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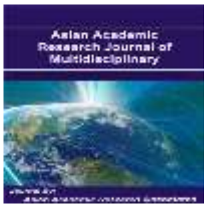

MICROBIOLOGICAL AND PHYSICOCHEMICAL EVALUATIONS OF SOME STREAMS ALONG ILISAN-AGO IWOYE ROAD, OGUN STATE, NIGERIA

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ABSTRACT

Microbial and physicochemical analyses were carried out on water samples collected from seven different streams from different communities along Ilisan, Ago-Iwoye Road, Ogun State, Nigeria, using standard procedures. The evaluated streams include Ome, Ogboni, Ogbe, Ona, Orisa, Oruken and Ayanyelu. The physicochemical parameters assayed for include temperature, pH, taste, appearance, odour, presence or absence of particles. Mannitol salt agar medium was used for the isolation of *Staphylococcus aureus*, while Salmonella-Shigella was used for isolation of *Salmonella* sp. The coliform test was employed to detect the faecal organisms while the Most Probable Number (MPN) was by the multiple tube fermentation technique. Oruken stream had the highest number of bacterial isolates with total count of 5.73 x 10^5 cfu/ml while Orisa stream had the lowest bacterial isolates with total count of 0.29 x 10^5 cfu/ml. The microorganisms isolated include *Escherichia coli, Proteus mirabilis, Bacillus firmus, Salmonella typhi and Staphylococcus aureus.* Most of the stream waters were unsafe for drinking as they were of low quality thresholds. Thus, the stream waters require further purification to ensure their suitability for human consumption.

Key words: Coliform bacteria, stream water, evaluation, microbes.

1.0 Introduction

Water fit for consumption is called drinking water or potable water. Sometimes, the term safe water is applied to potable water of a lower quality threshold (i.e., it is used effectively for nutrition in humans that have weak access to water cleaning processes, and does more good than harm)(Baffico, 2004; Brooks, 2006). Microbial indicators have been used worldwide to indicate if human wastes have contaminated a water body. Microbes typically utilized are those that are found in elevated concentrations in human faeces (Baffico, 2004). The typical indicators used include total coliform, faecal coliform, *Esherichia coli*, and enterococci (Brooks, 2006). Total and faecal coliforms were recommended by the US Environmental Protection Agency (EPA) in 1976. In 1986, the US EPA modified the guideline to specify the use of *E. coli* and enterococci as the indicators of choice. Many, however, have chosen to utilize the older US EPA guideline (USEPA, 2000).An additional indicator, *C. perfringens*, can be used for monitoring stream water quality (Fujioka and Shisumura, 1985). The choice of which indicator to utilize for monitoring recreational water bodies has been a source of considerable debate among the public health community. It has long been recognized that total coliform proliferate in nature (Mark, 1977). Data have also shown that faecal coliform, *E. coli*, and entorococci are found in the environment in the absence of a known sewage source of contamination (Wright, 1986; Rivera *et al.*,1988; Gücker *et al.*, 2006) and have been shown to multiply within warm tropical environments (Roll and Fujioka, 1997; Solo-Gabriele, 2000; Desmarais *et al.*, 2002). *C. perfringens* is not capable of re-growth in aerobic environments but persists for long periods of time and, its detection may not be an indicator of recent sewage contamination. Pollution of water resources by untreated or poorly treated domestic wastewater is an increasing concern for human health and for the negative effect on the worldwide environment. Aquatic microorganisms play a vital role in the cycling of nutrients within their environment, and thus are a crucial part of the food chain/web . Many microorganisms obtain their nutrition by breaking down organic matter in dead plants and animals. As a result of this process of decay, nutrients are released in a form usable by plants. These aquatic microorganisms are especially important in the cycling of the nutrients nitrogen**,** phosphorus , and carbon. Without this recycling, plants would have few, if any, organic nutrients to use for growth. The dramatic population growth is affecting the natural ecosystems, and the coral reefs are declining on a global scale and sewage pollution is a main factor (Paul *et al.*, 1997; Mckenna *et al.*, 2001; Sanchez-Gil *et al.*, 2004; Elmir *et al.*, 2007).

