



Bacteriological and Physicochemical Quality Assessment of a Segment of Asa River Water, Ilorin, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FAJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OMK read the protocol, managed the analyses of the study and approved the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The existence of all living organisms depends on water resource which is continually polluted, and is therefore of public health importance. This study investigated river water samples for physicochemical and bacteriological quality of post-office segment of Asa river in Ilorin using standard procedures and the isolates were also identified with standard methods. Eight selected antibiotics used were in this study to determine the trend of susceptibility of the microorganisms to some of the antibiotics. The values recorded for physicochemical parameters of the water samples were within the limits of WHO standard for safe drinking water. The temperature of the water samples ranged between 21.0 and 28.4°C while water pH ranged from 7.1 to 7.5. The total heterotrophic count values ranged between 1.2×10^4 and 7.8×10^4 cfu/ml, total coliform count values ranged between 4.0×10^2 and 1.0×10^4 cfu/100ml, total fecal count values were between 0 and

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5.9×10^3 cfu/100ml some of which were found to be higher than the WHO stipulated values prescribed for river water. Large amount of the bacteria isolated from this water indicated that the water source is a reservoir for many waterborne pathogens; this includes *Escherichia coli*, *Enterococcus faecalis*, *Salmonella* sp., *Shigella* sp., *Pseudomonas aeruginosa*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus* sp. Approximately 75% of the isolates were sensitive to ofloxacin and 50% of the isolates were sensitive to gentamicin, while the entire organisms were resistant to augmentin, cefuroxime, ceftazidime, ceftriaxone. It was inferred that, the selected segment of the river analyzed revealed high levels of pollution with antibiotic resistant organisms hence not suitable for human consumption without implementing appropriate purification and treatment regimen.

Keywords: Colony forming unit; total coliform count; fecal coliform count; Asa River.

1. INTRODUCTION

The survival and sustenance of all the biological entities in the earth crust might not be possible without availability of quality and accessible water but unfortunately only few percentage (0.3%) is utilizable by man [1]. As part of the significance of water in supporting the ecosystem functionality are nourishing and supporting the growth of plant, animal and microorganisms both in terrestrial and aquatic environment [2]. Likewise, water plays an important role for the sound health of every human being and human existence depends on adequate availability of water in terms of quality and quantity. However, due to increase in population and urbanization consequently, there had been more pressure on the availability of portable water for industrial, recreational and domestic activities as result of incessant pollution of the water system which continually threatened this valuable resource [1].

The potable water provision to the urban and rural populace is necessary in prevention of health-related issues. A potable water is the water devoid microbial agents such as bacteria and viruses, and toxic chemicals which adversely affect human-health [3]. Consumption of unsafe drinking water and microbial contamination results in water-related diseases affecting a relatively large percentage of the population in underdeveloped countries. According to WHO, unsafe water kills more than five million people annually as a result of contaminated drinking water systems. In most developing world including Nigeria, surface waters serve as one of the main sources of drinking water, irrigation of farms as well as of disposal waste by the populace which may result in sudden outbreak of diseases and later death.

Due to prevailing environmental conditions and anthropogenic activities, safe and clean water only exists briefly in nature in most region of the

world including Nigeria, as the surface water is continually polluted by large quantity of effluent discharged to the water body and provision of which is a major challenge. Therefore, some kind of water treatment should be given to water from these polluted sources to avert adverse health risk of the consumer of surface water and the aquatic ecosystem [4].

According to National Bureau of Statistics as reported by Adesakin et al. [1], more than 25% of rural occupants in north central of Nigeria such as Kwara state rely solely on surface waters such as rivers, streams, rainwater and dams as well as ground water as major sources for domestic purposes due to non availability of potable water.

