



SJMLS - 5 (1) - 015

Bacteriological and Physiochemical Analysis of Water from River Ethiope, Abraka.Akpo, C.O.^{1*} and Mukoro, J.¹*Department of Pharmaceutical Microbiology, Delta State University, Abraka¹.**Author for Correspondence *: +234- 803- 225- 5534***Abstract**

Water is a free gift of nature which is important to plants, animals and humans. The aim of this research is to assess the quality of water samples obtained from River Ethiope in Abraka. Water samples were analyzed for physiochemical properties as well as for the presence of bacteria. Physiochemical analyses showed a pH range of 7.40-7.70, dissolved oxygen (DO) of 3.8 – 5.0 mg/L, temperature (°C) 29.0 -30.0, alkalinity (mg/L) 1.26-1.40. Total bacterial counts ranged from 2.0×10^6 – 1.7×10^7 bacteria/ ml of water sample. Laboratory investigations showed the presence of *E. coli*, *S. aureus*, *Bacillus sp*, *S. epidermidis*, *Actinomyces* and *Enterobacter sp*. The result of this research shows that water from River Ethiope met WHO standard for some physiochemical parameters but has a low amount of dissolved oxygen. *Bacillus sp* are the commonest bacteria isolated from water. The presence of gram-positive bacteria such as *S. aureus*, *S. epidermidis* and *Actinomyces* indicate bathing and other human activities which are carried out in the water, since they are normal human flora. However, the presence of *E. coli* and *Enterobacter sp* indicate recent faecal contamination. Most of the bacteria isolated are associated with food poisoning. The people of Abraka should be educated on the need to avoid defaecating in the river and water from River Ethiope would need to be treated before human consumption to avoid the spread of infection.

Keywords: *Physiochemical, Water, River Ethiope, Bacteria*

Introduction

Water is found almost everywhere on earth but may be seen as liquid, which is the commonest

form; ice, the solid state and vapour, present in the air. It is common knowledge that water is the colourless, odourless and tasteless liquid used for many purposes. Man, animals and plants, are dependent on water for their existence. Water is important to man for many activities such as production in industries; in agriculture for irrigation, herbicide and insecticide formulations; transportation; sports (swimming, water skating); medicinal purposes and domestic purposes (cooking, bathing, washing, etc). Water is susceptible to contamination with microorganisms and organic matter among other pollutants regardless of the source (Gangil *et al.*, 2013).

Abraka is a community in Ethiope East local government area of Delta State, Nigeria. It is the most popular community in the Local Government area because the main campus of Delta State University is sited there. River Ethiope, a natural flowing river which serves as a national and international tourist attraction, runs through Abraka. This freshwater river takes its source from a tree in Umuaja, a community in Ukwani Local Government area of Delta State and runs through Ethiope East, Okpe, Ethiope West, and Sapele Local Government Areas. The river has served the communities through which it runs, a number of valuable uses such as water supply, food, medicine, spiritual purposes, research and recreation.

In light of the many activities which take place in River Ethiope, the water is prone to contamination by microorganisms and may not be safe for human consumption. Idise and Owhe-Ureghe (2015) isolated about seven bacterial genera from different locations along

River Ethiopie, Delta State. These isolates include *Bacillus sp.*, *Klebsiella sp.*, *Escherichia sp.*, *Micrococcus sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, and *Streptococcus sp.*

The United Nations General Assembly declared in 2010 that safe and clean drinking water and sanitation is a human right, essential to the full enjoyment of life and other human rights (World Health Organization, 2017). It has been reported that more than 1.2 billion people worldwide do not have access to safe water (Maduka *et al.*, 2014). Millions of people die yearly from diarrheal diseases and a larger proportion are children aged below 5 years (Muhammad *et al.*, 2012). Besides causing death, water-related diseases also prevent people from working and living active lives (Memon *et al.*, 2011). This research was carried to assess water from River Ethiopie for physiochemical properties (such as pH, dissolved oxygen, temperature, acidity and alkalinity) as well as the presence of bacteria.

Materials and Methods

Collection of Water Samples

The WHO sampling method for microbiological analysis (2017) was employed with modifications. The bottles used for collection were sterilized using an autoclave at 121°C for 20 minutes before proceeding to the river. Water samples were collected aseptically from different areas of the Ethiopie river behind Ethiopie hall, Campus 2, Delta State University, Abraka. These areas include: fast flowing (clear), slow flowing (slightly turbid) and stagnant, (turbid) regions. The water samples were labelled accordingly and immediately transported to the laboratory in an ice pack for analysis.

Physiochemical Analysis of Water Samples

A number of physiochemical parameters were carried out on the raw water samples using specified methods. These parameters include temperature, dissolved oxygen, pH, turbidity, alkalinity, acidity.

