 | 18.6 | 0.02 | 14.66 | 4.10 | 2.33 | 0.71 |
| Brew Cold | 6.7 | 29.6 | 134 | 0.06 | 1.0 | 2.0 | 67 | 10 | 36.0 | 30.5 | 6.88 | 15.22 | 4.14 | 1.66 | 0.65 |
| C.T.W | 8.3 | 29.8 | 170 | 0.08 | 1.6 | 3.8 | 85 | 8.1 | 34.2 | 28.8 | 4.01 | 71.39 | 14.30 | 0.12 | 0.12 |
| | **Brewery effluent samples** | | | | | | | | | | | | | | |
| **Effluent samples** | 5.8 | 29.2 | 80 | 0.04 | 5.1 | 6.0 | 40 | 18 | 61.0 | 30.5 | 7.14 | 20.9 | 9.10 | 1.59 | 0.78 |
| | **Effluent samples in contact with Ikpoba River** | | | | | | | | | | | | | | |
| Contact point (CP) | 4.8 | 29.7 | 498 | 0.23 | 63.8 | 90.1 | 249 | 410 | 101.2 | 61.0 | 5.12 | 86.19 | 11.48 | 1.58 | 1.20 |
| 10m before CP | 6.5 | 30.0 | 36 | 0.02 | 1.4 | 2.4 | 18 | 5.73 | 36.2 | 30.5 | 2.11 | 6.64 | 1.7 | 0.27 | 0.15 |
| 5m before CP | 5.6 | 29.7 | 24 | 0.01 | 6.1 | 8.4 | 12 | 19 | 34.4 | 36.6 | 2.32 | 6.41 | 1.8 | 0.09 | 0.17 |
| 5m after CP | 5.3 | 29.6 | 40 | 0.02 | 3.8 | 5.0 | 20 | 18 | 31.0 | 36.6 | 2.30 | 13.38 | 3.38 | 0.27 | 0.16 |
| 10m after CP | 5.5 | 29.9 | 52 | 0.02 | 7.3 | 8.4 | 26 | 22 | 28.8 | 30.5 | 2.14 | 22.77 | 10.88 | 0.17 | 0.32 |

*Turb=turbidity, TSS=total soluble solids, TDS=total suspended solids, THC=total hydrocarbon content, COD=chemical oxygen demand*

The surface water temperature range of non-effluent samples materials was 29.6 – 29.9ºC. The electrical conductivity of the condensate was 80µs/cm, compared to 600µs/cm in the glycol, and 4.8µs/cm at contact point. Salinity of all materials sampled ranged from 0.01-0.2g/l with glycol being the most. Turbidity was high in glycol (70.8 NTU) followed by that at the contact point (63.8 NTU). Turbidity was least in both boiler feed and brew cold (1.0 NTU). The value range for manganese (Mn) in liquid materials ranged from 0.03mg/l in the glycol to 0.18mg/l at the contact point (Table 2). The content copper (Cu) was 0.07mg/l in the brewery effluent, compared to 0.04 – 0.53mg/l in the non-effluent sampled materials. At 10m distance before contact point, the value 0.03mg/l was increased to 0.06mg/l after contact point (Table 2). Although lead (Pb) was present in the non-effluent sampled materials (0.01- 0.16mg/l) and 0.01mg/l in the brewery effluent discharge, it was however below detectable limit in the water samples collected from Ikpoba River. The heavy metal Ni, Pb, and V were undetected in the brewery effluent and non-effluent sampled materials collected, but present in the brewery effluent discharge. This may be as a result of metal corrosion in pipe that convey brewery effluent discharge (Table 2).

