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Full Length Research Paper

Study of the bacteriological and physicochemical indicators of pollution of surface waters in Zaria, Nigeria

Vincent N. Agbogu[1], Veronica J. Umoh[1]*, Charles A. Okuofu[2], Stella I. Smith[3] and Joseph B. Ameh[1]

[1]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria, Nigeria.
[3]Genetics Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.

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This study investigates the pollution level of surface waters in Zaria, Nigeria. The bacteriological and physicochemical analyses performed were in accordance with standard procedures. Out of 228 samples from different sites, 128 (56.1%) had counts higher than the standards. Samaru stream was the most polluted. The frequency of contamination of samples with *Escherichia coli* O157 was only 2.2%. There was a positive correlation between faecal coliform count with most of the physicochemical parameters. The use of the surface waters as raw water for drinking, irrigation of food crops for raw consumption and for recreational activities may be hazardous. The study therefore, stresses on the need to control the faecal pollution of the bodies of water.

Key word: Surface waters, indicators of pollution, *Escherichia coli* O157.

INTRODUCTION

Non-pathogenic bacteria present in faeces are used to indicate the occurrence of faecal contamination of water and hence the possibility that pathogens may be present. The most frequently used indicator organisms are faecal coliforms, faecal streptococci, and *Clostridium perfringens*. Routine monitoring of water samples also involves screening for the presence of the waterborne parasite *Cryptosporidium parvum* (Fricker, 2000; Gale, 2001). However, in all cases faecal coliform counts and *Escherichia coli* detection in particular, remains the major and most reliable tool in the assessment of the health risks posed by pathogens in water (Byamukama et al., 2000).

Exposure to contaminated water through ingestion as drinking water, recreation or irrigation is a significant mode of transmission of gastrointestinal infections.

Outbreaks have been associated with swimming in a crowded lake (Ackman et al., 1997), contaminated drinking water (CDC, 1999; Olsen et al., 2002) as well as surface water (Effler et al., 2001). Other modes of transmission include human-to-human contact, contact with inanimate surfaces and with animals or their faeces (Payment and Riley, 2002; Pruss et al., 2003).

The major sources of contamination of surface water are urban and farm runoffs, discharges from sewage treatment facilities, failing septic systems, wildlife, farm animals and direct faecal contamination by humans and animals (Payment and Riley, 2002; Okafo et al., 2003). This study is significant because it is common practice in Samaru village to discharge sewage directly from residential houses into gutters and drains and thus into the stream. This study is therefore aimed at studying the indicators of pollution in surface waters, the suitability of the water for herd watering, irrigation and recreational activities and to isolate and characterize enteropathogenic *E. coli* from the surface waters and diarrhoeal stool of children in the study area.

*Corresponding author. E-mail: veroumoh@yahoo.com. Tel: 2348036053022.

