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Physico-chemical and microbiological profile of bacterial and fungal isolates of Ikpoba River in Benin City: Public health implications

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This study examined the physico-chemical and microbiological profile of bacterial and fungal isolates of Ikpoba River between February 2013 and March 2013. The mean bacterial count for upstream water sample obtained in February was $2 \times 10^2 \pm 1$ cfu/ml while $1.09 \times 10^4 \pm 3.6$ was the count for treated industrial effluent sample collected in March. The mean fungal counts for the downstream water sample in February was $2 \times 10^2 \pm 1$ cfu/ml while the count collected at the point of discharge of effluent into the river in March was $2.0 \times 10^3 \pm 7$ cfu/ml. There was a significant statistical difference observed in the mean bacterial and fungal counts ($P < 0.05$). The total coliform counts recorded for samples obtained from downstream was 2 MPN/105 ml while 20 MP/105 ml was for sample collected at the point of effluent discharge respectively. Several bacterial and fungal genera were isolated from the River water samples. Water samples collected upstream and downstream points on the river were colorless while samples collected at the point of effluent discharge were light brown in color. The mean pH, turbidity and conductivity of the respective samples ranged from 5.63 ± 0.05 to 6.78 ± 0.05 , 4.1 ± 0.21 to 6.81 ± 0.55 NTU and 3.3 ± 0.25 to 73.3 ± 6.56 $\mu\text{s/cm}$. The biological oxygen demand (BOD), dissolved oxygen (DO) and chemical oxygen demand (COD) varied from 2.6 ± 0.5 to 305.19 ± 43.2 mg/l, 5.5 ± 0.3 to 6.1 ± 0.6 mg/l and 15.8 ± 0.6 to 883.8 ± 28.5 mg/l respectively. The quality of Ikpoba River is being negatively impacted by the disposal of effluent as well as human activities around the area rendering the water unsafe for consumption.

Key words: Physico-chemical, microbiological profile, bacterial, fungal isolates.

INTRODUCTION

Water is abundant in the planet as a whole, but fresh potable water is not always available at the right time or the right place for human or ecosystem use and is,

undoubtedly the most precious natural resource, vital to life (Karikari and Ansa, 2004). Rivers are open systems, which have come under increasing pressure from human

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activities, often affecting their ecological integrity over the last century throughout the world (Skoulidakis et al., 2002). The physicochemical impact on water quality of rivers have been indicated by rise in conductivity, pollution of water bodies with nitrate, nitrite and soluble reactive phosphorus, by the appearance of tannin and lignin, and by the steady accumulation of inorganic and organic suspended matter along the river (Whitehead et al., 1997). The role of the river is not primarily to carry industrial waste but their ability to do so is hugely exploited. There has been significant impairment of rivers with pollutants, rendering the water unsuitable for beneficial purposes (Filkersilasié, 2011). Rivers provide a variety of services for human populations, including water for drinking and irrigation, recreational opportunities, and habitat for economically important fisheries (Leroy et al., 2002). The growing problem of pollution of river ecosystem has necessitated the monitoring of water quality (Ravindra et al., 2003). Regions with dense human populations are the areas at risk. The earliest anthropogenic threats to water resources were often associated with human health, especially disease causing organisms and oxygen-demanding wastes (Meybeck and Helmer, 1996). Rajaram and Ashutosh (2008) opined that industrial wastes were one of the major causes of irreversible degradation occurring in surface water system. Organic pollution caused by oxygen demanding wastes is common amongst surface water (Masson, 1990). The natural processes of chemical oxidation and biological decomposition that occur within water courses, consume dissolved oxygen. Decomposition of materials is a normal process in all aquatic ecosystems and is a function of decomposers such as aerobic bacteria and fungi (Filkersilasié, 2011). Nonetheless, serious consequences to aquatic biota may result if the natural mechanisms that clean the water are overloaded by large influx of pollutants. Severe oxygen depletion can result in the loss of many desirable aquatic biota and also produce an odorous anaerobic system (Zimmerman, 1993). Majority of the inhabitants that live in riverine areas rely on water from the river for domestic and drinking purposes due to the scarcity of portable water supply by the government (Shuaib, 2004). Wu et al. (1999) reported that in China, approximately 700 million people, over half the population, consume water contaminated with different levels of animal and human excreta with total coliform bacteria exceeding maximum permissible levels by as much as 86% in rural areas and 28% in urban areas. Rapu (2003) stated that in South Africa, over 15% of urban dwellers depend on polluted river waters for their domestic needs. Khalil (2005) reported that over 70% of people in Sudan get their water supply from surface waters, which in most cases are polluted by agricultural chemicals and industrial effluents. Shuaib (2004) stated that over 40% of Nigerians depend on either polluted surface waters or wells for their domestic activities. The constant use of heavily polluted