Concerns about the effect of farming and other anthropogenic activities on the quality of water supplies in the area and the potential hazards associated with the use of untreated stream waters by most rural areas prompted a survey of the microbial and physicochemical qualities of the stream waters in the communities within IIisan and Ago Iwoye, Ogun State, Nigeria which receives a fair amount of rainfall (750 – 1000 mm) annually. These microbe concentrations were also measured in support of a pilot-scale epidemiologic study designed to evaluate relationships between microbial water quality and human health (Crowther *et al.*, 2001; Byappanahalli *et al.,* 2003). Only the water quality data are presented in this current paper.

2.0 Materials and Methods

2.1 Study area

Ogun State is a state in western Nigeria. It borders Lagos State to the South, Oyo and Osun states to the North, Ondo State to the east and the republic of Benin to the west. Ilisan lies closely with Irolu and Ikenne. The old Ijebu Ode - Abeokuta road passed through Ilisan. The Sagamu - Benin Express road passes very closely by Ilisan in the outskirt South - Western side. The Sagamu - Benin Express road puts Ilisan on the left hand side. Ilisan is about ten kilometres to Sagamu. Ilara and Irolu are on the Northern side of Ilisan. Sagamu - Ago-Iwoye - Ijebu Igbo road passes through Ilisan. Irolu, Ilisan and Ikenne are bounded on the Western side by Odogbolu Local Government Area. Ago-Iwoye is a rural community in Ijebu North local government area of Ogun State. Geographically, it lies between latitudes 6^{o}56’N and 7^{o}00’N and longitudes 3^{o}54’E and 4^{o}00’E, with neighboring towns such as Ilisan Remo, Ijebu-Ode, Oru, Awa and Ijebu-Igbo. The drainage system in the area is provided by tributaries and sub tributaries derived from River Omi and River Osun, with an undulating topography and moderate relief. The study area is within the tropical region, usually marked with alternating wet and dry season with mean temperature of 30^{o}C and average humidity of 5% throughout the year. The mean monthly rainfall varies from 5mm in January to more than 200 mm in June and July. The relative humidity in lower amount of about 140 mm is recorded in August. On seasonal basis, the wind velocity is higher during the wet season, with a rapid change in percentage frequency distribution. The entire area is underlain by igneous and metamorphic rocks of the Older Granite Suite and Gneiss Complex respectively, which outcrop in most parts of the study area. Presence of groundwater in the area reflects a characteristic hydro-geological situation typical of the

Basement Complex Aquifer. This is governed by the extent of weathered overburden and presence of fractures and joints.

2.2 Collection of samples

Stream water samples were collected aseptically from seven different streams in different communities along Ilisan - Ago Iwoye road. The samples were collected in sterile container and analysed within 24 hrs.

2.3 Microbiological analysis of water samples

Water samples were serially diluted to obtain dilution factors of up to $10^{-6.}$ Plates were inoculated with 1 ml of dilution factors employing the spread plate technique. The media used for the bacteriological analysis of water were prepared according to the manufacturers' specifications. Mannitol salt agar medium was used for the isolation of *Staphylococcus aureus*, while Salmonella-Shigella was used for isolation of *Salmonella* sp. MacConkey broth was used for the determination of coliform counts while the multiple tubes method was used as the presumptive i.e. Most Probable Number (MPN). Discrete colonies, after 48 hrs of incubation at 37^{o}C, were sub-cultured to obtain pure cultures and were identified by biochemical procedures as described by Cowan and Steel (1985) and Klein and Bickmell (1995).

2.4 Physicochemical Parameters

The physicochemical parameters properties assayed for include temperature, pH, taste, appearance, odour and presence or absence of particles employing the methods described by Hewitt (2001) and Osho *et al.* (2010). The pH readings of the water samples were taken using pH meter Wag WT 3020. The pH meter was standardized with buffer 4, 7 and 9 before being used. Temperature of each sample was determined using mercury-bulb thermometer and this was recorded at the point of collection of sample before being transported to the laboratory. Both colour and turbidity of each of the samples were determined using WagWT3020 turbidimeter.