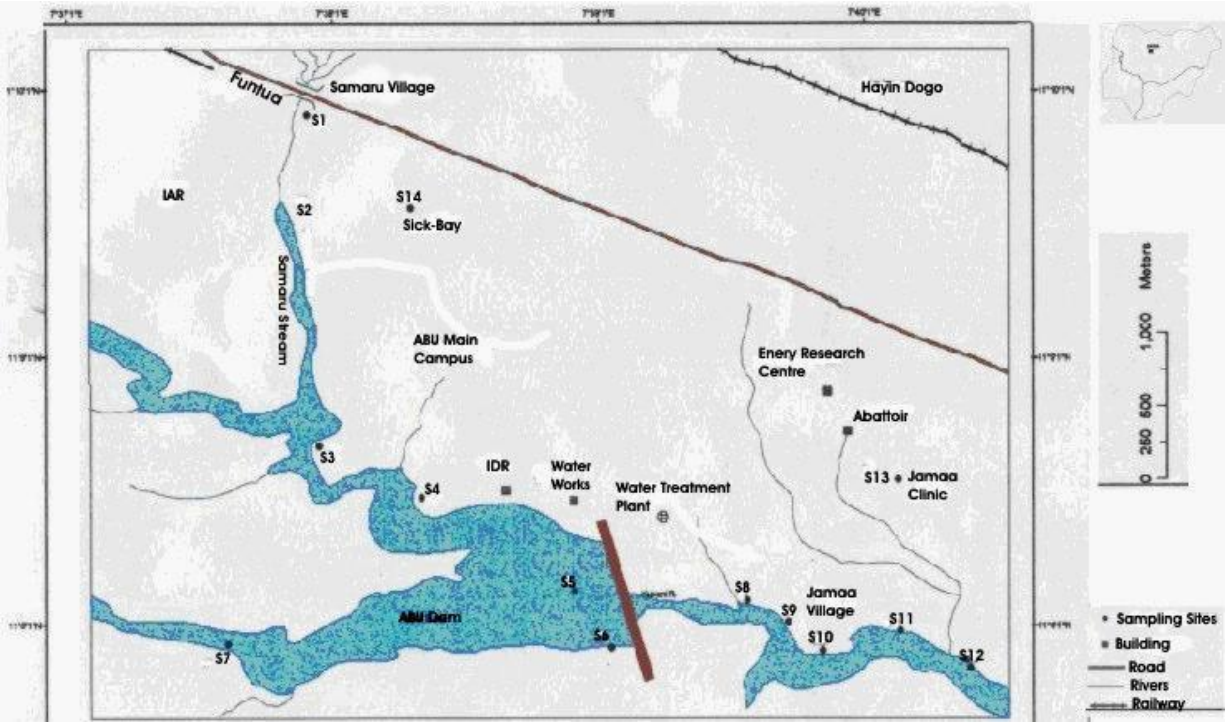

Figure 1. The study area and sampling sites, Zaria, Nigeria.

METHODOLOGY

Study area

The study area (Figure 1) is a suburban University settlement comprising of Samaru and Jama'a in Kaduna state of Nigeria. It is located within latitudes 11° 7', 11° 12' N and longitudes 07° 41' E. The entire area is highly influenced by the presence of the University. The population has increased from 20,000 in 1975 to about 100,000 in 1991 (1991 census) and even higher now. It is characterized by a tropical climate with two main reasons; a rainy season of about 210 days (May to October) and a dry/harmattan season (November to April). The monthly mean temperature records show a range from 13.8 to 36.7°C and an annual rainfall of 1092.8 mm.

Samaru stream discharges into the University dam which is the source of drinking water for the University community. Similarly Kubanni river discharges into Zaria dam the source of raw water for Zaria water works. Kubanni River is largely used by local farmers for the irrigation of commercial crops (tomatoes, lettuce, cabbage, onions, spinach, sugarcane etc) and Samaru stream for recreation, bathing and herd watering. The surface waters are sources of raw water treated for drinking.

Sampling sites

Twelve sampling sites were selected, three along Samaru stream (S1 – recreational site and point source contamination from domestic sewage draining from Samaru village, S2 – near student hostel septic tanks, S3 – site where Samaru stream meets with Kubanni River), four at the dam (S4 – site of anthropogenic influence, S5 – point of abstraction of raw water from the dam for purification, S6 – site at a shallow zone where herdsmen water their animals and S7 – point where another tributary empties into the dam), five sites along Kubanni river (S8 – site where University sewage treatment effluent enters Kubanni river, S9, S10 and S11 were 300 m intervals down stream from S8 and S12 – is located 10 m down stream, at the point where the gutter that drains effluent from Jama'a village and Abattoir meets the river) (Figure 1).

Sample collection

Sampling was according to the procedure recommended by American Public Health Association (APHA, 1992). A total of 228 water samples were collected; 58 from the stream, 74 from the dam and 96 from the river. All the samples for microbiological analysis were collected aseptically and transported in ice pack to the Department of Microbiology, Ahmadu Bello University, Zaria for microbiological analysis. About 2.5 litres of water was also collected in a clean plastic can and transported to the Public Health Laboratory for physicochemical analysis.

A total of 112 diarrhoeal and 11 non diarrhoeal stool specimens from age – matched children of age less than 15 years were collected in sterile screw-capped specimen containers. Specimens were transported in ice pack to laboratory for analysis within two hours of collection.

Bacteriological analysis

The Most Probable Number (MPN) technique was used to determine the faecal coliform counts of the water samples (APHA, 1992). This involved the presumptive test using MacConkey broth (Bioteck Suffolk, UK) with durham tube, confirmatory test using brilliant green lactose broth and completed test using Eosin Methylene Blue (EMB) agar (LAB M, Lancashires, UK). The tubes and plates were incubated at 44.5°C for 24 to 48 h. Gas and turbi-

Table 1. Range of counts, mean and median of faecal coliform counts at different sites.