water for a long time usually results in health problems. Researchers in different parts of the world have reported health problems associated with prolong time use of polluted river water, which range from dysentery, diarrhea, abortion, premature birth, viral hepatitis and gastric and duodenal ulcers amongst others (Shuaib, 2004; Odjugo, 2004; Purnamitta, 2004). Ikpoba River also called the Oken River is a fourth order stream situated within the rainforest belt of Edo State, southern Nigeria. The river is particularly important to the people of Benin City which is the capital and largest city of Edo state, estimated to have a population of 1,086,882 people according to 2006 Census. One of the major dams in the Edo State was constructed across the river in Okhoro Community. Industrial effluents and water from drainage channels are discharged into the river at various points as well. Ikpoba River is subject to pollution from storm-water run-off in the rainy season as it flows through inhabited areas and in particular, through Benin City. Most of the activities around the river in its upper reaches are agricultural such as crop farming and fishing (Atuanya et al. 2012). Nonetheless, industrial effluents, and drainage system are channeled to the river. Government abattoir managed by the Local government where about 50 cows and goats are slaughtered daily is also situated by the river (Atuanya et al. 2012). The aim of this study was to verify if physico-chemical and microbiological parameters of analyzed water are below the regulatory limits to ensure the water quality.

MATERIALS AND METHODS

Study area

The area experiences an equatorial climate. Although there is hardly any month without some elements of rainfall, rains concentrate within the months of March and October (rainy season) while the dry season with little rainfall prevails between November and February (Odjugo and Konyeme, 2008). The mean annual rainfall total is constantly above 2000 mm, relative humidity is above 80% and the mean air temperature is 28°C (Odjugo and Iweka, 2005). Industrial effluent (brewery waste) is conveyed by means of an underground metal pipe which is discharged into the river. In spite of this, people living around this section of the river use the water from the river for drinking, washing of clothes and bathing purposes. Furthermore, fishing activities are conducted by some people living around the river bank close to point of discharge.

Sample collection

Water samples were collected twice a month from three sampling points along Ikpoba River close to the discharge pipe conveying treated effluent from the nearby brewery plant using plastic containers sterilized in 5-10% bleach water thereafter rinsed with boiled water and allowed to dry. Samples of surface water were sourced from about 3 m upstream from the discharge point. Fresh water samples were obtained at the point of discharge (but before effluent discharge) of treated brewery effluent into the river. About 3 m downstream from the point of discharge of the effluent, water

samples were also obtained. Also, samples of the treated effluent were also obtained with the aid of sterile plastic containers. The sampling period was conducted from February 2013 to March 2013. The plastic containers were appropriately labeled and were immediately transported to the laboratory for analysis and those that could not be analyzed immediately were stored at 4°C for 6 h in a refrigerator for subsequent analyses.

Total heterotrophic bacterial and fungal counts

The total heterotrophic bacterial and fungal counts of the respective surface water samples were evaluated according to the methods delineated by Harley and Prescott (2002) and Pepper and Gerba, (2004). The total heterotrophic bacterial count for each sample was then determined using nutrient agar as general purpose medium. About 1 ml aliquot of the serially diluted sample was transferred onto sterile labeled plates before the addition of 15 ml of cooled molten nutrient agar under aseptic conditions. Plating were done in triplicates and incubated at 30°C for 48 h. The mean count of the resultant bacterial colonies in triplicate plates were enumerated and recorded. The same procedure was applied in respect of the total fungal count for the respective samples. However, 1 ml of an antibiotic solution (500 µg of chloramphenicol dissolved in 20 ml of distilled water) was pipetted onto each plate before the pouring of 15 ml of cool molten Potato dextrose agar (PDA) (Ogbuile et al., 1998). The PDA plates were incubated at ambient temperatures (28 ± 2°C) for 5 days, after which the mean count of the fungal colonies on replicate plates was also recorded.

Determination of the total coliform and fecal coliform counts

The total coliform and fecal coliform (*Escherichia coli*) counts of the water samples were evaluated according to methods stated by Cheesebrough (2001). Both tests were conducted in three stages namely: Presumptive stage, confirmatory stage and completed stage.

Presumptive stage

Fifty (50) ml of the sample was dispensed to a labeled, sterilized 100 ml conical flask containing 50 ml of sterile MacConkey broth and an inverted Durham tube (for detection of gas production). Also 5 test tubes containing 10 ml sterilized MacConkey broth and inverted Durham tubes were prepared and 10 ml of the respective water samples were added under aseptic conditions. Another 5 test tubes containing 9 ml of sterilized MacConkey broth and inverted Durham tubes were also arranged and 1 ml of the corresponding water samples added also under sterile conditions. These tubes and conical flasks were incubated at 30°C for 48 h. The procedure was repeated for the fecal coliform count but the inoculated test tubes and conical flasks were incubated at 44°C for 24 h. At the end of the respective incubation periods, the test tubes and conical flasks were examined for both acid production and gas production, and reference was made to statistical tables to ascertain the most probable number (MPN) of both the total coliform and fecal coliform count in 10 ml of the respective surface water samples (Cheesebrough, 2001).

