International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **7(5),** 1-6, May **(2018)** *Int. Res. J. Biological Sci.*

Physico-chemical and microbiological analysis of Ikpoba River Water in Benin City, Edo State, Nigeria

Hilda Abiola Akatah[1], Solomon Omonigho[2], Azeez God's Gift Ibrahim[1] and Doris Onolenmen Seyinde[3]
[1]Department of Biological Sciences, Faculty of Natural and Applied Sciences, Anchor University, Lagos, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
[3]Department of Chemical Sciences, Faculty of Natural and Applied Sciences, Anchor University, Lagos, Nigeria
akatahhilda@gmail.com

Available online at: www.isca.in, www.isca.me
Received 10th January 2018, revised 30th April 2018, accepted 5th May 2018

Abstract

Surface waters are always polluted because they are exposed directly to contaminants and therefore do not usually meet required standards. The physico-chemical and microbiological analyses of Ikpoba River was examined to determine the specie composition, microbial load and the antibiotic susceptibility pattern of the isolated strains. Analysis of the physicochemical parameter shows that all except manganese and iron were within WHO permissible limit. The total microbial population counts ranged from $3.3x10^3$ to $6.7x10^3$ CFU/ml and $4.75x10^4$ to $9.7x10^4$ CFU/ml for the fungal and bacterial isolates respectively. Total coliform counts spans from 434 MPN/100 ml to 819 MPN/100 ml. The microorganisms isolated were Aspergillus flavus, Geotrichum sp, Penicillium sp., Aspergillus niger, Mucor sp., Aspergillus fumigatus, Trichoderma sp., Escherichia coli, Staphylococcus aureus, Salmonella sp., Pseudomonas sp., Shigella sp., Vibrio sp., Proteus sp., Bacillus sp and Klebsiella sp.. The percentage of resistance of the antibiotics ranged from amoxicillin (20%) to ciprofloxacin (5%). The variations in the bacteriological and physicochemical parameters of various stations of the river water samples when compared had no significant difference (P>0.05). Therefore, Ikpoba river water is contaminated with substances which may be harmful to human health.

Keywords: Antibiotics, microbiological, physico-chemical, surface water, water quality.

Introduction

Water is a means of disseminating human associated bacteria[1]. Potable water is essential for humans and if it is contaminated, may pose serious threat to consumers[2]. The effect of river pollution on human health is dependent on the water use and the concentration of pathogens in the water[3]. Most streams in Nigeria are exposed to pollution from contaminated urban run-off, water from health care institutions, industrial, agricultural, residential, commercial and recreational areas[4].

The quality of water is determined by its physical, chemical and biological parameters[5,6]. Surface water sources do not always meet required standards and as such have to undergo various treatment processes to improve the quality of the water in a bid to ensure and protect public health[7]. Conformation to acceptable standard is of importance because of the ability of water to disseminate diseases within a large population[8].

Antimicrobial resistance has therefore become a major public health issue[9] and its presence in waste water, rivers, streams, ground water and drinking water is well recorded[10,11]. Water has an essential influence on ecological life support systems on which economic and sustainable social development rely[12]. Ikpoba River is located in Benin City and is highly disturbed, due to the high population density and the dependence on the stream, downstream riparian communities depend on the river for domestic purposes, hence the need to determine the state of Ikpoba River water. It is a stream, located in Benin City, Edo State, Nigeria[13]. Its major source is from the North West of Benin City, and flows through the city. The present study was patterned to analyze the physico-chemical and microbiological parameters of Ikpoba river in Benin City. A further objective was to determine the antimicrobial resistance profile of the isolates.

Methodology

Samples were collected from three sampling points around the Ikpoba River, namely, Station 1, Station 2, Station 3. Station 1 was established upstream of the river, station 2 was established at the downstream of the river and the station 3 was established middle point of the river. Water samples were collected in the months of May and June, at the three stations. The stations were each at a distance of at least 1km apart to ensure homogeneity and proper representation of the water. For physicochemical analysis, samples were collected midstream at depths 20–30 cm directly into the sterilized plastic and bottle containers. A total of 18 samples was collected and brought to the Microbiology laboratory in an icebox at 4°C for analysis[14].