**Table 2. Heavy metal content of waste water samples collected**

| | Fe | Mn | Cu | Zn | Pb | Cd | Ni | V |
|---|---|---|---|---|---|---|---|---|
| | mg/l | | | | | | | |
| **Non-effluent samples** | | | | | | | | |
| Glycol | 8.68 | 0.03 | 0.53 | 0.11 | 0.16 | ND | ND | ND |
| Condensate | 1.62 | 0.04 | 0.05 | ND | ND | 0.01 | ND | ND |
| Boiler feed | 0.43 | 0.13 | 0.04 | ND | 0.01 | 0.07 | ND | ND |
| Brew Cold | 0.37 | 0.13 | 0.07 | 0.02 | 0.04 | 0.05 | ND | ND |
| C.T.W | 0.39 | 0.10 | 0.05 | ND | ND | 0.08 | ND | ND |
| **Brewery effluent samples** | | | | | | | | |
| **Effluent** | 1.36 | 0.13 | 0.07 | 0.04 | 0.01 | 0.14 | 0.11 | 0.09 |
| **brewery effluent samples in contact with Ikpoba River** | | | | | | | | |
| Contactpoint (CP) | 11.20 | 0.18 | 0.10 | 0.06 | ND | 0.07 | ND | ND |
| 10m before CP | 6.75 | 0.08 | 0.03 | ND | ND | 0.06 | ND | ND |
| 5m before CP | 6.40 | 0.05 | 0/03 | ND | ND | 0.05 | ND | ND |
| 5m after CP | 6.93 | 0.07 | 0.06 | 0.10 | ND | 0.07 | ND | ND |
| 10m after CP | 6.97 | 0.13 | 0.06 | 0.07 | ND | 0.09 | ND | ND |

*ND = not detected, ≤ 0.001mg/l*

Hazard quotient was calculated to assess the level of ecological risk of the water samples to plants (Table 3), invertebrate (Table 4), fish (Table 5) and all organism (Table 6). The results showed that apart from HQ value of Cu in glycol (Table 6), HQ value for all other heavy metal in the samples was less than 1, the implication being that at any giving point of collection the material did not contain heavy metals in concentration of ecological concern. In the non-effluent sampled materials, total bacteria count ranged from 0.2 – 2.8 x $10^3$cfu/ml, the highest being recorded in glycol (Table 7). Total bacteria count in brewery effluent discharge was 2.6x$10^3$cfu/ml, compared to 0.5 - 4.6x$10^3$cfu/ml in the water sample collected from Ikpoba River. Total fungi count in the non-effluent samples material ranged 0.9 – 2.2x$10^3$cfu/ml, 1.2x$10^3$cfu/ml in the brewery effluent discharge and 1.2 – 1.5x$10^3$cfu/ml in water collected from Ikpoba River. Total coliform was absent, not detected in brew cold, boiler feed, as well as 10m before and after contact point. *Shigella/Samonella* count was negligible in non-effluent samples materials and water sample in Ikpoba River. Bacterial isolated in the glycol included *Pseudomonas aerogenosa* and *Escherichia coli*, compared to brewery effluent discharge which had additional *Vibro* sp (Table 8). Fungi isolate in the

brewery effluent samples were *Aspergillus niger, fusavium solani* and *penicillium* sp. The most predominant fungi species in all water samples collected were both and *Penicillium* sp.