Sources and Uses	Site	counts (MPN/100 ml)		
		Range	Mean	Median
Samaru Stream				
Recreation	S1	$1.6\times1^{5} - 1.6\times10^{6}$	7.8×10^{5a}	4.3×10^{5}
	S2	$3.0\times10^{4} - 3.5\times10^{5}$	1.2×10^{5b}	6.7×10^{4}
Herd watering	S3	$2.0\times10^{1} - 1.6\times10^{4}$	3.2×10^{3c}	4.0×10^{2}
Dam				
Fishing	S4	$4.0\times10^{1} - 3.5\times10^{3}$	7.8×10^{3a}	1.9×10^{3}
Raw water treated for drinking	S5	$0.2\times10^{1} - 1.7\times10^{2}$	4.9×10^{1b}	1.8×10^{1}
	S6	$6.0\times10^{1} - 2.2\times10^{4}$	3.3×10^{3a}	1.4×10^{3}
Herd watering	S7	$2.0\times10^{1} - 1.7\times10^{3}$	1.2×10^{3a}	2.7×10^{2}
Kubanni River				
Recreation	S8	$2.0\times10^{2} - 1.7\times10^{4}$	2.9×10^{4}	9.0×10^{3}
Irrigation	S9	$6.0\times10^{1} - 3.5\times10^{4}$	4.5×10^{3}	1.3×10^{3}
Herd watering	S10	$2.0\times10^{2} - 3.5\times10^{3}$	1.2×10^{3}	1.3×10^{3}
	S11	$4.0\times10^{2} - 2.6\times10^{3}$	1.7×10^{3}	3.3×10^{2}
Flows into Zaria Dam	S12	$2.0\times10^{2} - 1.6\times10^{5}$	3.0×10^{4}	1.1×10^{4}

*Means with different superscript are significantly different (P<0.05) using Duncan's Multiple Range Test.

dity in the tubes as well as metallic sheen or pink with dark centre colonies on EMB agar indicated positive. All isolates that produced gas at 44.5°C, stained Gram negative and were nonspore forming and rod-shaped were regarded as faecal coliform and the counts calculated from a standard probability table (APHA, 1992).

E. coli was isolated from water samples using the Tryptic Soya Broth (TSB) enrichment and high temperature (44.5°C) incubation methods described by LeJeune et al. (2001), followed by plating for isolation on EMB agar. Pure colonies of the isolates were subjected to motility-indole-urease test, methyl-red-voges-proskauer (MRVP) test, citrate utilization and Kligher's iron agar tests. All confirmed *E. coli* isolates from the biochemical tests were screened on sorbitol MacConkey (SMAC, OXOID, Basingstoke UK) agar plates. After incubation at 37°C for 24 h all non-sorbitol (colourless) colonies were recorded as presumptive aquatic *E. coli* O157 (Singleton, 1997).

Stool specimens were cultured for *E. coli* isolation on EMB agar and incubated at 44.5°C as described by Okeke et al. (2000). Pure colonies were confirmed as presumptive *E. coli* O157 as above. All sorbitol-negative colonies from SMAC agar were tested for agglutination with *E. coli* O157 antiserum (Robert Koch Institute, Burgstrasse, Germany) at the Nigerian Institute for Medical Research, Lagos, Nigeria. Appearance of a strong agglutination was reported as a positive result.

Physicochemical analysis

The physical parameters determined were temperature, pH, electrical conductivity, turbidity, total dissolved solids and total soluble solids using field kits as described in American Public Health Association (APHA, 1992). The chemical analyses performed include chlorides and ammonia-nitrogen using titration method, phosphorus and nitrate levels using colorimetric method and biochemical oxygen demand (BOD) by seeding and dilution methods (APHA, 1992).

Statistical analysis of data

The means of data obtained form counts and physicochemical analysis were compared using one way analysis of variance (ANOVA) and Duncan's multiple range tests. The coefficient of correlation between faecal coliform counts and the physicochemical parameters was calculated by the Pearson correlations test. Statistical significance was set at P<0.05 (Colton, 1974).

RESULTS

The highest faecal coliform count was recorded at sampling sites S1 and S2 (mean 105 FCC/100 ml) and S1 had significantly higher FCC than S2 (Table 1). All the samples obtained and from the two sites and 90% of the samples collected from site S12 were higher than 1000 MPN/100 ml, the limits for faecal coliform in surface waters used as raw water for drinking and for irrigation of food crops for raw consumption and for recreational activities. However, the samples obtained from site S5 (the abstraction point at the dam) had counts less than 200 MPN/100 ml (Table 2). On the whole Samaru stream was the most polluted, having faecal coliform counts of >1000MPN/100 ml in 82.8% (48/58) of the samples tested from the three sites. This was closely followed by Kubanni River with 45.8% (44/96) from the five sites and the dam with 31.1% (23/74) of the samples from four sites (Table 2).