Determination of physico-chemical parameters: The physico-chemical parameters were assessed according to the procedure

in the standard methods for the examination of water and wastewater[15]. Turbidity was determined with a HACH 2100P Turbidimeter. The dissolved oxygen and salinity was analyzed using dissolved oxygen (DO) meter. The total dissolved solids and suspended solids were measured gravimetrically after drying in an oven. Nitrate, phosphate, sulphate, manganese, ammonium aluminium, iron and lead were determined using the photometer (model spectroquant). Ammonia was analyzed using a comparator (HANNA), alkalinity by strong acid titration method. Conductivity was analyzed with a conductivity meter. Calcium and magnesium was measured by EDTA titration[15].

The temperatures were determined instantly at the study site using mercury-in-glass thermometer. The pH of the samples was determined using pH meter immediately the samples was brought to the laboratory. All samples was transported to the laboratory within 2 hours of collection and stored in the fridge[14].

Isolation and Characterization of colonies: The media used are: MacConkey broth, Brilliant Green Lactose Bile (BGLB) broth and Eosine Methylene Blue (EMB) agar for enumeration of coliform, nutrient agar for total heterotrophic bacterial count, potato dextrose agar for total heterotrophic fungal count, *Salmonella/Shigella* agar (SSA) for isolating *Salmonella* and *Shigella* and Thiosulphate citrate bile salt (TCBS) agar for isolating *Vibrio spp*. All the media were prepared according to the manufacturers' instructions. Each sample was analyzed in triplicate and was serially diluted. 1ml of the 5 fold dilutions was spread on to the surface of nutrient agar plates. The plates were incubated at 37°C for 24 hours. The colonies were enumerated, characterized, and recorded[16].

Purification of Colonies: Colonies were purified by twice sub-culturing on Mueller Hinton agar, using the streaking plate method. All the isolates were subjected to Gram staining and biochemical tests[17].

Antimicrobial Susceptibility Testing: Antibiotic susceptibility test was performed by the Kirby-Bauer disk diffusion method using the Mueller-Hinton agar[16]. The following antimicrobial discs were used: septrin, 30µg; ciprofloxacin, 10µg; amoxicillin, 30µg; streptomycin, 30µg; chloramphenicol, 30µg; gentamycin, 10µg; pefloxacin, 30µg and augumentin, 30µg. Colonies were selected for susceptibility tests, after incubation at 37°C for 24 hours, the susceptibility of each organism was measured and the results were interpreted according to National Committee for Clinical Laboratory Standards[18].

Statistical Analysis: The results obtained was subjected to Kruskal-Wallis test (Nonparametric ANOVA), the analysis was done using SPSS version 16.0.

Results and discussion

Determination of the total heterotrophic bacteria (THB) of the samples obtained from the three (3) stations of Ikpoba River showed that it ranged from $4.75x10^4$ CFU/ml (Station 3) to $9.7x10^4$CFU/ml (Station 2) as shown in Table-2, Station 2 having the highest THB counts. Result revealed that the lowest total coliform and faecal coliform counts (434MPN/100ml and 64MPN/100ml respectively) were observed in Station 1, while the highest counts of 819MPN/100ml and 150MPN/100ml total coliform and faecal coliform respectively was obtained from Station 2. There was no significant difference ($P>0.05$) in the parameters analyzed as seen in Table-2. The determination of total heterotrophic fungi (THF) of the samples obtained from the three (3) stations of the Ikpoba River showed that the THF counts (Table-2) ranged from $3.3 x10^3$CFU/ml (Station 3) to $4.0 x10^3$CFU/ml (Station 2), Station 2 having the highest THF counts. The fungal isolates obtained and their occurrence in percentage (Figure-2) are *Aspergillus flavus* (22.85%), *Geotrichum sp* (20%), *Penicillium sp* (17.14%), *Aspergillus niger* (14.29%), *Mucor sp* (11.43%), *Aspergillus fumigatus* (11.43%) and *Trichoderma sp* (2.86%) were observed in the water samples from all stations. The bacterial isolates identified belonged to Enterobacteriaceae group amongst which was *Escherichia coli* (25%), *Salmonella sp* (12.5%), *Shigella sp* (10%), *Pseudomonas sp* (10%), Vibrio sp (10%), *Proteus sp* (7.5%), *Klebsiella sp* (5%), were found. Others were *S. aureus* (12.5%) and *Bacillus sp* (7.5%). The results of antibiotic susceptibility are presented in Table-3. There was multiple antimicrobial resistance by the isolates. Resistance to β-lactam antibiotics (Amoxicillin) was major among all isolates. The Gram negatives were also resistant to Septrin, Augumentin and Chloramphenicol, while the Gram positives were susceptible to Quinolones (Pefloxacin and Ciprofloxacin).