**Table 3. Hazard quotient of heavy metals in water samples for metal toxicity to aquatic plants**

| Code | Fe | Mn | Cu | Zn | Pb | Cd | Ni | V |
|---|---|---|---|---|---|---|---|---|
| **Non-effluent samples** | | | | | | | | |
| Glycol | NA | NA | 0.53 | 0.0037 | 0.00030 | $<10^{-3}$ | $<10^{-3}$ | $<10^{-3}$ |
| Condensate | NA | NA | 0.05 | $<10^{-3}$ | $<10^{-3}$ | 0.005 | $<10^{-3}$ | $<10^{-3}$ |
| Boiler feed | NA | NA | 0.04 | $<10^{-3}$ | 0.0001 | 0.035 | $<10^{-3}$ | $<10^{-3}$ |
| Brew Cold | NA | NA | 0.07 | 0.0007 | 0.0001 | 0.025 | $<10^{-3}$ | $<10^{-3}$ |
| C.T.W | NA | NA | 0.05 | $<10^{-3}$ | $<10^{-3}$ | 0.04 | $<10^{-3}$ | $<10^{-3}$ |
| **Brewery effluent samples** | | | | | | | | |
| **Effluent samples** | NA | NA | 0.07 | 0.0013 | 0.0002 | 0.07 | 0.022 | NA |
| **Brewery effluent samples** in contact with Ikpoba River | | | | | | | | |
| Contact point (CP) | NA | NA | 0.10 | 0.002 | $<10^{-3}$ | 0.035 | $<10^{-3}$ | $<10^{-3}$ |
| 10m before CP | NA | NA | 0.03 | $<10^{-3}$ | $<10^{-3}$ | 0.03 | $<10^{-3}$ | $<10^{-3}$ |
| 5m before CP | NA | NA | 0.03 | $<10^{-3}$ | $<10^{-3}$ | 0.025 | $<10^{-3}$ | $<10^{-3}$ |
| 5m after CP | NA | NA | 0.06 | 0.0033 | $<10^{-3}$ | 0.035 | $<10^{-3}$ | $<10^{-3}$ |
| 10m after CP | NA | NA | 0.06 | 0.0002 | $<10^{-3}$ | 0.045 | $<10^{-3}$ | $<10^{-3}$ |

*NA = not available*

**Table 4. Hazard quotient of heavy metals in water samples for metal toxicity to invertebrates**

| Code | Fe | Mn | Cu | Zn | Pb | Cd | Ni | V |
|---|---|---|---|---|---|---|---|---|
| **Non-effluent samples** | | | | | | | | |
| Glycol | NA | NA | 0.0873 | 0.0001 | 0.0063 | $<10^{-3}$ | $<10^{-3}$ | $<10^{-3}$ |
| Condensate | NA | NA | 0.0082 | $<10^{-3}$ | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |
| Boiler feed | NA | NA | 0.0066 | $<10^{-3}$ | 0.0004 | NA | $<10^{-3}$ | $<10^{-3}$ |
| Brew Cold | NA | NA | 0.0115 | 0.0001 | 0.0016 | NA | $<10^{-3}$ | $<10^{-3}$ |
| C.T.W | NA | NA | 0.0082 | $<10^{-3}$ | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |
| **Brewery effluent samples** | | | | | | | | |
| **Effluent samples** | NA | NA | 0.0115 | 0.0001 | 0.0004 | NA | 0.0009 | - |
| **Brewery effluent samples in contact with Ikpoba river** | | | | | | | | |
| Contact point (CP) | NA | NA | 0.0165 | 0.0001 | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |
| 10m before CP | NA | NA | 0.0049 | $<10^{-3}$ | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |
| 5m before CP | NA | NA | 0.0049 | $<10^{-3}$ | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |
| 5m after CP | NA | NA | 0.0099 | 0.0001 | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |
| 10m after CP | NA | NA | 0.0099 | 0.0001 | $<10^{-3}$ | NA | $<10^{-3}$ | $<10^{-3}$ |