In most countries of the world, the major risk to human health is associated with the consumption of polluted water that are of microbiological in nature as water serve as reservoir for many of these waterborne pathogens such as bacteria, viruses and protozoa [5]. In Nigeria, the increase in the trend of surface water pollution as becomes a matter of great concern. According to WHO as reported by Khan et al. [6] up to 80% of illnesses are waterborne and are caused by consumption of unclean water and almost 3.1% mortality arise due to unsafe and deteriorating water quality [7]. Hence, the need for effective monitoring of river water in terms of physicochemical and microbiological parameters, this is crucial in prevention of river pollution [8,9]. Therefore, the bacteriological and physicochemical examination of this water source is important in pollution studies as a measurement of damaging effect on the health of the consumers and aquatic ecosystem.

2. MATERIALS AND METHODS

Study area and Water sampling: This study was conducted on a segment of Asa river; post-office

(8°29'18.0"N 4°33' 39.7"E) within Ilorin, Kwara state. The study area (Asa river) is one of the major rivers that runs through the township of Ilorin (Nigeria). The studied site receives effluent from different sources such as agricultural fields as well as domestic wastes along the bank of the river.

Sampling of water was done weekly for a period of eight weeks on Asa River in Ilorin, Nigeria. The inhabitants of this area rely absolutely on water from the streams, rivers, dams and ground water for irrigation and domestic uses due to scarcity of water in this location. Water samples were collected into pre-sterilized bottles, kept on ice-box, transported immediately to the laboratory and analyzed within 3 hours for both physicochemical and bacteriological analyses.

2.1 Physicochemical Parameters

The water samples collected were based on the following parameters; temperature, turbidity, pH, total dissolved solids, total suspended solids and total solids.

Temperature of the water samples were determined on-site using mercury thermometer. Total suspended solids and total dissolved solids were analyzed by filtration techniques [8,9].

2.2 Isolation and Identification

Water samples were serially diluted and inoculated on Nutrient agar, MacConkey agar and Eosin methylene blue agar and incubated at 37°C for 18–24 hours, this represent total bacterial count, total coliform count and fecal coliform count respectively and, reflect the general hygiene condition of the samples. Pure cultures of the recovered bacterial isolates were characterized and identified using standard methods.

2.3 Antibiotic Susceptibility Test

Antibiotic susceptibility testing was done on young culture of 16-18 hours. This was spread evenly on an already solidified Mueller Hinton Agar and was allowed to dry. Ofloxacin (OFL): 5µg, Gentamycin (GEN): 10µg, Ciprofloxacin (CPR): 10µg, Augmentin (AUG): 30µg, Nitrofurantoin (NIT): 20µg, Ceftazidime (CAZ): 20µg, Cefuroxime (CRX): 30µg disc were placed and pressed firmly on the medium. The plates were then, incubated at 37°C for 18-24 hours. Zone of inhibitions (mm) were measured and recorded accordingly [10].

2.4 Statistical Analyses

One way Analysis of variance (ANOVA) was used for data collected to determine the bacterial and physicochemical variations for the period of sampling using SSPS version 22.

3. RESULTS

The results obtained from the physicochemical characteristics of the analyzed water samples are presented in Table 1. The data show that all the tested parameters are within the WHO standard for drinking water. Water temperature ranged from 21.0 – 28.4°C. The colour of all the samples was yellowish- brown (amber). The pH values of samples were between 7.1 – 7.5. Total suspended, total dissolved and total solids ranged between 0.120 -1.340, 0.160 – 3.100 and 0.39 - 4.44 respectively all measured in mg/L.

Table 2 shows the bacterial load of the water samples analyzed in term of total bacterial count (1.4×10^3 - 7.8×10^4 cfu/ml), total coliform counts (4.0×10^2 - 1.0×10^4 cfu/100ml) and total fecal counts (0 – 2.5×10^3 cfu/100ml). The results obtained indicated that all the water samples collected for all the weeks exceeded WHO limits of microbial quality of drinking water except in week 5 for total fecal coliform count where 0 cfu/100ml was obtained.