Discussion: The determination of the physico-chemical parameters revealed that the pH of the samples from Station 2 and 3 were below WHO permissible limit of 6.5-8.5[14,19]. Comparable range of 6.1-7.0 was gotten by Edjere O. et. al[12]. The temperature, dissolved oxygen (DO) and total dissolved solutes (TDS) of the samples were within WHO standard for surface water. The color was objectionable and was not within WHO standard except for Station 2. Station 1 and Station 3 had turbidity values of 20NTU both were higher than the WHO permissible limits of 15.0NTU, this could be attributed to the erosion due to heavy rainfall. Nitrate and alkalinity were within WHO permissible limit. Values of the total hardness, calcium and magnesium of the three stations studied were within permissible limit. Manganese values of the stations were not within WHO standard values, except for Station 1 (0.012mg/l). The value of iron was within WHO guideline of 0.3mg/l in the other stations except for Station 2 (0.32mg/l)[20] revealed that the presence of corrosive materials in a water body could increase the iron content of the water. Ammonium, phosphate, sulphate and aluminum were within WHO guideline value. Conductivity was found to be within WHO permissible limit, the low conductivity values of the samples imply that the dissolved salts are minimal. Biological Oxygen Demand (BOD) is the amount of oxygen to be used by bacteria to decompose organic matter present within the samples under aerobic conditions[5]. Chemical

Oxygen Demand (COD) is a measure of the total quantity of oxygen required to oxidize organic matter into carbon dioxide and water. The COD and BOD of these water samples were within WHO permissible limit. The level of nitrate in the samples of all the stations was low. The level of total dissolve solids (TDS) are also within the recommended range of 500mg/l and above. The values of lead and phosphate from the table showed that it was within WHO permissible limits[19]. Statistical analysis of the physico-chemical parameters of the three stations showed no significant difference (P> 0.05).

Table-1: Physico-chemical parameters of the samples.

Parameter (unit)	Station 1	Station 2	Station 3	WHO limits
pH	6.6	6.1	6.0	6.5 – 8.5
Temperature (^oC)	26	24	25	25 – 30
Colour (CTU)	20	15	20	15
Turbidity (NTU)	18	18	18	5
Chloride	10.6	10.6	14.1	200-250
Dissolved Oxygen (mg/l)	2.1	4.4	4.0	14
Biochemical Oxygen Demand ($B.O.D_5$)	1.0	2.2	1.6	4.0
Chemical Oxygen Demand (C.O.D)	5.25	5.9	5.7	80
Total Dissolved Solids (mg/l)	46.8	10.8	5.4	500
Suspended Solids (mg/l)	39	7	6	Not Specified
Total Solids (mg/l)	85.8	17.8	11.4	500
Nitrate (mg/l)	16.3	4.5	6.4	50
Alkalinity (mg/l)	20	6	4	200
Hardness (mg/l)	34	12	8	500
Calcium (mg/l)	1.6	1.6	1.6	50
Magnesium (mg/l)	1.46	1.46	1.46	30
Manganese (mg/l)	0.012	0.084	0.076	0.05
Iron (mg/l)	0.19	0.32	0.26	0.30
Lead (mg/l)	0.01	0.008	0.003	0.05
Ammonia (mg/l)	0.015	0.022	0.01	0.64
Phosphate (mg/l)	0.15	0.16	0.14	200
Sulphate (mg/l)	7	6	6	250
Conductivity (µs/cm)	90	20	10	500

Table-2: Profile of total coliform and faecal coliform, total heterotrophic bacteria (THB) and total heterotrophic fungal (THF) counts obtained from the water samples.