*NA = not available*

**Table 5. Hazard quotient of heavy metals in water samples for metal toxicity to fish**

| Code | Fe | Mn | Cu | Zn | Pb | Cd | Ni | V |
|---|---|---|---|---|---|---|---|---|
| **Non-effluent samples** | | | | | | | | |
| Glycol | 0.007 | 0.00002 | 0.1394 | 0.0030 | 0.0005 | $<10^{-3}$ | $<10^{-3}$ | $<10^{-3}$ |
| Condensate | 0.001 | 0.00002 | 0.0132 | $<10^{-3}$ | $<10^{-3}$ | 0.0059 | $<10^{-3}$ | $<10^{-3}$ |
| Boiler feed | 0.0007 | 0.00007 | 0.0105 | $<10^{-3}$ | 0.0005 | 0.0411 | $<10^{-3}$ | $<10^{-3}$ |
| Brew Cold | 0.0002 | 0.00007 | 0.0184 | 0.0005 | 0.0021 | 0.0210 | $<10^{-3}$ | $<10^{-3}$ |
| C.T.W | 0.0003 | 0.00006 | 0.0132 | $<10^{-3}$ | $<10^{-3}$ | 0.0471 | $<10^{-3}$ | $<10^{-3}$ |
| **Brewery effluent samples** | | | | | | | | |
| **Effluent samples** | 0.0010 | 0.0000 | 0.0184 | 0.0011 | 0.0005 | 0.0824 | 0.0031 | 0.0011 |
| **Brewery effluent samples in contact with Ikpoba river** | | | | | | | | |
| Contact point (CP) | 0.0086 | 0.0001 | 0.0263 | 0.0016 | $<10^{-3}$ | 0.0412 | $<10^{-3}$ | $<10^{-3}$ |
| 10m before CP | 0.0052 | 0.0001 | 0.0079 | $<10^{-3}$ | $<10^{-3}$ | 0.0353 | $<10^{-3}$ | $<10^{-3}$ |
| 5m before CP | 0.0050 | 0.0001 | 0.0079 | $<10^{-3}$ | $<10^{-3}$ | 0.0241 | $<10^{-3}$ | $<10^{-3}$ |
| 5m after CP | 0.0053 | 0.0001 | 0.0158 | 0.0027 | $<10^{-3}$ | 0.0411 | $<10^{-3}$ | $<10^{-3}$ |
| 10m after CP | 0.0054 | 0.0001 | 0.0158 | 0.0019 | $<10^{-3}$ | 0.0529 | $<10^{-3}$ | $<10^{-3}$ |

*NA = not available*

**Table 6. Hazard quotient of heavy metals in water samples for metal toxicity to all organisms**

| Code | Fe | Mn | Cu | Zn | Pb | Cd | Ni | V |
|---|---|---|---|---|---|---|---|---|
| **Non-effluent samples** | | | | | | | | |
| Glycol | 0.0549 | 0.0001 | 2.3043 | 0.0037 | 0.0130 | $<10^{-3}$ | $<10^{-3}$ | $<10^{-3}$ |
| Condensate | 0.0103 | 0.0001 | 0.2174 | $<10^{-3}$ | $<10^{-3}$ | 0.0667 | $<10^{-3}$ | $<10^{-3}$ |
| Boiler feed | 0.0027 | 0.0001 | 0.1739 | $<10^{-3}$ | 0.0008 | 0.4667 | $<10^{-3}$ | $<10^{-3}$ |
| Brew Cold | 0.0023 | 0.0001 | 0.3045 | 0.0007 | 0.0033 | 0.3333 | $<10^{-3}$ | $<10^{-3}$ |
| C.T.W | 0.0025 | 0.0001 | 0.2314 | $<10^{-3}$ | $<10^{-3}$ | 0.5333 | $<10^{-3}$ | $<10^{-3}$ |
| **Brewery effluent samples** | | | | | | | | |
| **Effluent samples** | 0.0086 | 0.0001 | 0.3043 | 0.0013 | 0.0008 | 0.9333 | 0.022 | 0.0011 |
| **Brewery effluent samples in contact with Ikpoba river** | | | | | | | | |
| Contact point (CP) | 0.0709 | 0.0002 | 0.4348 | 0.0002 | $<10^{-3}$ | 0.4667 | $<10^{-3}$ | $<10^{-3}$ |
| 10m before CP | 0.0427 | 0.0001 | 0.1304 | $<10^{-3}$ | $<10^{-3}$ | 0.4 | $<10^{-3}$ | $<10^{-3}$ |
| 5m before CP | 0.0405 | 0.0001 | 0.1304 | $<10^{-3}$ | $<10^{-3}$ | 0.3333 | $<10^{-3}$ | $<10^{-3}$ |
| 5m after CP | 0.0439 | 0.0001 | 0.2609 | 0.0033 | $<10^{-3}$ | 0.4667 | $<10^{-3}$ | $<10^{-3}$ |
| 10m after CP | 0.0441 | 0.0001 | 0.2609 | 0.0023 | $<10^{-3}$ | 0.6 | $<10^{-3}$ | $<10^{-3}$ |

