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RESEARCH PAPER

BACTERIOLOGICAL POLLUTION INDICATORS IN OGUN RIVER FLOWING THROUGH ABEOKUTA METROPOLIS

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ABSTRACT
Water resources are significant part of integrated community development policy and good health. Hence, the need to reduce the impact of natural and anthropogenic pollution causes so as to enhance water quality. The bacteriological quality of the Ogun River was investigated to determine the sanitary conditions of the water body between March and August, 2014. Total heterotrophic bacteria counts (THBC), total coliform counts (TCC) and total Escherichia coli counts (TEC) using standard plate count and Most Probable Number (MPN) techniques were determined. The isolates were identified using 16SrRNA gene. Total heterotrophic bacteria counts varied between 1.13 × 10^6 and 4.1 × 10^7 CFU/ml, TCC ranged between 2.5 × 10^5 and 2.33 × 10^7 CFU/ml and TEC was between 5 × 10^4 and 1.3 × 10^6 CFU/ml. Most Probable Number of coliforms in all samples varied between 120 and 1600 MPN/100 ml. Isolated microorganisms include Escherichia coli strain SUS9EC, Escherichia coli O157:H7 strain SSI7, Escherichia coli strain BW25113, Escherichia coli strain C-X1B, and Klebsiella oxytoca strain KU-5. One-way analysis of variance showed significant difference within the samples at (P<0.05).The results revealed high bacteria counts which is higher than the recommended value of 1.2 × $10^2$for THBC, a zero E. coli count and not more than 10 coliforms per 100 ml by World Health Organization standards for drinking water.

Keywords: *Water, bacteria, Escherichia coli, pollution*

INTRODUCTION

Water is vital to our existence in life and its importance in our daily life makes it imperative that thorough microbiological and physico-chemical examinations be conducted on water (Shittu *et al.*, 2008). The quality of water influences the health status of any population, hence, analysis of physical, biological and chemical properties are very important for public health studies (Shalom *et al*., 2011).

The health of a population, depends on the

quality of water within their domain, which is of great environmental concern (Taiwo *et al.*, 2014). The most important characteristic of good quality water is obviously absence of pathogenic organisms (Richard *et al*., 1977). According to Nouri *et al.* (2008) water quality is determined by both natural and anthropogenic forces. The natural forces include precipitation rate, weathering process and soil erosion, while the anthropogenic forces are urban and industrial activities such as domestic, municipal and agricultural wastes.

Water contamination is the introduction or release into water organisms or toxic substances that render it unfit for human consumption or domestic use (Olabisi *et al*., 2008). Water bodies polluted by faecal discharge from man and other animals may transport a variety of human pathogens. These agents include pathogenic bacteria viruses, protozoa and several more complex multicellular organisms that can cause gastro-intestinal illness. Other organisms are opportunistic in nature infecting susceptible individuals through body contact with contaminated film or by inhalation of poor quality water droplets in aerosols of various origins (Meybeck *et al*., 1989).

Nutrient loading has been a major threat to the stability of fresh water worldwide, with an expected tendency to worsen in the future (Olayinka *et al*., 2013). Despite concerted efforts at reducing the anthropogenic impacts, many water resources such as rivers and streams are still faced with the problem of pollution and in many instances rendered useless for drinking, fishing and other uses (St Laurent and Mazumder, 2012). The discharge of effluent and wastewater into rivers, lakes, estuaries and the sea is of great concern in most developing countries (Danazumi and Bichi, 2010; Bao *et al*., 2013). Globally, rivers and streams are increasingly under pressure due to direct human use and pollution that often overwhelm the capacity of rivers to assimilate these water bodies (Olayinka *et al*., 2013). Drinking of water contaminated with pathogens has manifested in diseases like typhoid fever, amoebic dysentery, cholera and so on, which has resulted in the deterioration of health and in some cases death (Isikwue *et al*., 2011). The bacterial qualities of groundwater, pipe borne water and other natural water supplies in Nigeria have been reported to be unsatisfactory with coliform counts far exceeding the level recommended by WHO (Dada *et al*., 1990; Edema *et al*, 2001).