Parameters	Station 1	Station 2	Station 3
Total coliform (MPN/100ml)	434	819	553
Faecal coliform (MPN/100ml)	64	150	93
THB (cfu/ml)	9.3×10^4	9.7×10^4	4.75×10^4
THF (cfu/ml)	4.0×10^3	6.7×10^3	3.3×10^3

Table-3: Antibiotic sensitivity of bacteria isolated from water sample.

Isolates	Antibiotics							
	SXT	CIP	AM	S	GN	PEF	AU	CH
Salmonella sp	–	+	+	–	+	+	–	–
E. coli	–	–	–	+	–	–	–	–
Pseudomonas sp	–	–	+	–	+	–	–	–
Vibrio sp.	–	+	–	+	–	+	–	–
Proteus sp.	+	–	–	+	–	–	+	+
Shigella sp.	–	–	–	+	–	+	–	–
Staplylococcus aureus	+	–	–	–	+	+	–	–
Bacillus sp.	–	+	–	–	–	+	+	–

Where: ⁻ = represents resistance, + = susceptible to antibiotic, CPX = ciprofloxacin, GN/CN = gentamycin, AU = augumentin, SXT = septrin, CH = chloramphenicol, AM = amoxicillin, PEF = perfloxacin, S = streptomycin.

This study showed the occurrence of total and faecal coliforms and heterotrophic bacteria and fungi in the samples analyzed, which reveals water contamination as some of these species are indicators of faecal contamination[4]. These organisms may harbor potential pathogens which may endanger the health of potential consumers and immune-compromised individuals. *Staphylococcus* species are known to produce enterotoxin[21]. *Proteus* is an intestinal flora, majorly found in soils and water[22].

The genus *Aspergillus* was the most isolated in this study, this is also similar with the record of Hageskal G.[23] that *Aspergillus* is the most occuring genera isolated in river water. *Aspergillus* produce aflatoxins (B1, B2, G1 and G2), which are toxic and hepato-carcinogenic[24]. *A.flavus* was also isolated in this study, and is known to cause invasive and non-invasive aspergillosis[25]. *A. niger* also causes opportunistic invasive infections in immunocompromised patients[26]. *Penicillium* causes allergy, asthma and some respiratory problems[23]. The fungi isolated in this study may have allergic ability, if immunocopromised persons are exposed. *Mucor* causes of thrombosis, nasal infections and GI disorders. *Trichoderma* species are carried by soil particles and have immense ability in production of spores[23], and are known to cause mycosis in humans[27,28].

Most of the isolates which were resistant to ß–lactam antibiotics (amoxicillin), were also resistant to non-ß–lactam antibiotics, the rate of occurrence of these microorganisms recommends that characterization of the antimicrobial resistance genes and the plasmids on which they reside could provide information about sources of antimicrobial resistance in the environment. In addition, most of isolates were Gram negative bacteria, and also showed greater resistance towards the antimicrobials tested than Gram positive bacteria. Many studies[9,29,30] showed that different water bodies serve as source for antimicrobial resistant genes and microorganisms. A large number of the isolates were resistant to chloramphenicol, trimethoprim (septrin) and amoxicillin. The trend was in harmony with prior studies which showed resistance towards chloramphenicol, β-lactam, macrolides, and phenicols[30,31]. A large percentage of isolates were also resistant to augumentin, aminoglycosides