*NA = not available*

**Table 7. Total microbial counts of water samples collected from study area**

| | Bacterial counts (x $10^3$Cfu/ml) | Hydrocarbon degrading bacterial counts (x $10^3$Cfu/ml) | Fungal counts (x $10^3$ Cfu/ml) | Hydrocarbon degrading bacterial counts (x $10^3$Cfu/ml) | Total coliforms | Salmonella /Shigella (x $10^3$ Cfu/ml) | Vibro counts (x $10^3$ Cfu/ml) |
|---|---|---|---|---|---|---|---|
| | **Non-effluent samples** | | | | | | |
| Glycol | 4.5 | 0.6 | 2.2 | 0.2 | 1.5 | 0 | 0 |
| Brew Cold | 2.8 | 0.4 | 1.2 | 0.4 | 0 | 0 | 0 |
| Condensate | 2.2 | 0.7 | 0.9 | 0.1 | 0.5 | 0 | 0 |
| Broiler field | 0.8 | 0.002 | 1.2 | 0.1 | 0 | 0 | 0 |
| C.T.W | 0.2 | 0.005 | 1.1 | 0.3 | 0.05 | 0 | 0.8 |
| | **Brewery effluent samples** | | | | | | |
| **Effluent samples** | 2.6 | 0.9 | 1.2 | 0.4 | 1.2 | 0.3 | 0.6 |
| | **Brewery effluent samples in contact with Ikpoba River** | | | | | | |
| Contact point (CP) | 1.4 | 0.5 | 0.5 | 0.9 | 0.05 | 0 | 0 |
| 10 m after CP | 3.8 | 0.4 | 1.5 | 1.3 | 0 | 0 | 0 |
| 5m after CP | 1.7 | 0.7 | 0.9 | 0.5 | 1.1 | 0 | 0 |
| 10 m before CP | 4.6 | 0.8 | 0.8 | 0.1 | 1.5 | 0 | 0 |
| 5m before CP | 0.5 | 0.005 | 0.2 | 0.1 | 0 | 0 | 0 |

**Table 8. Microbial composition of water samples collected from study area**

| | Bacteria | | | | | Fungi | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Peudomonas aureginosa** | **Bacillus substilis** | **Enterobacter aerogene** | **Vibrio sp** | **Esherichia coli** | **Aspergillus niger** | **A. flavus** | **Fusarium solani** | **Penicillum sp** |
| | Non-effluent samples | | | | | | | | |
| Glycol | + | + | + | - | + | + | - | + | - |
| Brew Cold | + | + | - | - | - | + | - | - | + |
| Condensate | + | + | + | - | - | + | - | - | + |
| Broiler field | - | + | - | - | - | - | + | - | - |
| C.T.W | + | + | + | + | - | + | - | - | + |
| | Brewery effluent samples | | | | | | | | |
| Effluent samples | + | + | + | + | + | + | - | + | + |
| | Brewery effluent samples in contact with Ikpoba River | | | | | | | | |
| Contact point (CP) | + | + | - | - | - | + | + | - | - |
| 10 m before CP | + | + | - | - | - | + | - | - | - |
| 5m before CP | + | + | - | - | - | + | - | - | + |
| 10 m after CP | + | + | - | - | - | + | - | - | + |
| 5m after CP | + | + | - | - | - | + | - | - | + |