The morphological and biochemical characteristics of the isolated bacteria from water samples are shown in Table 3. All isolated bacterial species were identified using standard methods (Bergey's manual of bacteriology). Eight different bacterial species were obtained of which five of them is Gram negative and the rest are Gram positive bacteria.

Frequency occurrence of the identified bacteria is presented in Fig. 1. From the result, it was observed that *Pseudomonas aeruginosa* has the highest frequency of occurrence 5(9%), *Salmonella* spp. and *Shigella* spp. have the same number of occurrence 6(11%), *Escherichia coli* 7(13%) while *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus* spp and *Klebsiella* spp also have the same number of occurrence 8 (14%) respectively.

Antibiotic susceptibility testing is presented in Table 4 using agar diffusion methods. Eight different types of antibiotic discs were used; gentamicin, cefuroxime, ceftazidime, ceftriaxone, erythromycin, cloxacilin, ofloxacin and

Table 1. Physicochemical parameters of water samples from Post-office segment of Asa River for eight weeks

Period (Weeks)	Temperature (C)	Colour	pH	Total suspended solids (mg/L)	Total dissolved solids (mg/L)	Totalsolids (mg/L)	Turbidity (NTU)
1	27.0	Amber	7.2	0.920	0.160	1.08	0.335
2	21.5	Amber	7.4	0.740	1.360	2.10	0.327
3	23.7	Amber	7.3	0.120	0.279	0.39	0.328
4	21.0	Amber	7.5	0.900	1.760	2.66	0.331
5	24.2	Amber	7.4	1.240	0.399	1.64	0.351
6	28.4	Amber	7.2	0.300	0.899	1.19	0.363
7	25.7	Amber	7.5	0.700	2.040	2.74	0.349
8	23.0	Amber	7.1	1.340	3.100	4.44	0.376

WHO 2011 Standard: pH - 6.5 to 8.5; Turbidity - 5 NTU; TSS - 500mg/l Values represent means of triplicates

Table 2. Total bacterial counts of the water samples from Post-office segment of Asa River for eight weeks

Weeks	Total bacterial count (cfu/ml)	Total coliform count (cfu/100ml)	Total fecal coliform count (cfu/100ml)
1	7.7×10^4	7.9×10^3	1.7×10^3
2	7.8×10^4	7.5×10^3	1.9×10^3
3	1.5×10^4	1.0×10^4	2.5×10^3
4	2.2×10^4	3.5×10^3	1.1×10^3
5	5.0×10^3	4.0×10^2	0
6	1.7×10^3	1.3×10^3	1.2×10^3
7	1.2×10^4	1.8×10^3	1.5×10^3
8	1.4×10^3	1.2×10^3	1.0×10^3

Values represent means of triplicates

Table 3. Biochemical tests carried out on pure isolates from the water samples for eight weeks

Isolates	1	2	3	4	5	6	7	8
Shape	Irregular	Irregular	Irregular	Circular	Irregular	Circular	Circular	Circular
Elevation	Raised	Umbonate	Flat	Convex	Flat	Umbonate	Raised	Convex
Margin	Curled	Filiform	Entire	Entire	Undulate	Entire	Entire	Entire
Surface	Wavy	Rough	Wavy	Smooth	Glistening	Smooth	Wavy	Smooth
Colour	Green	Black	Cream	Pink	Greenish metallic sheen	Golden yellow	Cream	Pink
Grams reaction	- rods	- rods	-rods	- rods	- rods	+ cocci	+ cocci	+ve cocci
Citrate	+	+	-	+	-	+	+	-ve
Indole	-	-	+	-	+	-	-	-ve
Coagulase	-	-	+	+	+	+	-	-ve
Oxidase	+	-	-	-	-	-	-	-ve
Catalase	+	+	+	+	+	+	-	-ve
Methyl red	-	+	+	-	+	+	+	-ve
Voges	-	-	-	+	-	+	-	+ve
proskauer								
Spore staining	-	-	-	-	-	-	-	-ve
Urease	-	-	-	+	-	+	-	-ve
TSI	-	+	+	+	+	-	-	-ve
Capsule staining	-	-	-	+	-	-	+	+ve
Glucose	-	+	+	+	+	+	+	+ve
Sucrose	-	-	-	+	+	+	+	+ve
Maltose	-	+	+	+	+	-	+	+ve
Fructose	-	-	-	-	-	+	+	+ve
Lactose	-	-	-	+	+	+	+	+ve
Galactose	-	-	+	-	-	+	+	-ve
Mannose	+	+	+	+	-	+	-	
Suspected organism	<i>P. aeruginosa</i>	<i>Salmonella</i> species	<i>Shigella</i> species	<i>Klebsiella</i> spp.	<i>E. coli</i>	<i>S. aureus</i>	<i>Streptococcus</i> Species	<i>Enterococcus faecalis</i>