Confirmatory stage

About 0.1 ml of the positive presumptive test tubes was pipetted onto test tubes containing 10 ml of sterile MacConkey broth and inverted Durham tubes to detect gas production. The tubes were

incubated at 30°C for 48 h for total coliform count and 44°C for 24 h in respect of fecal coliform count.

Completed stage

The contents of the positive confirmatory test tubes (the inoculated test tubes which displayed visible color change at the end of the incubation period) were streaked on freshly prepared Eosin methylene blue agar plates with the aid of a sterile inoculating loop, under aseptic conditions. The colonial morphology of the streaked colonies were observed and further biochemical tests such as methyl red, indole production, citrate utilization, Voges Proskauer and urease production tests were done to further identify the various sub cultured colonies. For the completed stage of the fecal coliform test, indole test was carried out to differentiate between streaked colonies of *Enterobacter aerogenes* and *E. coli* (Cheesebrough, 2001).

Identification and characterization of microbial isolates

Pure cultures of the heterotrophic bacterial isolates were identified and characterized on the basis of cultural, morphological and biochemical characteristics according to the methods of Cullimore (2000), Aneja (2003) and Sharma (2009). The fungal isolates were identified through macroscopic observation of their sub-cultured colonies, microscopic examination of their respective spores and hyphal appendages using wet mount technique (Sharma, 2009). Distilled water and lactophenol cotton blue were utilized as mountants (Ogbuile et al., 1998). The results of the microscopy were compared with illustrations contained in Barnett and Hunter (1972).

Determination of physico-chemical parameters

pH and temperature

The hydrogen ion concentration (pH) of each sample and temperature were measured using a HACH digital pH/temperature meter. The electrode probe was inserted into a glass beaker containing about 20 ml of the sample and the result was read from the screen and recorded. The pH meter was calibrated before and after each readings using freshly prepared pH buffers (7.00), (4.00) and (9.00).

Alkalinity

Total alkalinity was determined by titrimetric method using standardized sulphuric acid, phenolphthalein and methyl orange indicator. The development of a pink color indicates the presence of carbonate. Then 2 drops of 0.1% methyl orange indicator was added and titrated with standard 0.25 N sulphuric acid. A colourless reaction gave the end point (APHA, 1993).

Total solids

The total solid of each water and effluent sample was derived from the addition of both the total suspended solid (TSS) and total dissolved solid (TDS) values (Ademoroti, 1996).

Electrical conductivity

The electrical conductivity of each water and effluent sample was

determined using a portable conductivity meter. 50 ml of the sample was collected with a beaker and the plastic electrode probe was inserted into the sample and the result in microsiemens ($\mu\text{s}/\text{cm}^{-1}$) read from the screen. The meter was calibrated using distilled water after each measurement (APHA, 1993).

Colour

Fifty (50) ml of the water and effluent sample was dispensed into a clean conical flask. The color of the sample was observed and recorded.

Turbidity

The turbidity of the respective water and effluent samples were determined using a spectrophotometer. Twenty five milliliters of the sample was dispensed into a curvette and placed in the light chamber and the absorbance was measured at a specific wavelength using distilled water as blank. The turbidity values were recorded in nephelometer turbidity unit (NTU) (APHA, 1993).

Phosphate

Twenty (20) ml of the water and effluent sample was dispensed onto a clean curvette. About 4 ml of phosphate reagent containing ammonium molybdate, antimony potassium tartate and ascorbic acid was also added to the curvette containing the effluent sample. 1 ml of 95% ethanol and 1 ml of concentrated H_2SO_4 was then added. It was shaken, and then left for 5 min to allow for color development. The absorbance was determined at a specific wavelength using a spectrophotometer (Radojevic and Bashkin, 1999).

Nitrate

Ten (10) ml of the sample was then placed in a test tube, followed by the addition of 2 ml NaCl solution, this mixture was swirled and 10 ml of H_2SO_4 solution was also added. The resultant solution was also swirled and allowed to stand. A sample blank was also prepared. To the first test tube containing the mixture of the sample, NaCl and H_2SO_4 , 0.5 ml of brucine –sulphanilic acid reagent was added and the test tube was swirled and left to stand for about 20 minutes. The test tubes were allowed to develop color and the absorbance reading of the solution was taken using a spectrophotometer at a specified wave length (Ademoroti, 1996).

Sulphate

Ten (10) ml of the water sample was decanted onto a clean curvette. 1 ml of 95% isopropyl alcohol, 0.5 ml of glycerol and 5 ml of conditioning reagent which consist of NaCl, BaCl and Citric acid were added to the curvette containing the sample. The solution was left to stand for 5 min to allow colour development, after which the absorbance was read at a specific wavelength using a spectrophotometer (APHA, 1993).