3.0 Results

Table 1: Distribution of bacteria in the different water sources

Isolates	Ome	Ogboni	Ogbe	Ona	Orisa	Oruken	Ayanyelu
Escherichia coli	+	-	-	-	-	+	-
Proteus mirabilis	-	+	-	-	-	+	-
Bacillus firmus	-	-	+	-	-	-	-
Salmonella typhi	-	-	+	-	-	-	-
Staphylococcus aureus	-	-	-	+	+	-	+
Total microbe per Sample	1	1	2	1	1	2	1

Key: - = Absent; + = Present

S. aureus occur in three of the seven streams under investigation indicating that it is the most predominant organism while each of *Bacillus firmus* and *Salmonella typhi* occurred in only one stream (Ogbe). *E. coli* and *Proteus mirabilis* occurred in two of the seven streams (Table 1).

Table 2: Physicochemical characteristic of the stream water samples

Sample source	Temperature (°C)	pH	Taste	Appearance	Odour	Presence or absence of particles
Ome	30.0	7.4	Tasteless	Light brown	Odour	Present
Ogboni	29.0	6.4	Tasteless	Colourless	No odour	Absent
Ogbe	29.5	6.2	Tasteless	Brown	No odour	Present
Ona	30.0	6.6	Tasteless	Creamy	No odour	Present
Orisa	30.0	6.1	Tasteless	Brown	No odour	Absent
Oruken	28.0	5.8	Tasteless	Colourless	No odour	Present
Ayanyelu	29.0	6.4	Tasteless	Colourless	No odour	Absent

The pH of the stream waters ranged from 5.8 to 7.4 while the temperature ranged from 28 to 30^0C. All the stream water samples were tasteless but with different colours except for Ogboni, Oruken and Ayanyelu Streams that were colourless. Ome stream water sample was found to give some foul smell (Table 2).

Table 3: The Most Probable Number (MPN) and Total Viable Count of the water sample

Sample source	Most Probable Number (MPN)	Coliform Count/ 100 ml of water	Total viable count Cfu/ml
Ome	3 - 3 – 0	240	2.99 x 10^5
Ogboni	3 – 0 – 2	64	0.98 x 10^5
Ogbe	3 – 0 – 1	39	0.49 x 10^5
Ona	3 – 2 – 2	210	2.62 x 10^5
Orisa	3 – 0 – 0	23	0.29 x 10^5
Oruken	3 – 3 – 3	460	5.73 x 10^5
Ayanyelu	3 – 2 – 2	210	2.62 x 10^5

Coliform counts ranged from 23 to 460 cfu/100 ml while total bacterial count ranged from 0.29 x10^5 to 5.73 x 10^5 cfu/ml (Table 3).

Table 4: Biochemical characteristics of the bacteria present in the stream samples

TEST	*E. coli*	*Proteus mirabilis*	*Bacillus firmus*	*Salmonella typhi*	*Staphylococcus aureus*
Gram stain	-	-	+	-	+
Shape	Rod	Cocco bacilli	Rod	Rod	Cocci
Catalase test	+	+	+	+	+
Indole test	+	+	-	+	-
Methyl red test	+	+	-	-	-
Voges proskauers test	-	-	+	+	+
Citrate utilization test	-	-	-	+	-
Motility test	-	+	+	-	-
Glucose fermentation	AG	AG	A+	AG	AG
Fructose fermentation	AG	AG	A	NA	NA
Lactose Fermentation	AG	NA	A+	NA	AG
Sucrose fermentation	AG	NA	A+	NA	AG
Maltose fermentation	AG	AG	A	NA	NA

KEY: AG = Acid and Gas; A+ = Weak acid; A= Acid; + = positive; - = Negative; NA = No action

Five genera of bacteria were identified in the stream waters sampled and these include *E. coli, P. mirabilis, B. firmus, S. typhi* and *S. aureus* (Table 4).