Escherichia coli has been recognized as the most appropriate bacteriological indicator of water pollution by human excrement, and the most probable number (MPN) of coliform group has been universally used as an indicator (Godd, 1998). The World Health Organization suggests a zero *E. coli* count as an appropriate standard (Richard *et al*., 1977). Therefore by determining the level of bacteria pollution and identifying the bacteria present in the Ogun River in Abeokuta can be used to predict the health hazards associated with the use of such waters.

MATERIALS AND METHODS

Study Area

The study areas were the Federal University of Agriculture, Abeokuta (FUNAAB) reservoir, which served as a control sample, (latitude 7^{o} 13′N and longitude 3^{o} 02′ E), the Ogun River at Lafenwa (latitude 7^{o} 09.7338′ N and longitude 3^{o} 19.7344′ E) and Adigbe (latitude 7^{o} 09.7144′ N and longitude 3^{o} 19.6904′ E). All these locations were within Abeokuta, Ogun State (see fig 1). Abeokuta has a prevailing tropical climate with mean annual rainfall and temperature of about 1,270 mm and 28^{o}C respectively (Olabisi *et al*., 2008).

Sample collection

Water samples were collected in sterile one litre plastic bottles. Sampling was done in the middle of the stream by dipping each sampling bottle at approximately 10 - 20 cm below the water surface, projecting the mouth of container upstream against the flow direction. The bottle was opened to allow the container to fill up. The sampled bottles were then covered with the caps under water. The samples were

collected in triplicates twice in a month for six months (March to August, 2014), and transported to the laboratory on ice packs where the bacteriological analysis were carried out.

Isolation and characterization of bacteria

Total heterotrophic bacterial counts

Enumeration of heterotrophic bacteria was carried out as described by APHA (1998). Plate count agar (Lab M, UK) was used for the enumeration of THBC and was prepared following the manufacturer's instruction. Ten-fold serial dilution was carried out and 1 ml of each of dilution factors 10^{-3} and 10^{-5} were plated out using pour plate method. The plates were incubated at 37 ^{o}C for 24 h and the THBC of the samples was recorded.

Total coliform bacterial counts

Enumeration of total coliform counts was carried out on Eosine methylene blue agar (Lab M, UK). Ten-fold serial dilution was carried out and 1 ml of each of the dilution factors 10^{-3} and 10^{-5} were plated out using pour plate method. The plates were incubated at 37 ^{o}C for 24 h and the TCC of the samples was recorded. Colonies showing typical *E. coli* characteristics (green metallic sheen) were also counted for total *E. coli* counts.

Most probable number method

The most probable number method (MPN) was carried out as described by Richter *et al*. (1992) using lactose broth as a medium of growth. Serial dilution was carried out on the water samples, 10 mL of the sample was put into tubes (3) each with double strength. One milliliter was put into each single strength tubes (3), and 0.1 ml sample was put into each one of the other three tubes.