Iron (Fe), Nickel (Ni), Copper (Cu) and Lead (Pb)

The concentration of the respective heavy metals (iron, nickel, copper and lead) present in the water and effluent samples were determined with the aid of an Atomic absorption spectrophotometer (Buck Scientific model 210 VGP USA). Appropriate standards of

known concentrations of the respective metals were prepared and used to calibrate and auto zero the electrode. The water samples were dispensed onto sterile plastic bottles. After calibrating and auto zeroing the electrodes, the samples were read at specific wavelengths and printed results sheets were examined. The final concentration of the respective metals was deduced (Ademoroti, 1996).

Dissolved Oxygen (DO)

The dissolved oxygen content is the amount of available oxygen present in the water (Venkatesharajuik et al., 2010). The dissolved oxygen value depends on a number of physical, chemical, biological and microbiological processes (Abida and Harikrishna, 2008). 250 ml DO bottles were filled to the brim with samples, taking care to minimize contact with air. 100 ml of the sample solution was measured to which 2 drops of starch indicator was added. The resulting dark blue solution was titrated against a colourless 0.0125 M Thiosulphate solution (Ademoroti, 1996).

Biological oxygen demand (BOD)

Biological oxygen demand is a measure of the oxygen in the water that is required by the aerobic organisms (Abida and Harikrishna, 2008). The water sample was aerated using an air pump. A measured dilution of the water sample was done and seeding of the water sample was also conducted. Determination of the Dissolved Oxygen (DO_1) using Wrinkler's method on a suitable portion of the seeded water was carried out. An incubation bottle was filled to the brim with the remainder of the diluted water sample. The bottle was screw capped and incubated in the dark for 5 days at 20°C . On the 5th day, the DO value was determined. The BOD value was the result of the difference of the respective DO values divided by the percentage dilution (Ademoroti, 1996).

Chemical oxygen demand (COD)

The chemical oxygen demand is a measure of the oxygen equivalent of organic matter in a sample that is susceptible to oxidation by a strong oxidizing agent (Radojevic and Bashkin, 1999). The COD values for the water and effluent samples were determined using the colorimetric procedure as described by Ademoroti (1996). HACH COD reagents (high range), COD reactor (HACH) and HACH DR 2010 Spectrophotometer were utilized. A measured volume of the sample was added to 5 ml of high range COD reagent (HACH). This mixture was placed in a COD reactor for about 1 h. and upon cooling, the absorbance of the mixture was read at a specified wavelength using a HACH DR 2010 Spectrophotometer.

Statistical analysis

The analysis of variance of the mean microbial counts was conducted ($\alpha=0.05$). Duncan Multiple Range (DMR) tests were conducted to locate the cause of any significant differences in the analyzed mean counts (Ogbeibu, 2005).

RESULTS

The mean bacterial count ranged between $2 \times 10^2 \pm 1$ cfu/ml for upstream water sample obtained during the second week of February, 2013 to $1.09 \times 10^4 \pm 3.6$ cfu/ml



Map 1. Ikpoba River in Benin City. Industrial effluent (brewery waste) discharged into the river.

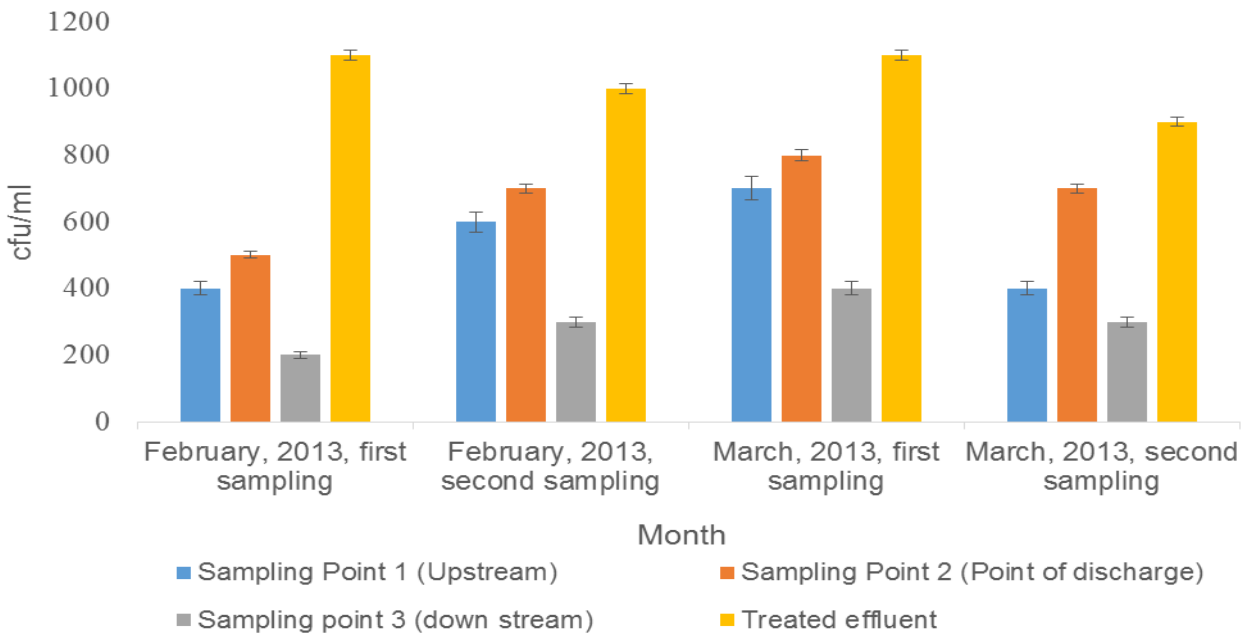


Figure 1. Mean heterotrophic bacterial counts for the water and effluent samples sourced from the respective sampling points from February, 2013 to March, 2013.