A total of 228 samples of water were tested and 96 *E. coli* isolates confirmed. Out of the 96 isolates, 20 (8.8%)

Table 2. Counts of faecal coliforms at different sites falling into recommended standard limits.

Source	Site	No (%) of samples with counts (MPN/100 ml)			
		<200	200 – 1000	1000 – 2000	>2000
Samaru Stream					
	S1 (n = 20)	0(0.0)	0(0.0)	0(0.0)	20(100.0)
	S2 (n = 20	0(0.0)	0(0.0)	0(0.0)	20(100.0)
	S3 (n = 18)	5(27.8)	5(27.8)	2(11.1)	6(33.3)
Dam					
	S4 (n = 18)	1(5.6)	6(33.3)	4(22.2)	7(38.9)
	S5 (n = 20)	20(100.0)	0(0.0)	0(0.0)	0(0.0)
	S6 (n = 20)	6(30.0)	4(20.0)	4(20.0)	6(30.0)
	S7 (n = 16)	6(37.5)	8(50.0)	2(12.5)	0(0.0)
Kubanni River					
	S8 (n = 20)	0(0.0)	6(30.0)	3(15.0)	11(55.0)
	S9 (n = 20)	4(20.0)	5(25.0)	7(35.0)	4(20.0)
	S10 (n = 18)	3(16.7)	5(27.8)	6(33.0)	4(22.2)
	S11 (n = 18)	6(33.3)	8(44.5)	2(11.1)	2(11.1)
	S12 (n = 20)	0(0.0)	2(10.0)	4(20.0)	14(70.0)

n = Number of samples tested per site

Table 3. Distribution of *E. coli* isolates from the three sources of water.

Source of water	No. (%) of samples tested	No. of *E. coli* isolates	No of NS *E. coli*	No. (%) of *E. coli*
Samaru Stream	58(25.4)	`25	7	2
Dam	74(32.5)	26	7	6
Kubanni River	96(42.1)	45	6	6
Total	228(100.0)	96	20(20.8)*	14(14.6)*

No.(%) = Number and percent of samples tested.
*No(%) of the isolates.
NS = Non sorbitol *E. coli*.

were non-sorbitol fermenting *E. coli* (Table 3). Only 5 (2.2%) of the 228 surface water samples contained *E. coli* O157. The frequency of contamination of water samples with *E. coli* O157 were one out of 58 samples from Samaru stream, two out of 74 from the dam and two out of 96 from Kubani River. A total of 112 diarrhoeal and 11 non-diarrhoal stool specimens were collected and tested for the presence of *E. coli.* None of the 11 control stool specimens had non-sorbitol fermenting *E. coli*. However, of the 16 (18.2%) non-sorbitol fermenting *E. coli* from diarrhoeal specimens, 6 (6.8%) were confirmed as *E. coli* O157 (Table 4). Of these confirmed isolates two were from female and four from male children.

Table 5 shows the comparison of the quality of surface waters from the dam, Kubanni River and Samaru stream. The stream had significantly higher values of conductance total dissolved solids (TDS), total suspended solids (TSS), chloride, phosphate and biochemical oxygen demand (BOD) than the water samples from the dam and Kubanni River ($P < 0.05$) (Table 5). However, all the values obtained were below the standards (Tebbut, 1990; WHO, (1996). Results of the statistical analyses of possible relationships between the bacteriological and physiochemical parameters using Pearson correlation test revealed a positive correlation between faecal coliform count and each of conductance ($r = 0.55$), TDS ($r = 0.31$), TSS (0.27), chloride ($r = 0.51$), phosphate ($r = 0.30$), nitrate ($r = 0.25$) and BOD ($r = 0.51$).

DISCUSSION

The presence of faecal coliform is an index of the bacteriological quality of water. The European community and WHO limits for surface waters used as raw water for drinking is 200 MPN/100 ml and less than 1000MPN/100 ml for irrigation of food crops consumed raw (Tebbut, 1990; WHO, 1996). All the samples obtained from site S5, the abstraction point in the dam had counts less than 200 MPN/100 ml and could be said to meet the limits for raw water treated for drinking.

Table 4. Distribution of *E. coli* isolates from diarrhoeal stools of children from study area.