Water pH

A pH meter was used to measure the pH of each water sample. The pH meter was calibrated by preparing buffers of pH 4.0 and 7.0. The pH electrode was rinsed in distilled water, dipped into each buffer to read the pH and when stable,

the pH meter is set to allow for more accurate and tuned readings. After each reading in the pH meter had been recorded, the probe was rinsed in distilled water to avoid cross-contamination among the samples.

Turbidity

One hundred milliliters (100ml) of each water sample was measured into a sterile measuring cylinder, standing on a clean white paper marked with black ink crosses. If water sample is turbid, it prevents light from reaching the marked black crosses indicating that the water contains foreign matter that makes it turbid and not pure.

Temperature

The temperature of each water sample was measured using mercury-in-glass thermometer which was dipped into each water sample in a sterile beaker.

Alkalinity

Fifty milliliters (50ml) of the water sample was measured into a 250ml conical flask and 2 drops of methyl red indicator was added and the solution was titrated against 0.01M H₂SO₄ to a pink end point (Sawyer *et al.*, 2000). The volume of acid used was noted and recorded. The alkalinity of the water sample was thus calculated.

Dissolved Oxygen (DO)

This was carried out using the Winkler method (Numako and Nakai, 1995) of dissolved oxygen present in the water samples. In this procedure, 300ml DO bottles were filled with the water samples respectively and 2ml of Manganese sulphate and 2ml of alkali-iodide azide solution was added by inserting a pipette just below the liquid. The bottles were then covered using a stopper to avoid the introduction of air and were mixed by inverting severally. The bottles were left to stand for a few minutes. The presence of oxygen is determined by the formation of a brownish-orange precipitate. Thereafter, 2ml of H₂SO₄ was added and mixed again by inverting to dissolve the precipitate. The resulting solution was then measured (200ml) into a 250ml conical flask and titrated against Sodium thiosulphate solution using the starch indicator until the solution turned colourless. The DO contained in the water sample was thus calculated.

Bacteria Plate counts

Water samples aseptically collected from River Ethiopie were diluted serially to obtain 10^{-5} dilution and 0.1ml cultured by spread plate technique in media (these are MacConkey agar, Simmon citrate agar, Mannitol salt agar, Salmonella-Shigella agar, Sabouraud dextrose agar and blood agar) and incubated at 37°C for 24 hours. Total bacterial counts were done after 24-hour incubation of the water samples in nutrient agar. The number of colonies were counted and the number of bacteria per milliliter of water sample was calculated.

Isolation of Bacteria

Samples of dirty/stagnant water, slightly turbid water and very clear/fast flowing water collected from River Ethiopie were plated in blood agar, MacConkey agar, Mannitol salt agar and Simmon Citrate agar. Plates were incubated for 24 hours at 37°C . The cultural appearance in each medium was recorded. Gram staining procedure was also carried out and noted. Biochemical tests done to further identify the bacteria present in the water samples are fermentation (galactose, maltose, sorbitol, glucose, sucrose and lactose), H_2S , indole, urease, oxidase, catalase and coagulase.

Results

Table 1: Physiochemical Analysis of Water Samples

Parameters	WHO standard	Sample 1	Sample 2	Sample 3
pH	6.5 – 8.5	7.6	7.4	7.7
Turbidity (NTU)	5	4.5	2.9	0.55
DO (mg/L)	5- 9.5	4.2	5.8	6.5
Temperature (oC)	27 – 30	29.90	29.00	30.00
Alkalinity (mg/L)	100-200	1.4	1.26	1.30

Key: Sample 1: Stagnant/ turbid water); Sample 2: Slow flowing/ slightly turbid and fast flowing/ clear water.

Table 2: Mean Bacteria Count in Water Samples (Bacteria/millilitre)

Agar media	Dilution factor	Sample 1	Sample 2	Sample 3
MacConkey agar	10^{-5}	1.3×10^7	1.7×10^7	8.0×10^6
Simon citrate	10^{-5}	3.0×10^6	2.0×10^6	N/G
Manitol salt	10^{-5}	2.0×10^6	N/G	N/G
Salmonella Shigella agar	10^{-5}	N/G	N/G	N/G
Sabouraud dextrose agar	10^{-5}	N/G	N/G	N/G
Blood agar	10^{-5}	5.0×10^6	7.0×10^6	2.0×10^6

Key: Sample 1: stagnant, (turbid) water, Sample 2: slow flowing (slightly turbid) water and Sample 3: fast flowing (clear) water. NG: No Growth