for the treated effluent sample collected during the second week of March, 2013 (Figure 1). The mean fungal counts varied from $2 \times 10^2 \pm 1$ cfu/ml for the downstream water sample collected during the third week of February, 2013 to $2.0 \times 10^3 \pm 7$ cfu/ml for the water sample sourced from the point of discharge of the effluent stream into Ikpoba River during the first week of March, 2013 (Figure 4). The observed differences in the mean bacterial counts was statistically significant ($P < 0.05$) and counts recorded for the treated effluent were responsible for the difference. The differences in the mean fungal counts were also statistically significant ($P < 0.05$) and counts

recorded for both the treated effluent and point of discharge were responsible for the difference. The total coliform counts ranged from 2 MPN/105 ml recorded for samples obtained from downstream and the treated effluent during the first and second week of March, 2013 and the third week of February, 2013 to 20 MPN/105 ml for sample collected at the point of discharge during the second week of March 2013 (Figure 2). *Escherichia coli* counts varied from 1 MPN/105 ml recorded for upstream sample collected during the third week of February, 2013 to 5 MPN/105 ml recorded for sample collected at the point of discharge during the second week of February,

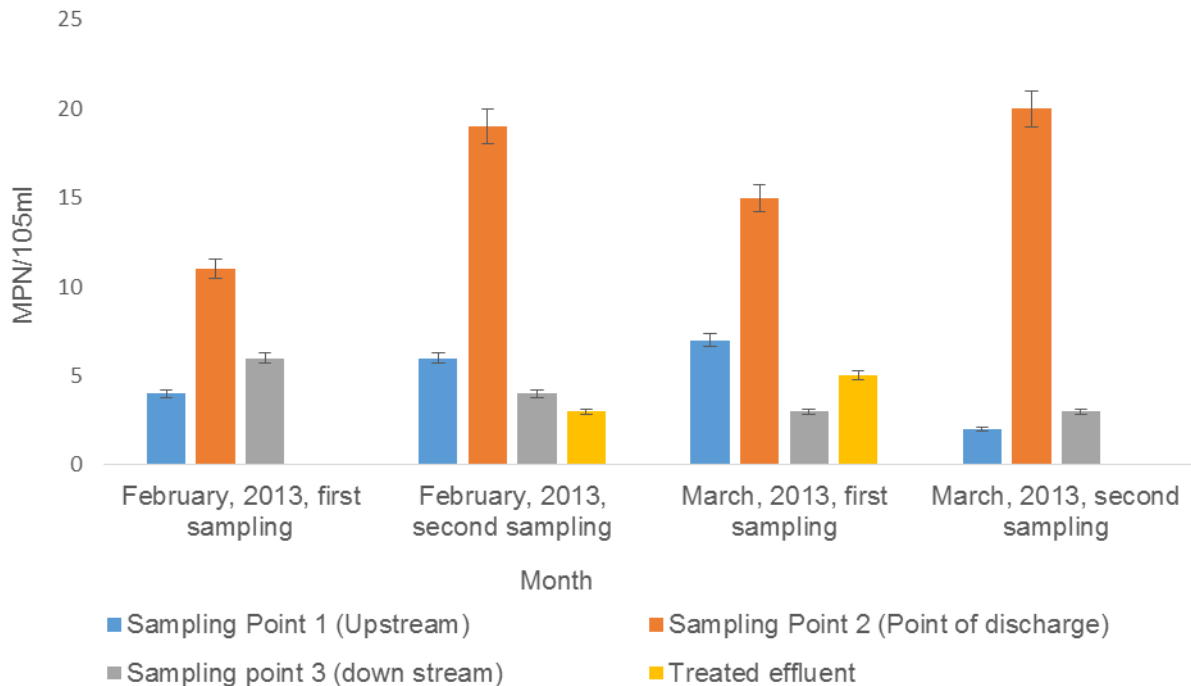


Figure 2. Total coliform counts for the water and effluent samples obtained from the respective sampling points from February, 2013 to March, 2013.