(gentamycin) and ciprofloxacin (quinolones). Broad dissemination of antimicrobial resistant organisms in surface and ground waters has been recorded in prior studies[3,5] and the result of this study is not dissimilar. These resistances could be caused by heavy contamination from effluents, runoffs, agricultural activities, animal or industrial pollution. The result from this study reflects the need to conduct studies to enumerate the dominance of antimicrobial resistant genes among environmental isolates and the spread of resistant genes among the pathogens[8]. This would channel information about the risks associated with the intake of contaminated water. It has been recorded that antimicrobial resistant genes encoded with plasmids were distributed among pathogenic and non-pathogenic Gram negative bacteria in the environment which assumed the high antimicrobial resistance action[31]. Sidhu A.K. et al[32] also reported that some of the cured isolates from water were resistant to antibiotics, and that the resistant genes seems to be encoded by genes of bacterial chromosome.

Conclusion

Ikpoba river water receives brewery, hospital, and abattoir effluents, therefore it could be a source of antimicrobial resistant bacteria, with sewage contamination contributing to the spread of antimicrobial resistant bacteria in the environment. Antibiotic resistance surveillance, regulation of the usage of drugs, education of the public on the consequences of the misuse of antibiotics can be used as tool to control the problem of antibiotic resistance. Regular monitoring of Ikpoba River water microbiological quality and non-consumption of water from Ikpoba River, are vital to ensure public health protection.

References

1. Ababiaka T.O. and Sule I.O. (2011). Bacteriological assessment of selected borehole water samples in Ilorin Metropolis. *International Journal of Applied Biological Research,* 2(2), 31-37.

2. Erah P.O. and Akujieze C.N. (2002). The Quality of Groundwater in Benin City: A baseline study on inorganic chemicals and microbial contaminants of health importance in boreholes and open wells. *Tropical Journal of Pharmaceutical Research*, 1(2), 75-82.

3. Edema M.O., Omemu A.M. and Fapetu O.M. (2001). Microbiology and Physicochemical Analysis of different sources of drinking water in Abeokuta. Nigeria. *Nigerian Journal of Microbiology*, 15(1), 57-61.

4. Uzoigwe C.I. and Agwa O.K. (2012). Microbiological quality of water collected from boreholes sited near refuse dumpsites in Port Harcourt, Nigeria. *African Journal of Biotechnology*, 11(13), 3135-3139.

5. Omezuruike O.I., Damilola A.O., Adeola O.T. and Enobong A. (2008). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. *African Journal of Biotechnology*, 7(5), 617-6721.

6. Okpokwasili G.C. and Akujobi T.C. (1996). Bacteriological indicators of tropical water quality. *Environmental Toxicology*, 11(2), 77-81.

7. Kumarasamy P., Vignesh S., Arthur James R., Muthukumar K. and Rajendran A. (2009). Enumeration and identification of pathogenic pollution indicators in Cauvery River, South India. *Research Journal of Microbiology*, 4(12), 540-549.

8. Akatah H.A. (2016). Physico-chemical and microbiological analyses of Ikpoba River, Benin City, Edo State, Nigeria. master's thesis, University of Benin, Benin City, Edo State, Nigeria.

9. Kim J., Kang H.Y. and Lee Y. (2008). The identification of CTX-M-14, TEM-52, and CMY-1 enzymes in Escherichia coli isolated from the Han River in Korea. *The Journal of Microbiology*, 46(5), 478-481.

10. Kümmerer K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy*, 54(2), 311-320.

11. Iversen A., Kühn I., Franklin A. and Möllby R. (2002). High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Applied and Environmental Microbiology*, 68(6), 2838-2842.

12. Edjere O., Ibezute A.C. and Ibezute S.U. (2016). Impact of Brewery Factory Effluent on the Physicochemical Characteristics of Ikpoba River in Edo State, Nigeria. *Resources and Environment*, 6(3), 53-62.

13. Benker-Coker M.O. and Ojior O.O. (1995). Effects of slaughter House waste on the quality of Ikpoba River, Benin city, Nigeria. *Bioresource Technology*, 52, 5-12.

14. World Health Organization (2003). Guidelines for drinking water quality. WHO press, Geneva, Switzerland, 685.

15. American Public Health Association (APHA) (1998). Standard Methods for the Examination of Water and Wastewater. 20th edition (American Public Health Associated Incorporation, New York. Washington, 1998) D.C., 213.