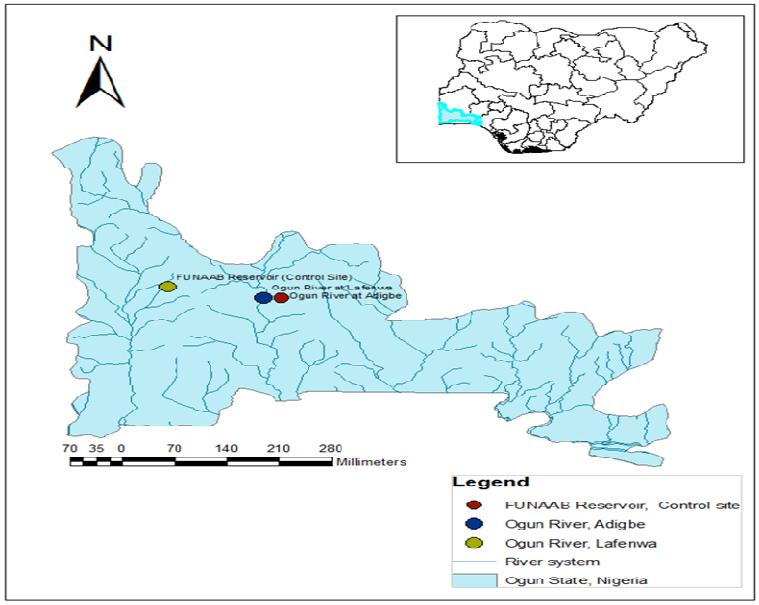

Fig. 1: Map showing the study area locations

The tubes were incubated at 37 ^{o}C and those, that showed gas production in Durham tubes after 24h were presumptively positive for coliforms. The negative tubes were further incubated for another 24h. Aliquot of 0.1 ml of each of the gas positive tubes were transferred to a separate tube and incubated at 37 ^{o}C for 48h. The formation of gas confirms the presence of coliform bacteria. Coliform counts were determined using the MPN index (Veissman and Hammer, 1993).

Isolates were identified using their Gram's reaction, indole production, methyl red, Voges Proskauer, oxidase, catalase, urease, coagulase, citrate utilization, sugar fermentation, motility, lactose, mannitol, and hydrogen sulfide production using Bergey's manual of determinative bacteriology (Holt *et al.*,1994).

DNA isolation and sequencing

DNA isolation was carried out using the Norgen DNA isolation kit (Norgen Biotek Corporation Canada). One millitre of bacterial culture was centrifuged at 14000 rpm for 30 s to pellet the cells. The pellets were suspended in lysis buffer and vortexed. For Gram negative bacteria, 250 µl of lysis solution and 12 µl of proteinase K were added to the cell suspension. It was mixed by vortexing and incubated at 55^{o}C for 30 min. For Gram positive bacteria, 12 µl of previously prepared lysozyme stock solution was added. Then, 250 µl of lysis solution and 12 µl of proteinase K were added to the cell suspension. The cell suspension was mixed by vortexing and incubated at 37^{o}C for 2 hr. Pellet of the bacterial colony was resuspended in binding buffer and vortexed. Then the sample was centrifuged (14 000 rpm, 2 min), washed twice with 500 µl washing buffer and then dried. DNA was preserved by elution with 200 µl buffer and frozen at 20^{o}C for later usage.

For visualizing the DNA extracts, 10 µl of each extract was electrophoresed on 1% agarose gels in TAE buffer, which were then stained with ethidium bromide and examined under UV light. The PCR amplification of 16SrRNA gene, from the purified genomic DNA was carried out using universal primer sets. Sequencing of the gene was done and identification of the sequence was by Basic Local Alignment Search Tool (BLAST) with the National Center for Biotechnology Information (NCBI) gene data base.

RESULTS

Total heterotrophic bacteria count (THBC) from various sampling points ranged from 1.13 × 10^{6} to 8.75 × 10^{6} CFU/ml in the month of March while in June, it varied between 7.5 × 10^{6} and 1.21 × 10^{7} CFU/ml and in August it was between 1.27 × 10^{7} and 3.01 × 10^{7} CFU/ml (Table 1). Generally, high THBC was observed between June and August, at the peak of rainy season compared to other months. The highest count of 3.1 × 10^{7} CFU/ml was obtained from sample E in the month of August and the lowest count of 1.13 × 10^{6} CFU/ml were obtained in the month of March from sample C (Table 1).