KEY: -/-ve = negative, +/+ve = positive

Table 4. Antibiotic susceptibility test results for bacterial isolates from Asa river Post-office segment

Antibiotics	<i>P. aeruginosa</i>	<i>Salmonella</i> species	<i>Shigella</i> species	<i>E. coli</i>	<i>Klebsiella</i> species	<i>S. aureus</i>	<i>Streptococcus</i> sp.	<i>E. faecalis</i>
Gen 10µg	R	S	R	R	S	S	S	R
Crx 30µg	R	R	R	R	R	R	R	R
Caz 30µg	R	R	R	R	R	R	R	R
Ctr 30µg	R	R	R	R	R	R	R	R
Ery 5µg	R	R	R	R	I	R	S	R
Cxc 5µg	R	R	R	R	R	R	R	R
Ofl 5µg	S	R	S	S	S	S	S	R
Aug 30µg	R	R	R	R	R	R	R	R

Key: Gen = Gentamicin, Crx = Cefuroxime, Caz = Ceflazidime, Ctr = Ceftriaxone, Ery= Erythromycin, Cxc = Cloxacilin, Ofl=Ofloxacin, Aug = Augmentin, S= Susceptible, R = Resistance, I=Intermediate; Zone diameter interpretation : Resistance: 13mm; Intermediate: 14-16mm; Susceptible: 17mm or more