*+ present, - absent.*

## 4. DISCUSSION

The problems associated with the dispersal of industrial and urban wastes generated by human activities are the contamination of the soil, controlled and uncontrolled disposal of wastes, accidental and process spillage, mining and smelting of metalliferous ores and sewage sludge application to agricultural soils. These are responsible for the migration of contaminants onto non – contaminated sites as dust or leachates, and therefore contribute towards contamination of our ecosystem [17]. Hence, this study was to find out the physicochemical and microbial composition of water samples collected in the study area. The pH range for the non-effluent samples in this study was between 5.5 to 8.3 as shown on Table 1 above, while the pH range of the brewery effluent samples was 5.8, and that of the brewery effluent samples in contact with the river fluctuated between 4.80 and 6.50. The low pH of 4.8 at the contact point which is acidic can be attributed to the concentration of the waste coming out of the brewery, and were not within the WHO and FMEnv regulatory unit of 6.5 to 8.5 set for drinking water. The acidic nature of African rivers had earlier been recorded by various workers [18,19,20]. This indicated that the water is moderately acidic with little fluctuations in pH values recorded. The pH of waters usually determines the nature of carbon dioxide in water, free carbon dioxide is known to be present at lower pH ranges of 4.8-5.5, the carbonate and bicarbonates dominate at higher pH [21].

The temperature range of the non-effluent sample fluctuated between 29.6ºC and 29.9ºC, while those of the brewery effluent samples and various samples collected from the impacted river was 29.2ºC and 29.6ºC to 30ºC, respectively. These results are within the FEPA permissible limit of less than 40$^{o}$C [22].

Electrical conductivity (EC) is a measure of the total ionic composition of water and therefore its overall chemical richness. It is primarily determined in water by the presence and levels of concentration of sodium and magnesium ions and to some extent calcium ions. Their ions help buffer the effect of bicarbonate and carbonate ions, thus maintaining the pH. The EC of the water samples ranged between 30 to 600 µs/cm for the non-effluent samples and 80 µs/cm for the brewery effluent samples while the range of 24 to 498 us/cm were observed at various contact points. The EC of water is a useful and easy indicator of its salinity or total salt content. In the present study the salinity values are less than 100 mg/l set by the World Health Organization [6] and Standard Organization of Nigeria (SON). This implies that the water samples were not saline.

Water on the earth can be said to be enormous in quantity, when it is considered that more than two-thirds of the earth surface is covered by water [23], but UNEP and WHO [5] argued that it is not sufficient merely to have access to water in adequate quantities, the water also needs to be of adequate quality, to maintain health and it must be free from harmful biological and chemical contamination.

Drinking water must be free of disease-causing organisms, poisonous substances and excessive amount of minerals and organic matter, and certain levels of minerals and dissolved substances are allowed [24].

It is known that calcium and magnesium along with their carbonates, sulphates and chlorides naturally confer temporary and permanent hardness. Water having 0-75mg $CaCO^{3}$ $l^{-1}$ was describe as soft, 75-15075mg $CaCO^{3}$ $l^{-1}$ as hard water while samples having total hardness of over 300mg $CaCO_3$L as hard according to Adeyeye and Abulude [25]. Samples of water collected at various points and brewery effluent samples were below hard water

concentration limits, as the amount of calcium and magnesium was low as compared within the FMENV permissible limit of 100mg/l. The low values of calcium and magnesium in the various samples collected from the river may have resulted from the rapid dissolution of calcium and magnesium in the flowing river. Brewery effluent samples contain organic materials like spent grains, waste yeast, spent hops and grit. Total suspended solids (TSS) range for the non-effluent samples fluctuated between 3.4 and 401.2 mg/l, while that of the brewery effluent samples was 18mg/l. The value of 410mg/l obtained for water samples collected at the contact point was above the 30mg/l permissible limit set by FMENV. This can be attributed to the fact that as the waste water permeated from the brewery, most of the wastes were deposited at the contact point (see Plate 2). If this waste water is applied directly to agricultural field or discharged into rivers and stream, this could make it unsuitable for aquatic life. For the total dissolved solids (TDS), values obtained for all samples assayed were within the permissible limit set by FMENV. Polluted water contains low levels of dissolved oxygen (DO) as a result of heavy biological oxygen demand(BOD) and chemical oxygen demand(COD) placed by brewery effluent samples waste materials discharged into surface water. This makes water unsuitable for drinking and irrigation (Hari *et al*., 1994) or any other use. Similarly the COD for all the samples ranged from 31.0 to 117.6. The highest value of 101.2 of COD for the contact point was below the 150mg/l set by FMENV. Some treatments such as addition of coagulants may be required to make this water suitable for domestic purposes Ipeayada and Onianwa [26], suggest that brewery effluent samples on entering nearby stream will cause oxygen depletion and may cause suffocation of fish and other aquatic organisms.