Table 2 shows the total coliform counts obtained from various sampling locations. Total coliform counts ranged between 2.5 × 10^{5} and 4.75 × 10^{6} CFU/ml in March while it varied between 1.85 × 10^{6} and 1.3 × 10^{7} CFU/ml, 1.15 × 10^{6} and 7.0 × 10^{6} CFU/ml and 9.15 × 10^{6} and 2.3 × 10^{7} in April, May and August respectively (Table 2). It was observed that TCC increased as the rainy season progressed and peaked in the months of July and August.
Total *Escherichia coli* counts in August varied between 4.5 × 10^{5} and 1.3 × 10^{6} CFU/ml and 4.5 × 10^{5} and 8.5 × 10^{5}CFU/ml in July while it ranged between 5 × 10^{4} and 3.9 × 10^{5} CFU/ml, 5 × 10^{4} and 6.69 × 10^{5}CFU/ml and 1.25 × 10^{5} and 6 × 10^{5} CFU/ml in the months of March, April and May respectively (Table 3). TCC values were higher than TEC values in all samples.

The MPN values ranged between 430 MPN/100 ml and 1600 MPN/100 ml in the month of June. It varied between 920 MPN/100 ml and (greater than) 1600 MPN/100 ml in July and between 430 MPN/100 ml and greater than 1600 MPN/100 ml in August (Table 4). The MPN values were higher in August than July and June respectively.

DISCUSSION

Enumeration of total coliform and *E. coli* are reliable assessment of microbial faecal pollu-

Table 1: Total heterotrophic bacteria counts from various sampling points

Sampling location	March (10^6)	April (10^6)	May (10^6)	June (10^6)	July (10^6)	August (10^6)
FUNAAB water reservoir	2.85±0.05^c	2.3±0.0^a	6.9±0.1^d	7.5±0.1^a	13±0.1^a	21.9±0.1^b
FUNAAB fish pond	1.98±0.02^b	2.55±0.15^a	9.6±0.1^e	9±0.1^b	17.65±0.25^b	21.85±0.15^b
Lafenwa market	1.13±0.07^a	2.61±0.015^a	1.99±0.01^a	9.2±0.2^b	19.7±0.2^c	37.8±0.1^d
Lafenwa abattoir	1.26±0.02^a	2.48±0.02^a	3.8±0.1^b	10.35±0.15^c	28.7±0.2^e	40.9±0.9^e
Adigbe abattoir	8.75±0.05^e	6.6±0.0^c	5.5±0.1^c	9.9±0.1^c	27.2±0.2^d	12.65±0.25^a
Adigbe sand dredging	6.45±0.05^d	2.95±0.15^b	12.55±0.15^f	12.05±0.15^d	26.4±0.4^d	30.5±0.5^c

Values are means of duplicate readings ±standard error of means
Mean values with the same letter within the column are not significantly different at p<0.05

Table 2: Total coliform counts obtained from various sampling units

Sampling location	March (10^6)	April (10^6)	May (10^6)	June (10^6)	July (10^6)	August (10^6)
FUNAAB water reservoir	0.25±0.15^a	1.85±0.15^a	1.15±0.15^a	2.55±0.05^a	5.5±0.0^b	9.15±0.15^a
FUNAAB fish pond	1.65±0.05^b	12.95±0.05^a	1.55±0.05^b	2.48±0.025^a	3.72±0.03^a	16.15±0.15^c
Lafenwa market	2.95±0.05^d	4.73±0.03^a	4.05±0.05^d	6.95±0.05^c	18.9±0.1^e	18.0±0.0^e
Lafenwa abattoir	3.64±0.035^e	4.11±0.11^a	3.695±0.01^c	9.95±0.05^e	23.35±0.35^f	23.3±0.3^f
Adigbe abattoir	1.97±0.03^c	3.8±0.1^a	7.0±0.01^f	6.5±0.2^b	9.8±0.2^d	17.1±0.1^d
Adigbe sand dredging	4.75±0.05^f	3.43±0.03^a	5.9±0.1^e	7.5±0.095^d	9.1±0.2^c	14.35±0.35^b

Values are means of duplicate readings ±standard error of means
Mean values with the same letter within the column are not significantly different at p<0.05