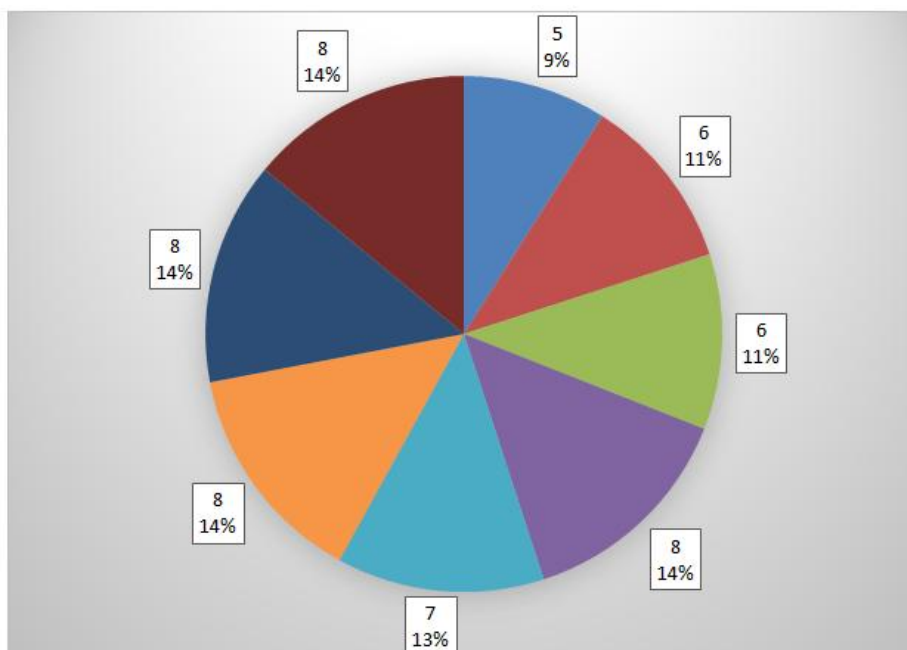


Fig. 1. Frequency of Occurrence of Isolated Bacteria

KEY:
■ *Pseudomonas aeruginosa* ■ *Shigella species*
■ *Salmonella species* ■ *Klebsiella species*
■ *Escherichia coli* ■ *Staphylococcus aureus*
■ *Streptococcus sp.* ■ *Enterococcus faecalis*

augmentin. From the study, it was observed that ofloxacin is the most effective against the test organism with 75% susceptibility while all the organisms are resistant to cefuroxime, ceftazidime, ceftriaxone and augmentin.

4. DISCUSSION

Water quality is defined by the measurement of several parameters, as it is not a stable condition of a system. There is a series of physical, chemical and biological components that affect water quality [11]. The results obtained in this study indicated that Asa river water samples are highly polluted with microorganisms. The physicochemical qualities of water reported in this study were linked with the standard of World Health Organization for drinking water guidelines and allowable limited is observed.

The physicochemical parameters of the water samples analyzed were presented in Table 1. Temperature values ranged between 21.0 and 28.4°C, this falls within the range of WHO standard [12]. The temperature at the various weeks can be attributed to the difference in the weather condition at the time of the sampling, the

range of the temperature observed, permits the growth of many of the bacteria isolated from the water samples. Temperature of water affect many activities in water body such as reduction in concentration of dissolved oxygen which in turn affect the taste and odour of the water, and microbial growth is also affected by change in temperature. This result is a bit lower compared to a study on Oyun river by Kolawole et al. [9]. This may be as a result of difference in weather condition and location of the river. All the colour of analyzed water is amber (yellowish-brown) which may as a result of dissolution of some materials in water body.

The pH recorded was within the WHO standard range, and pH values ranged between 7.1 and 7.5, which indicate variation of neutrality and it is the optimum pH range for proper plant growth and aquatic animals. The pH values recorded in this study falls within range reported for some rivers in Nigeria; for the same river by Ootobo, [13] (6.8-8.9), for Foma river by Agbabiaka and Oyeyiola, [14] (6.14- 7.97), as well as Mohammad et al.[9]. Though, values of the pH in surface waters may be associated with the type of soil surrounding the study area or probably the

kind of organic material brought into the river by the runoffs.

Turbidity values ranged between 0.327 and 0.376 NTU, due to the muddiness of the water which has been highly polluted, Turbidity rate of river is a product of suspended materials such as silt, clay, finely divided inorganic and organic matter, planktons and other microorganisms [15,16]. The mean turbidity values obtained in the current study was in contrary to the report of Amadi et al. [17].

The Total suspended solid ranged between 0.920-1.34 mg/l, total dissolved solids values ranged between 0.160-3.100 mg/L and total solids ranged between 0.39-4.44 mg/L. TSS and TDS of the sample analyzed are indicative of materials carried in solid/ suspension respectively, had value within the allowable limits for river water quality which is 500 mg/l for TSS [12]. TSS and TDS affect both the colour and the taste of the drinking water at high level beyond allowable limits.

The total bacterial count values ranged between 1.2×10^4 and 7.8×10^4 cfu/ml, total coliform count values ranged between 4.0×10^2 and 1.0×10^4 cfu/100ml and total fecal count values were between 0 and 5.9×10^3 cfu/100ml, which indicate the high level of fecal pollution of the water which potentially poses a high health risk for domestic use for human and animal and unfit for drinking without some form purification. A high total fecal count was obtained in all period except in week five. Microorganisms usually find their way in to the water body either through natural process such as flood water by rain fall as well as anthropogenic activities by direct or indirect disposal of domestic and industrial wastes. High level of coliform and fecal coliforms in the study site is an indication of the water is contaminated with potentially harmful organisms. In accordance to WHO standard, no coliform or fecal coliform must be present in 100 ml of drinking water. Since their presence is an indication of the presence of pathogens in water. Severe morbidity may results from consumption of these contaminated water source including, gastroenteritis, cholera, dysentery and typhoid.