16. Krieg N.R. and Hott J.G. (1994). Bergy's Manual of systemic bacteriology. 8th edition. 8th (William and Wilkins, Baltimore Ltd. USA, 1994), 353-514.

17. Cheesbrough M. (2000). District laboratory practice in tropical Countries. *Part 2*. (Cambridge University Press. U.K, 2000), 454.

18. National Committee for Clinical Laboratory Standards (NCCLS) (1997). Performance standards for antimicrobial disk susceptibility tests M2-A6. NCCLS M100-S14. 3rd edition., (Wayne (PA). Pennsylvania, USA, 2122.

19. World Health Organization (WHO) (2006). Guidelines for Drinking Water Quality. 3rd edn. (WHO Press Geneva, Switzerland, 2006), 1, 515.

20. Pelig-Ba K.B. (1989). A Report on an Investigation of Water Quality Problems on Borehole AP216 at Oyibi. (Water Resources Research Institute, Accra, Ghana), 3rd ed., 112.

21. Ayantobo O.O., Oluwasanya G.O., Idowu O.A. and Eruola A.O. (2013). Water quality evaluation of hand-dug wells in Ibadan, Oyo State, Nigeria. *Global Journal of Science Frontier Research Agriculture and Veterinary,* 13(10), 21-27.

22. Schwartz T., Kohnen W., Jansen B. and Obst U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS microbiology ecology*, 43(3), 325-335.

23. Hageskal G., Knutsen A.K., Gaustad P., de Hoog G.S. and Skaar I. (2006). Diversity and significance of mold species in Norwegian drinking water. *Applied and environmental microbiology*, 72(12), 7586-7593.

24. Bennett J. and Klich M. (2003). Mycotoxins. *Clinical microbiology reviews*, 16(3), 497-516.

25. Morgan J., Wannemuehler K.A., Marr K.A., Hadley S., Kontoyiannis D.P., Walsh T.J. and Warnock D.W. (2005). Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Medical mycology*, 43(sup1), 49-58.

26. Hoog G.S., Guarro J., Gene J. and Figueras M.J. (2000). Atlas of clinical fungi. Central bureau voor schimme cultures, Utrecht, The Netherlands, 321.

27. Jaakkola M.S., Nordman H., Piipari R., Uitti J., Laitinen J., Karjalainen A. and Jaakkola J.J. (2002). Indoor dampness and molds and development of adult-onset asthma: a population-based incident case-control study. *Environmental health perspectives*, 110(5), 543-547.

28. Tang P., Mohan S., Sigler L., Witterick I., Summerbell R., Campbell I. and Mazzulli T. (2003). Allergic fungal sinusitis associated with Trichoderma longibrachiatum. *Journal of clinical microbiology*, 41(11), 5333-5336.

29. Akinpelu A.T., Akinloye O.M., Olukemi B.C., Adegoke A. E. and Olayinka S. (2014). Antibiotic resistant pattern of isolated bacteria from Obere River in Orile-Igbon, Oyo State, Nigeria. *African Journal of Microbiology Research*, 8(12), 1318-1321.

30. Ash R.J., Mauck B. and Morgan M. (2002). Antibiotic resistance of gram-negative bacteria in rivers, United States. *Emerging infectious diseases*, 8(7), 713-716.

31. Ozgumus O.B., Sandalli C., Sevim A., Celik-Sevim E. and Sivri N. (2009). Class 1 and class 2 integrons and plasmid-mediated antibiotic resistance in coliforms isolated from ten rivers in northern Turkey. *The Journal of Microbiology*, 47(1), 19-27.

32. Sidhu A.K., Metkar G.J., Nandurikar S.P. and Patil S.N. (2015). An Investigation on Metal Tolerance and Antibiotic Resistance Properties of Bacterial Strains Isolated From Two Different Drinking Water Sources. *Int. J. Curr. Microbiol. App. Sci*, 4(2), 305-313.