Source	No. (%) of stools collected			No. of *E .coli*	No. of non-sorbitol	No.(%) of *E.coli*
	M	F	Total	Isolate	*E.coli*	0157
Sick Bay	15	20	35(31.2)	30	6	1(2.9)
Jama'a Clinic	28	49	77(68.8)	58	10	5(2.7)
Total	43	69	112(110)	88	16(18.2)*	6(5.6)**

*No.(%) = Number and percent of samples tested.
**No.(%) = Number and percent of total isolates.

Table 5. Mean values of water quality parameters for water obtained from the dam, Kubanni River and Samaru stream.

Parameter	Dam n = 4 sites	Kubanni river n = 5 sites	Samaru stream n = 3 sites
FCC (MPN/100ml)	$3.1\times10^{3}\pm1.1\times10^{3}$[a]	$1.3\times10^{4}\pm4.9\times10^{3}$[a]	$3.0\times10^{5}\pm8.4\times10^{4}$[b]
WT (^{0}C)	25.83±0.72	25.71±0.62	25.12±0.76
pH	6.64±0.03	6.73±0.03	6.70±0.06
NTU	139.68±24.63	106.4±21.11	128.74±39.14
EC (µs/cm)	152.17±12.68[a]	224.08±9.38[b]	457.61±45.21[c]
TDS (mg/l)	22.75±2.45[a]	26.20±1.76[ab]	30.33±3.13
TSS	18.36±2.06[a]	18.80±1.45[a]	29.33±3.29[b]
Chloride (mg/l)	25.93±2.38[a]	29.02±2.12[a]	71.46±7.14[b]
PO_4 –P (mg/l)	0.16±0.02[a]	0.16±0.01[a]	0.23±0.02[b]
NO_3 – N (mg/l)	1.72±0.12[a]	1.86±0.09[ab]	2.19±0.18[b]
BOD_5 (mg/l)	1.23±0.13[a]	1.83±0.28[a]	5.33±0.73[b]

FCC – faecal coliform count; EC – Electrical conductivity; TDS – Total dissolved solids ; PO_4-P – Phosphate – phosphorus; BOD_5 – Biochemical Oxygen Demand; WT – water temperature; NTU – nephelometric turbidity units; TSS – Total suspended solids; NO_3-N – Nitrate – nitrogen

However, sites S1 to S4 and S6 to S12, the sources of raw water discharged into the dams had significantly higher counts. The high counts were attributed to contamination by domestic sewage, the waste stabilization pond, recreational activities in the water bodies, herd watering and drain from an abattoir. It is common to observe high organic matter in drinking water obtained from the dams after a heavy rain storm. Elsewhere, significant increases in organic and bacterial load after a rain storm from point sources have been linked to increase risk of infectious disease transmission (Kistemann et al., 2002). Another study revealed that higher bacterial concentrations were strongly associated with rainfall and sewage sources were linked to total coliform and faecal coliform (Crowther et al., 2001). The isolation of non-sorbitol *E. coli* confirmed by agglutination to be *E. coli* O157 is particularly important given the low infectious dose of this pathogen (Prince et al., 2000). The risk here is further amplified by the various uses of these surface waters which include recreational activities by children, domestic activities by villagers especially during the dry season, drinking water by the fishermen and for irrigation of crops that are sometimes consumed raw. Also the isolation of these organisms from diarrhoeal stools of children attending nearby clinics shows the epidemiological significance of the presence of such a pathogen in the surface waters and the environment. *E. coli* O157 has earlier been isolated from diarrhoeal stools in Southern Nigeria (Ogunsanya et al., 1994; Olorunshola et al., 2000).

Although the physical and chemical parameters were below recommended standard for each were positively correlated with the faecal coliform content of the surface waters, thereby confirming coutinuous pollution from both point and diffuse sources. Negative health effects have been detected in association with the use of raw or poorly treated waste water for irrigation, recreational activities and other purposes (Shuval 1990, Crowther et al., 2001).

In conclusion, compared to standards the surface waters studied could be regarded as physically and chemically acceptable but bacteriologically unsafe for use as raw water for drinking, animal herding, recreational activities and the irrigation of food crops to be consumed raw. There is need to control the faecal bacteria, the indicator for the faecal pollution of the water bodies. Provision of sewerage systems in Samaru village and waste stabilization pond may aid in the reduction of the pathogens before discharging into the stream. Improvement in water quality and availability will aid hygienic practices and interrupt the transmission of enteric pathogens through contaminated water in the study area.

Public health education aimed at improving personal, household and community hygiene is imperative.

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