Kolawole et al. [8] and Adesakin et al. [1] reported high number of total coliform and fecal coliform counts which is above the allowable limits of WHO standards in Asa and Samaru river respectively. The introduction of these organisms in water body may be through different activities

around the site; open defecation, runoffs of agricultural sites as well as dumping of waste in the river . The abundance of different types of bacterial species in the studied area identified by standard methods is an indication that the water is fecally polluted and possibly contains dangerous biological agents, hence, not fit for human consumption. A total number of eight bacterial isolates were obtained including; *Salmonella* sp., *Staphylococcus aureus*, *Klebsiella* sp., *Shigella* species, *Streptococcus* sp., *E. coli*, *Enterococcus faecalis* and *P. aeruginosa*.

Escherichia coli serve as model indicator organism for *Salmonella* sp. and indicator of recent fecal contamination. The presence of fecal coliform bacteria or *E. coli* indicates water contamination with fecal materials that may contain other harmful or disease causing organisms, such as bacteria, viruses or parasite such as *Gardia*. *E. coli* have been implicated in causing diarrhea, gastroenteritis and urinary tract infections. *Salmonella* sp., *Shigella* and *Klebsiella* are known pathogen of human that cause waterborne diseases such as enteric fever, bacillary dysentery and pneumonia respectively. *E. faecalis* is also recognized as bacterial indicator of recent fecal pollution of water body and their presence could be used to predict the presence of pathogens in water.

The percentage of the bacterial isolates were; *Pseudomonas aeruginosa* 9% has the least percentage, while *Salmonella* sp. and *Shigella* sp. have 11%, *Klebsiella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp. and *Enterococcus faecalis* have 14% and they have the highest bacteria frequency. High abundance of *Enterococcus faecalis* in the studied site is an indication of the presence of other bacterial pathogens as well as recent fecal contaminations of the surface water. Municipal and farmland runoffs may introduce some of these agents to the water body.

The sensitivity and resistance of the isolates against some antibiotics were enumerated; *Pseudomonas aeruginosa*, *Shigella* sp. and *Escherichia coli* were sensitive to only Ofloxacin (12.5% susceptibility) and resistance against Gentamicin, Erythromycin, Cefuroxime, Ceftazidime, ceftriazone, Cloxacilin, and Augmentin. *Salmonella* sp. was also susceptible to only Gentamicin (12.5% susceptibility) and resistance to other antibiotics. *Klebsiella* sp. and *Streptococcus* sp. were susceptible to

Gentamicin, Erythromycin and Ofloxacin (37.5% susceptibility) while *Staphylococcus aureus* was susceptible to Gentamicin and Ofloxacin (25% susceptibility) and *Enterococcus faecalis* was resistance to all the antibiotics used which makes the organism more dangerous to health. Seventy-five percent of the organisms were susceptible to Ofloxacin and it signifies the strongest antibiotics against most of the isolated bacteria. All isolated bacteria were resistant to cefuroxime, ceftazidime, ceftriaxone and augumentin which make them ineffective against the organisms. Most of the isolated bacteria from this water source are resistant two or more of the tested antibiotics; this indicates circulation of multi drug resistance bacteria in the river may results in possible health consequences for the users.

5. CONCLUSION

The result obtained in this study shows that the Asa river can cause serious problems to the settlers, both human and animals, since Asa river is the main source of water in Ilorin, there is need of proper care of the water, the river does not meet WHO specification, regarding human use and consumption, since high number of fecal contaminations were recorded.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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