Table 3: Identification of Bacterial isolates

Test	<i>E. coli</i>	<i>S. aureus</i>	<i>Bacillus sp</i>	<i>S. epidermidis</i>	<i>Actinomyces</i>	<i>Enterobacter sp</i>
Microscopy	Gram negative rods	Gram positive cocci in clusters	Gram positive rods. which were	Gram positive cocci	Gram positive rods in chains	Gram negative rods
Growth on media	Pink colonies in MacConkey agar	Round, smooth, raised and glistening colonies which changed mannitol salt agar from red to yellow.	Colonies showed beta haemolysis on blood agar,		White, smooth and convex colonies in blood agar. Colonies appeared dusty in the agar plate.	Tiny, transparent haemolytic, raised colonies in blood agar. Pink colonies with a sharp smell in MacConkey agar.
Sucrose	Negative	Positive	Negative	Negative	Positive (A/G)	Negative
Lactose	Positive		Positive (A/G)	Positive (A)	Positive (A/G)	Positive
Glucose	Positive	Positive	Positive (A/G)	Positive (A)	Positive (A/G)	Positive
Mannitol	Negative	Positive	Negative	Negative	Negative	Positive
Galactose	ND	ND	ND	ND	ND	ND
Maltose	ND	ND	ND	ND	ND	Positive
Sorbitol	ND	Negative	ND	ND	ND	Variable
Citrate	Negative	Negative	Negative	Negative	Negative	Positive
H ₂ S	Negative	Negative	Negative	Negative	Negative	Negative
Indole	Positive	Negative	Negative	Negative	Negative	Negative
Motility	ND	ND	ND	ND	ND	ND
Urease	Negative	Negative	Negative	Negative	Negative	Negative
Oxidase	Negative	Negative	Negative	Negative	Negative	Negative
Catalase	Negative	Positive	Positive	Positive	Negative	Positive
Coagulase	Negative	Positive	Negative	Negative	Negative	Negative

Key: A/G- Acid and gas production; A- Acid production; ND- Not done

Discussion

Table 1 shows the result for the physiochemical analysis of the water samples tested. The pH of a water sample measures the degree of acidity or alkalinity of the sample. In this study, the pH of the three samples ranged from 7.4 to 7.7 and thus fall within the range of normal drinking water recommended by WHO as standard (World Health Organization, 2017). The slow flowing, slightly turbid water (sample 2) had the lowest

pH 7.4, while the fast flowing, clear water (sample 3) had the highest pH 7.7. This indicates that the clarity of the water sample may not bring about a significant difference in the pH range of the water sample from River Ethiopia.

Turbidity expresses the cloudiness of water which may be caused by a number of suspended particles and organic particles, including microorganisms from soil runoff. Turbidity is

also an important aesthetic parameter, with turbidities of 4 nephelometric turbidity units (NTU) and above being visible, and affecting the appearance and acceptability of drinking-water to consumers (WHO-FMC-, 2017). In this work (Table 1) the highest turbidity (4.5 NTU) was recorded for the stagnant water (sample 1) and the lowest (0.55 NTU) for the fast flowing, clear water (sample 3). This result is an indication that the water samples from River Ethiopie are below the WHO maximum standard limit of 5 NTU.

Dissolved oxygen is the amount of oxygen dissolved in a body of water- the amount of oxygen available for aquatic life. It is used as an indicator of the health of surface-water bodies (Rounds *et al.*, 2013). Fast flowing water such as streams and rivers contain a lot of dissolved oxygen while stagnant water contains less. In this work (Table 1), the lowest dissolved oxygen (4.2 mg/L) was observed in the stagnant sample 1 while the highest dissolved oxygen (6.2 mg/L) was recorded for the fast flowing, clear water. The result of the stagnant water did not meet the WHO standard.

Water temperature is a measure of the hotness or coldness of water. The temperature obtained in this study for all the water samples fall within the WHO standard. The highest, 30°C was obtained from the fast flowing, clear water sample (Table 1). The temperature of water is an important physical property of rivers, having a direct impact on water quality (e.g. concentrations of dissolved oxygen) and on the growth rate and distribution of freshwater organisms (Mohseni-Bandpei and Yousefi, 2013; Ozaki *et al.*, 2003).

Alkalinity is the ability of water to resist changes in pH that would make the water more acidic. It is also the protector of health and piping, with respect to drinking water. The result of this study (Table 1) shows that the alkalinity of all the water samples tested was very low with a range of 1.26 mg/L to 1.30 mg/L. It has been reported (Yashaswini *et al.*, 2019) that the estimated values of alkalinity in the Kabini River Basin area, India varied from 0.13 to 1.95 g/L with the geometrical mean value of 0.91 g/L. These results are relatively low compared to the WHO standard of 100-200 mg/L recommended for safe drinking water.