4.0 Discussion and Conclusion

Qualitative analysis of the water samples confirmed the presence of *E. coli, P. mirabilis, B. firmus, S. typhi* and *S. aureus* (Table 1). *E. coli* occurred in Ome and Oruken samples while *P.mirabilis* was found in Ogboni and Oruken samples. *B. firmus* was found in Ogbe sample only . *S. typhi* was found in Ogbe sample and *S. aureus* occurred in three stream samples, namely, Ona, Orisa and Ayanyelu samples (Table 1). The pH range of the streams varied from 5.8 in Orunken to 7.4 in Ome samples. The temperature ranged from 28^{o}C in Orunken to 30^{0}C in Ome, Ona and Orisa samples. All the stream samples were tasteless and odourless, except Ome sample that had an irritating smell. The physicochemical parameters' values were more uniform. However, the pH of the streams showed considerable variations of $\pm$2, while also exhibited different appearances in nature (Table 2). The most probable number (MPN) Technique was employed to determine the coliform count. The range in microbial indicator numbers were considerably large and the concentrations varied by orders-of-magnitude from one stream to the other. Orisa stream had 23 coliform count/100 ml of water, being the smallest while Oruken stream possessed the highest number of coliform count with 460 coliform count/100 ml of water. The total viable count revealed that Oruken stream had the highest total count of 5.73 x 10^{5} cfu/ml while Orisa stream had the lowest total count of 0.29 x 10^{5} cfu/ml. This indicated that Orisa stream is the safest for consumption among all other stream waters investigated in this study (Table 3). The biochemical characterization of the bacterial isolates revealed the presence of five genera of bacteria namely: *E. coli, P. mirabilis, B. firmus, S. typhi* and *S. aureus* in Ome, Ogboni, Ogbe, Ona, Orisa, Oruken, and Ayanyelu streams (Table 4).

The results in some points of the streams show coliform densities higher than recommended guidelines for recreational waters (<200 MPN/100 ml). This rendered four out of the seven evaluated streams grossly unsuitable for human consumption. Likewise, studies conducted by Fujioka *et al*. (1999), and Toranzos and Marcos (2000), have shown that in the absence of any known sources of human/animal waste, enterococci and *E. coli* are consistently present and recovered in high concentrations in the subtropical environment. The isolated bacterial species in this study have been identified to be the same with those commonly encountered in water and aquatic environments as was also reported in a study on streams surface water in Wyoming in U.S.A. as reviewed by Banwo (2006). Different types of pollution characterize the different stream waters in the different communities. Variations in water quality will increase the stress on available water resources and valuable ecosystems, especially the adjacent coral reef of the evaluated streams. Geologically, the communities whose streams were evaluated are characterized by karst aquifers with fractures and groundwater streams, therefore contaminants can be transported long distances with little dilution. As a consequence, water quality can deteriorate severely, which can result in very high economic and social costs in order to clean the polluted sites and restore the ecosystem (Nicod 1991; Parise and Pascali, 2003). According to Doerfliger *et al*. (1999), karst environments are highly vulnerable to a variety of degradation and pollution problems. Similar bacterial profile was described by Ibe and Okplenye (2005) and Bello *et al.* (2013) where *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Staphylococcus aureus* were isolated from borehole water samples. Streams generally transport three types of material: bed load (pebbles and sand which move along the stream bed without being permanently suspended in the flowing water), suspended load (silts and clays in suspension) and dissolved load (material in solution). The absolute quantities and the relative proportions of these types of stream load vary from one stream to another, and within a single stream from one time to another. Human activity adds large amounts of dissolved and solid material to streams. These added materials include fertilizers, animal waste, and soluble compounds that are the by-products of agriculture, forestry, and industry. Agriculture and forestry also add large amounts of solid sediment into streams because of their disturbance of vegetated surfaces.

Conclusively, the microbial qualities of the evaluated stream waters were averagely poor which may be due to direct contamination by animal and human excreta and other activities such as swimming, washing of clothes, etc., and thus, require further purification to ensure their suitability for human consumption. This research work showed that most stream waters are not safe for human consumption as they are of low quality thresholds. The results of this monitoring will provide baseline data to propose and/or improve protection, conservation measures as well as suggest further research on human health in the area.

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