Table 3: Total *Escherichia coli* counts obtained from each sampling location

Sampling Location	March (10^5)	April (10^5)	May (10^5)	June (10^5)	July (10^5)	August (10^5)
FUNAAB water reservoir	0.5 ± 0.5^{a}	2.5 ± 0.5^{cd}	2.3 ± 0.3^{ab}	3.25 ± 0.25^{a}	5.5 ± 0.5^{ab}	5.25 ± 0.25^{a}
FUNAAB fish pond	1.5 ± 0.5^{ab}	0.5 ± 0.5^{a}	4.25 ± 0.25^{c}	5.5 ± 0.5^{b}	4.5 ± 0.5^{a}	7.75 ± 0.25^{b}
Lafenwa market	3.5 ± 0.5^{c}	3.5 ± 0.5^{d}	3.0 ± 0.0^{bc}	6.5 ± 0.5^{b}	8.5 ± 0.5^{c}	10.5 ± 0.5^{c}
Lafenwa abattoir	2.5 ± 0.5^{bc}	0.9 ± 0.4^{ab}	1.25 ± 0.25^{a}	6.5 ± 0.5^{b}	4.9 ± 0.1^{a}	8.05 ± 0.05^{b}
Adigbe abattoir	2.5 ± 0.5^{bc}	2.0 ± 0.0^{bc}	6.0 ± 1.0^{d}	3.5 ± 0.5^{a}	6.5 ± 0.5^{b}	4.5 ± 0.5^{a}
Adigbe sand dredging	3.9 ± 0.1^{c}	6.69 ± 0.09^{e}	4.25 ± 0.25^{c}	9.95 ± 0.06^{c}	5.1 ± 0.1^{ab}	13.35 ± 0.35^{d}

Values are means of duplicate readings ±standard error of means
Mean values with the same letter within the column are not significantly different at $p<0.05$

Table 4: Most probable number (MPN/100 ml) obtained from various sampling locations

Sampling Location	March	April	May	June	July	August
FUNAAB water reservoir	120	220	430	920	920	430
FUNAAB fish pond	540	280	430	540	350	450
Lafenwa market	280	450	920	1600	>1600	>1600
Lafenwa abattoir	210	280	280	920	920	1600
Adigbe abattoir	210	120	920	1600	>1600	350
Adigbe sand dredging	430	450	1600	430	920	1600