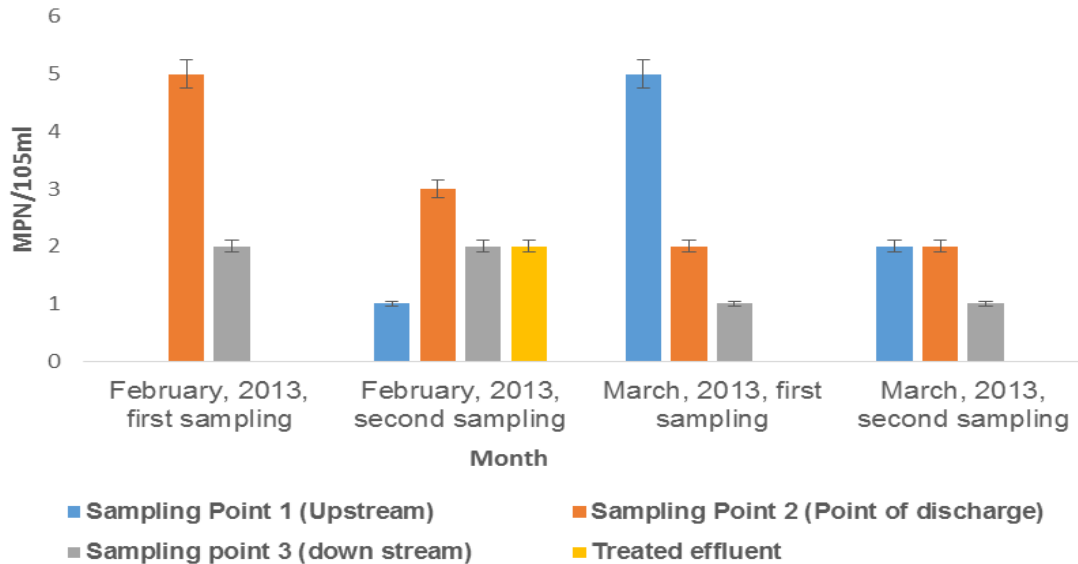


Figure 3. *Escherichia coli* (fecal coliform) counts for the water and effluent samples obtained from the respective sampling points from February, 2013 to March, 2013.

2013 and the first week of March, 2013 (Figure 3). Seven bacterial and four fungal isolates were identified; *Proteus vulgaris*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus* sp., *Pseudomonas* sp., *Enterobacter aerogenes*, *Saccharomyces cerevisiae*,

Candida tropicalis, *Penicillium* sp. and *Aspergillus niger*. *E. aerogenes* and *E. coli* were the most frequently occurring bacterial isolates (100%) while *P. vulgaris* was the least occurring bacterial isolate (25%). Amongst the fungal isolates, *A. niger* was the most dominant (100%)

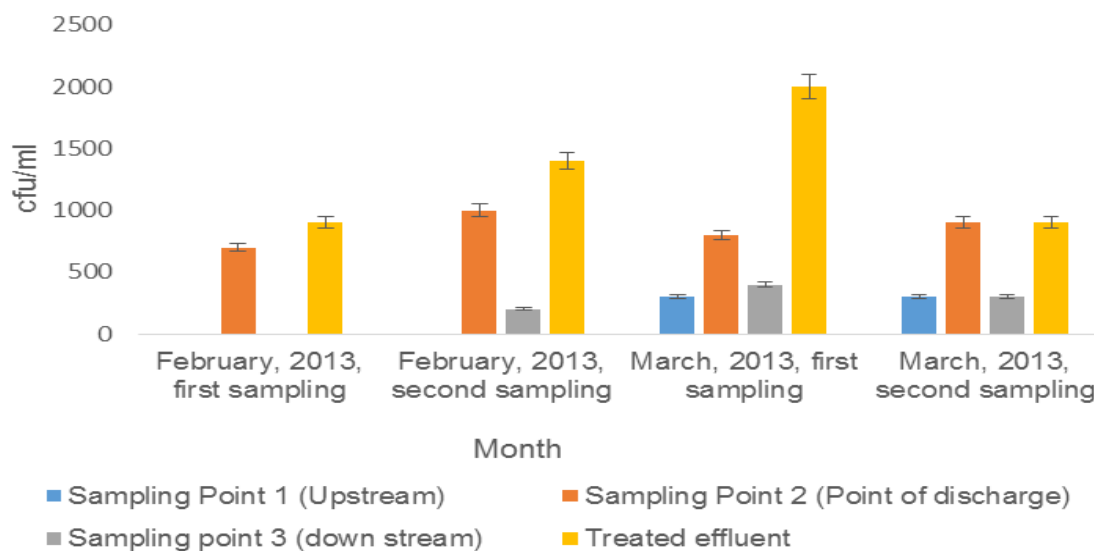


Figure 4. Mean fungal counts for the water and effluent samples abstracted from the respective sampling points from February, 2013 to March, 2013.

Table 1. Cumulative frequency of occurrence (%) of the microbial isolates.