The mean bacterial count (at a dilution of 10^{-5}) obtained from the three water samples is shown in Table 2. The number of bacteria per milliliter of water sample counted in MacConkey agar ranged from 8.0×10^6 to 1.7×10^7 . The total bacteria counted in this medium was highest in the slow flowing water sample and least in the fast-flowing sample. Mean bacterial count in blood agar was highest in the slow flowing water sample (7.0×10^6) and least in the fast-flowing water sample (2.0×10^6). From the result of this study, bacteria were more in MacConkey agar than in blood agar.

The mean bacterial count in Simmon Citrate agar (Table 2) were 3.0×10^6 and 2.0×10^6 in the stagnant and slow flowing samples, respectively. There was no growth from the fast-flowing water sample. The mean bacterial count obtained from Mannitol salt agar is 2.0×10^6 , in the stagnant water sample. The slow flowing and fast flowing water samples did not present any bacterial growth. In this study bacteria did not grow in Salmonella-Shigella agar as well as in Sabouraud Dextrose agar. It is known that Sabouraud Dextrose agar is used to grow fungi for an incubation period of 2 days to 4 weeks. The reason for the absence of growth in this medium may be attributed to the short incubation period of 24 hours.

Laboratory identification of the bacterial isolates from the water samples is expressed in table 3. The bacteria isolated are *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* species, *Staphylococcus epidermidis*, *Actinomyces* species and *Enterobacter* species. In this study, the gram-positive bacteria were more than the gram negative. A similar report, that gram positive genera, especially the genus *Bacillus* were the most abundant bacteria isolated from Kaduna River in Nigeria was reported by Oyeleke and Istifanus (2008). In the same vein, Idise and Owhe-Ureghe (2015) also isolated *Bacillus sp.*, *Staphylococcus sp.*, *Micrococcus sp.* and other bacteria from different locations along River Ethiopie.

Bacillus species seem to be the commonest bacteria present in rivers or other surface water. This may be due to their being a normal

microbial flora of soil and so must be present in water as a result of soil runoff. Recent research (Larrea-Murrell *et al.*, 2018) suggests that the genotypic and phenotypic diversity among *Bacillus* spp. strains isolated from a polluted Almendares River in Cuba. Previous report by Østensvik *et al.* (2004) revealed that some *Bacillus* species are cytotoxic. Their presence in water poses a great threat to human and animal health when consumed or used for other domestic purposes. *Bacillus* species are implicated in food poisoning, ocular infections, wound infections and a host of others (Tuazon, 2000). *Staphylococcus aureus* is known to cause food poisoning. In their research, Hennekinne *et al.* (2012) investigated the food poisoning toxins of *S. aureus*. The role of water activity in the growth of *S. aureus* and production of its toxins cannot be overemphasized. Previous report (Qi and Miller, 2000) shows that *Staphylococcus* Enterotoxin A and *Staphylococcus* Enterotoxin D production occurs under nearly all water activity conditions allowing growth of *S. aureus* as long as all other conditions are optimal.

Escherichia coli and *Enterobacter sp* were the coliforms isolated from the water samples from River Ethiope. They indicate recent faecal contamination of the water and pose a great health risk to those who consume the water. The World Health organization (1997) has recommended that ideally, drinking water should not contain any pathogenic microorganism or any bacteria indicative of faecal contamination. *E. coli* and *Enterobacter sp* are implicated in food poisoning, urinary tract infections, pneumonia and septicaemia.

Conclusion

This study has revealed low alkalinity in samples of water from River Ethiope, the source of domestic water for most farmers in Abraka. This water may need to undergo treatment for alkalinity for it to be used for some industrial purposes. Furthermore, a number of bacteria were isolated, which when consumed may result in waterborne diseases. This calls for disinfection of the water prior to consumption by individuals.

Recommendation

It is recommended that people of Abraka are educated on the danger of passing human and

animal wastes in River Ethiope. Farmers and indigenes should be educated on the risks involved when water from River Ethiope is consumed and encouraged to boil the water before consumption. Bacteria isolated from this water body may be studied to molecular level in order to ascertain the extent of their toxicity to humans and animals.

Acknowledgement

We appreciate the contributions of Mr. M. Oghenejobo and Mr E. O. Uvieghara, Technologists in the Departments of Pharmaceutical Microbiology and Pharmaceutical Chemistry respectively, Faculty of Pharmacy, Delta State University, Abraka.

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Citation: Akpo, C.O. and Mukoro, J. Bacteriological and Physiochemical Analysis of Water from River Ethiopia, Abraka. *Sokoto Journal of Medical Laboratory Science*; 5(1): 114 - 120.

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