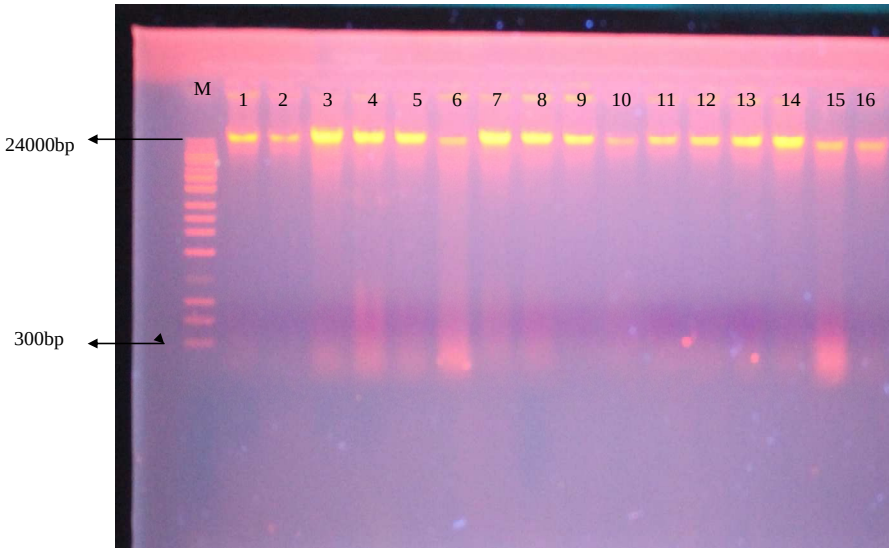

Plate 1: Genomic DNA extracted from bacteria isolated from water samples

Table 5: Isolated microorganisms with their accession number

Samples no	Sample Identification	% similarity	Accession number
2	*Escherichia coli* strain SUS9EC	100	KF991482.1
3	*Serattia liquefaciens* ATCC 27592	90	NC021741.1
4	*Escherichia coli* O157:H7 strain SSI7	100	CP008805.1
7	*Escherichia coli* strain BW25113	100	CP009273.1
8	*Klebsiella oxytoca* strain KU-5	100	JQ003982.1
9	*Escherichia coli* strain C-X1B	100	KJ806504.1

tion in tropical environments (Kei *et al.*, 2004). Total heterotrophic bacterial count of the water samples showed higher bacteria count. The ranges of THBC obtained from all samples were above the recommended value of 1.2×10^2 by WHO (1998) for drinking water. The result of total coliform counts also exceeded the WHO recommended standard for drinking water. This agrees with the work of Ewa *et al.* (2011) who reported that faecal coliform can be found in water contaminated by human and animal waste. The presence of enteric organisms (*Enterobacteriaceae*) indicates faecal contaminations by human and other animals. The occurrence of high coliform counts increases the probability of other pathogenic organisms

such as *Cryptosporidium* (FEPA, 2003) and other dangerous microorganisms (Richman, 1997). The presence of *E. coli* is also used as an indicator to monitor the possible presence of other harmful microbes such as *Cryptosporidium giardia*, *Shigella* and *Norovirus* (Taiwo *et al*., 2014). The result also agrees with the work of Obi *et al.* (2002); Bayoumi-Hamuda and Patko (2012) and Shafi *et al.* (2013) whose work on river water sources in Venda communities, Northern Province, South Africa; Danube River, Budapest, Hungary and Manasbal Lake of Kashmir respectively showed high bacterial counts as a result of pollution. They found elevated levels of faecal coliforms and *E. coli* in the rivers which are above the WHO permissible limits.

The very high contamination may be due to the non-hygienic disposal of faecal waste in water bodies as well as the period of the study which are months of rainy season, as a result water may be contaminated by run-off and seepages (Ibiene *et al*., 2012). This result compared favourably with the report of Banwo (2006) which indicated that the presence of bushes and shrubs makes it possible for smaller mammals to come around these water bodies to drink water, thereby passing out faeces into and around water bodies.

High MPN values in this study is higher than the standard recommended by WHO which are between 0 and 10 MPN/100 ml of coliforms (WHO, 2004; NAFDAC, 2004).This is an indication that these water bodies are prone to pathogenic organisms which were isolated in the course of the research and the water are unfit for drinking.

The high *E. coli* count is unacceptable by the WHO (1998) standard guideline which is supposed to be less than 10 CFU/ml. However, *E. coli* are observed to be greater than 160, which is above WHO permissible standard of zero. *Escherichia coli* count is used extensively as a basis for regulating the microbial quality of drinking water. Its presence in drinking water pose serious threat to health of consumers since it's an intestinal parasite indicating faecal contamination (Rose *et al*., 1993). The organism also poses ecological hazard in rivers where it increases the dissolved oxygen demand and biochemical oxygen demand (Olayinka *et al*., 2013).

CONCLUSION

Water quality is one of the main issues in the water sector all over the world and its improvement is a major concern. This study revealed that Ogun River at Lafenwa and Adigbe were polluted from different waste discharge, open defecation and washing of clothes in the areas. Therefore, it is essential to treat waste water before discharging it into surface water to prevent water borne diseases in the study area. Environmental regulatory bodies should be more effective in environmental assessment, monitoring and enforcement of environmental laws and regulations. Sustainable sanitation practices such as the careful disposal and storage of animal and human faecal waste and restriction of domestic washing along the streams and rivers are critical to maintain quality of surface water source.

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