Bacterial isolates	S.P 1 (Upstream)	S.P 2 (Point of discharge)	S.P3 (Downstream)	Treated effluent	% Cumulative freq. of occurrence
<i>Pseudomonas</i> sp.	+	-	-	+	50
<i>Klebsiella pneumoniae</i>	+	+	-	-	50
<i>Enterobacter aerogenes</i>	+	+	+	+	100
<i>Escherichia coli</i>	+	+	+	+	100
<i>Streptococcus</i> sp.	-	+	-	+	50
<i>Bacillus cereus</i>	-	+	+	+	75
<i>Proteus vulgaris</i>	-	-	+	-	25
Fungal isolates					
<i>Penicillium</i> sp.	-	+	+	+	75
<i>Saccharomyces cerevisiae</i>	-	+	-	+	50
<i>Candida tropicalis</i>	-	+	-	+	50
<i>Aspergillus niger</i>	+	+	+	+	100

SP, Sampling point; +, Present; -, Absent

while the yeast isolates *S. cerevisiae* and *C. tropicalis* had the least cumulative frequency of occurrence (50%) (Table 1). All water samples collected from upstream and downstream points on the river were colorless while the water samples collected at the point of effluent discharge were light brown in color. The mean pH, turbidity and conductivity of the respective samples ranged from 5.63 ± 0.05 to 6.78 ± 0.05 , 4.1 ± 0.21 to 6.81 ± 0.55 NTU and 3.3 ± 0.25 to 73.3 ± 6.56 $\mu\text{s}/\text{cm}$ respectively. The Biological Oxygen Demand (BOD), Dissolved Oxygen (DO) and Chemical Oxygen Demand (COD) varied from 2.6 ± 0.5 to 305.19 ± 43.2 mg/l, 5.5 ± 0.3 mg/l to 6.1 ± 0.6 mg/l and

15.8 ± 0.6 mg/l to 883.8 ± 28.5 mg/l respectively (Table 2). Heavy metals values are recorded in Table 3.

DISCUSSION

In Nigeria, especially in urban areas, surface waters have been used as the most expedient media of disposing wastes particularly effluents (Yakub, 2004). The microbiological quality of both the water samples and the treated effluent is very poor when compared with Federal Ministry of Environment limits for potable water, hence

Table 2. Mean physicochemical values for the effluent and surface water samples obtained from the respective sampling for the sampling period (February, 2013-March, 2013).

Parameters	Treated effluent	WHO standard	S.P 1 (Upstream)	S.P 2 (Point of discharge)	S.P 3 (Downstream)	FME limits
pH	5.63±0.05*	6.5-8.5	6.75±0.01	6.28±0.09	6.78±0.05	6.0-9.0
Temperature (°C)	29.5±0.14	30	24.8±0.17	26.5±0.17	25.8±0.06	20-33
Colour (TCU)	Light brown	15	Colorless	Light brown	Colorless	NS
Turbidity (NTU)	6.81±0.55	5	4.1±0.21	6.71±0.2	4.75±0.42	5
Conductivity (µs/cm)	73.3±6.56	8-10,000	3.3±0.25	59.9±5.43	3.6±0.57	4000
Alkalinity (mg/l)	33.84±2.4	150	11.97±0.5	41.69±3.7	13.64±1.3	NS
Total solids (mg/l)	147.9±3.3	1000	47.69±2.4	154.7±3.4	50.13±2.1	30
Biological oxygen demand (mg/l)	233.2±16.8	30	2.6±0.5	305.19±43.2	8.9±0.8	20-25
Chemical oxygen demand (mg/l)	752.5±35.9	80	15.8±0.6	883.8±28.5	23.6±3.7	50
Dissolved oxygen (mg/l)	5.5±0.3	3.0	6.1±0.6	5.5±0.5	5.6±0.6	7.5
Nitrate (mg/l)	1.9±0.3	5	0.4±0.3	2.4±0.5	1.0±0.1	10
Sulphate (mg/l)	3.8±0.7	400	1.14±0.3	3.4±0.5	2.3±0.6	500
Phosphate (mg/l)	0.4±0.1	6.5	0.1±0.03	0.3±0.1	0.2±0.1	>5

NS, Not Specified; S.P, Sampling Point; FME, Federal Ministry of Environment; WHO, World Health Organization Standard; * Mean ±Standard deviation.

Table 3. Mean heavy metal values for the effluent and surface water samples obtained from the respective sampling for the sampling period (February, 2013-March, 2013).

Parameters	Treated effluent	WHO standard	S.P 1 (Upstream)	S.P 2 (Point of discharge)	S.P 3 (Downstream)	FME limits
Lead (mg/l)	0.001±0.0005*	0.01	ND	0.003±0.001	0.001±0	0.002
Nickel (mg/l)	0.001±0.0005	0.02	0.001±0	0.004±0.001	0.002±0.0008	0.05
Iron (mg/l)	0.37±0.1	0.3	0.18±0.02	0.62±0.1	0.25±0.1	1.0
Copper (mg/l)	ND	2	0.001±0	0.001±0.0005	0.001±0	0.1

NS, Not Specified; S.P, Sampling Point; FME, Federal Ministry of Environment; WHO, World Health Organization Standard; * Mean ±Standard deviation. ND, not detected.