Lead (Pb) was only detected in the brewery effluent samples sample (0.01mg/l) and in the non-effluent samples sample (glycol, boiler feed and brew cold), but absent in all the other samples (See Table 2). The concentration of Pb obtained for the brewery effluent samples (0.01mg/l) was same as that of WHO permissible value of 0.01 mg/l and maximum contaminant level (MCL) of 0.015 mg/l for drinking water [27]. However adequate precautions should be taken to ensure that the Pb waste was reduced so that it does not exceed the WHO standards. Rivers also need to be treated so that the lead level meets WHO standards before it could be safe for drinking and useful for domestic activities. The nutrients, nitrate and phosphate which occur naturally in water are indices of organic pollution in water [28]. The levels of these nutrients vary seasonally in most African rivers and these variations are basically controlled by surface run-off and flooding [29].

The concentrations of Mn and Zn in all the samples were below the WHO limits of 0.1 and 5.0 mg/l respectively in drinking water. They also meet the 0.18 and 0.002 mg/l levels for Mn and Zn, respectively in water meant for aquatic ecosystem use [30]. On this basis, the water could support aquatic life if other conditions were favourable.

Cadmium concentration was in the range of 0.01mg/l to 0.08 mg/l in the non-effluent samples but not detected in glycol. Cadmium was detected in the brewery effluent samples (0.14mg/l). The range of 0.05mg/l to 0.09mg/l was detected in the various contact points. Factors such as the dumping of agricultural wastes, addition of impure brewery chemicals, leaching of metals from wastes site to the ground water plus rural and urban water run-off could be responsible for the observed high concentrations of Cd. WHO level of Cd in drinking water is 0.01 mg/l [27]. The level in water for aquatic system is 0.15 to 0.25 mg/l [30]. This suggests that the water sources from the studied area may pose threat to man and aquatic organism. The lowest concentration (1.36mg/l) obtained for Fe in all the samples assayed exceeded the 1.0 mg/l permissible level recommended by WHO for Fe in drinking water [30].

The composition of heavy metals in the effluent and water samples were lower than permissible limits as shown in significantly low values of hazard quotients (see Tables 2 – 6). When HQ value was less than unity, the implication was that the heavy metal of concern was adjudged to possess non-toxic concentrations. The bacteria isolates included *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes,* and *Pseudomonas sp.,* whereas the fungal isolates included *Aspergillus niger, A. flavus, Fusarium solani* and *Penicillium sp.*

## 5. CONCLUSION

Although to a very large extent, by comparing the physicochemical quality of the Ikpoba water body before, during and after contact with the brewery effluents, the quality of the water body was modified, the study however showed that contaminants sampled were within statutory limits. The water samples collected could not pose any environmental risk even when let into open waters. Although adjudged ecologically safe, the non effluent sample, glycol, was observed to contain more toxicants than the other samples assayed. It was however observed that when treated in the brewery, the brewery effluent discharged into the river contain relatively lesser pollutant amounts. This is further to ascertain the quality of waste water discharged into the environment.

## ACKNOWLEDGEMENTS

The researchers wish to express their profound gratitude to Mr. Stanley Elevochi of Justan Environmental And Laboratory Services Limited, Aba, Nigeria, for his diligent assistance in carrying out the samples analyses.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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