discouraging the direct consumption of the water sourced from the Ikpoba River at these sampling points by individuals living along the river banks. The samples obtained at the point of discharge of the treated effluent into the river had the highest concentration of viable microbial cells in comparison to both the upstream and downstream water samples. This trend is reflective of the high

organic, inorganic load of the treated brewery effluent introduced into the River which could have boosted the growth of both the resident and transient aquatic microflora. Kanu and Achi (2011) reported that brewery effluents are high in carbohydrates; nitrogen and the introduction of this wastewater, high in essential nutrients can bring about changes in the aquatic microflora

present in the receiving water body. The discharged brewery wastewater might have also served as a conduit through which a plethora of viable microorganisms were introduced into the river. The identification and prevalence of fecal coliforms especially *E. coli* and *E. aerogenes* in all the examined water and treated brewery wastewater samples is alarming as the presence

of these bacteria is indicative of fresh fecal contamination of the river and the potential presence of pathogens (Gerardi and Zimmerman, 2005). This trend is in tandem with an earlier observation by Bello-Osagie and Omoruyi (2012) who reported the isolation of high numbers of *E. coli* from water samples at the point of discharge of treated brewery effluent into Ikpoba River. A worrisome observation noticed during the sampling of both the treated effluent and the water sample at the point of discharge was the deliberate contamination of the treated effluent being conveyed within the pipe from the brewery plant at sections close to the river with human feces by certain individuals living around the vicinity of the river bank. This is suggestive of the abysmal level of public hygiene exhibited by people living around the river which is contributing to the fecal pollution alongside the deleterious effects of the treated brewery wastewater on the receptacle (Ikpoba River). However the total and *E. coli* counts recorded for the downstream water samples were lesser in comparison to those recorded for water samples obtained at the point of discharge. This phenomenon could be attributed to the self-cleansing activities of the river itself and the lesser anthropogenic activity occurring at this stretch of the river. More so, the effect of dilution cannot also be ruled out. This finding is in agreement with a report by Odjugo and Konyeme (2008), who investigated the impact of the urban environment and seasonality on the quality of the Ikpoba River. The isolation of *P. vulgaris* and *E. aerogenes* from the water samples is in agreement with a report by Belay and Sahile (2013) which identified these bacteria from Shinta River, which is a receptacle for Dashen brewery effluent, in Gondar town, Ethiopia.

The mean pH, temperature and electrical conductivity values recorded for the treated brewery wastewater were within the permissible limits stipulated by World Health Organization (2010) and Federal Ministry of Environment (2001). However, the chemical oxygen demand, dissolved oxygen and biochemical oxygen demand of the treated brewery effluent were above the limits prescribed by WHO (2010) and FME (2001). This trend is in agreement with an earlier study by Igboanugo and Chiejine (2012) which was a pollution survey of the Ikpoba River in Benin City, Edo State. They also stated that the Ikpoba River which is currently a receptacle for piped effluent stream from both Guinness and Bendel breweries was being subjected to effluent overloading. Apart from the dissolved oxygen mean values, the other mean values of the respective physicochemical parameters of the water samples sourced from the point of discharge were higher than those recorded for both upstream and downstream water samples. This phenomenon could be the direct result of the deliberate pumping of the treated brewery wastewater by the brewery into the river. The pH, temperature, dissolved oxygen, nitrate and phosphate mean values recorded for all the water samples were within the stipulated limits for

both drinking water and aquatic life as stated by the Federal ministry of environment (2001). However, the turbidity mean values were above the permissible limits for drinking water as indicated by the Federal ministry of environment (2001). The mean BOD, COD, electrical conductivity and alkalinity values contrasted with values reported by Ekhaize and Anyasi (2005) who assessed the bacteriological and physicochemical qualities of water samples obtained from several sampling points on the Ikpoba River.

All the mean heavy metal values recorded for the respective water samples with the exception of iron were within the limits stated by Federal ministry of environment (2001). The low concentrations of trace metals in all the analyzed water samples is in agreement with an earlier report by Oguzie and Okhagbuzo (2010) which evaluated the heavy metal concentration of fresh water samples obtained from several sampling points on the Ikpoba River, Benin City.

Conclusion

The piped effluent stream emanating from the brewery close to Ikpoba River is impacting negatively the quality of the river. Although the river possess the ability to heal itself through self-purification, there is need to proffer and implement remedial measures which would reduce or eliminate the deliberate pollution of the river by activities of the brewery. The operators of the brewery should be encouraged and mandated by relevant Government agencies to explore other cost effective ways of evacuating its wastewater generated in the course of its production activities. The brewery should also investigate and develop ways of treating effluent generated sufficiently to a standard that would make it suitable for reuse. Extensive public health enlightenment schemes aimed at educating the general public and residents living close to the discharge point on the dangers of deliberate fecal pollution of the river and direct consumption of water collected from the Ikpoba River should be conducted by both relevant Governmental and Non-Governmental agencies. Further research aimed at evaluating the levels of inorganic pesticides present in the river at specific sampling points should be conducted.

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