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Seasonal variation in the bacteriological quality of Ebutte river in ehor community EDO state, Nigeria

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ABSTRACT

The bacteriological quality of Ebutte River in Ehor Community was carried out to ascertain the variation in the quality of the river between August 2010 and January 2011.The bacteriological assessment was studied using the basic microbiological techniques. The bacterial counts were shown to be highest in the inhabited point (3) with downstream (points 4 and 5) showing dilution effects of human activities and upstream (point 1 and 2) showing lower counts due to absence of human activities. Bacterial counts were higher than the acceptable limit of the WHO standards. The total viable counts ranged from 3.40×10^5 to 3.71×10^6 TVC (cfu/ml) for the months of August 2010 to January 2011. The bacterial counts were shown to be highest in the rainy season and the least total viable counts were recorded in the month of January at sampling point 5. Total coliform counts ranged from 27MPN/100ml to 350MPN/100ml while the faecal coliform counts ranged from 5MPN/100ml to 26MPN/100ml. The faecal Streptococci counts were recorded to range from <2MPN/100ml to 14MPN/100, while the *Clostridium* counts ranged from <2MPN/100ml to 6MPN/100ml. The bacteria isolated and characterised included eleven(11) bacterial genera among which are *Escherichia*, *Klebsiella*, *Pseudomonas*, *Bacillus, Enterobacter, Streptococcus, Salmonella*, *Staphylococcus, Proteus, Clostridium* and *Shigella.* Analysis of variance showed that there was a high significant difference ($P<0.001$) between total viable counts obtained in the two seasons while a significant difference ($P<0.05$) was obtained for total coliform counts and faecal coloform counts. Significant difference ($P>0.05$) was obtained for faecal Streptococci and *Clostridium perfringens* counts. Correlation coefficient showed positive relationship between the total viable counts and some of the physicochemical parameters studied. Water quality assessment identified human, animal and agricultural activities as the major sources of water contamination, thus the water from Ebutte river was considered unsuitable for direct human use and it poses a serious threat to the health of the consumers.

{Citation: May N.O. Omoigberale, Jonathan O. Isibor, Joshua I. Izegaegbe, Mercy I. Iyamu. Seasonal variation in the bacteriological quality of Ebutte river in ehor community EDO state, Nigeria. American Journal of Research Communication, 2013, 1(7): 59-69} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Rivers are vital and vulnerable freshwater systems that are critical for the sustenance of all life. However, the declining quality of the water in these systems threatens their sustainability and is therefore a cause for concern. Rivers are waterways of strategic importance across the world,

providing main water resources for domestic, industrial and agricultural purposes (Farah, 2002). The maintenance of healthy aquatic ecosystem is depended on the physicochemical properties and biological diversity. A regular monitoring of water bodies would not only prevent the outbreak of diseases and occurrence of hazards but would check the water from further deterioration. Bacteriological assessment particularly for coliforms, the indicators of contamination by faecal matters is therefore routinely carried out to ascertain the quality and potability of water to ensure prevention of further dissemination of pathogens. One of the most important factors of water pollution is the microbial contamination especially with pathogenic microorganisms. Enteric pathogens are typically responsible for waterborne sickness (Bitton, 1994).Contamination of water is a serious environmental problem as it adversely affects the human health and the biodiversity in the aquatic ecosystem. The provision of good quality household drinking water is often regarded as an important means of improving health (Moyo *et al.*,2004).According to World Health Organisation (WHO,1992), there were estimated four billion cases of diarrhoea and 2.2 million death annually. The consumption of unsafe water has been implicated as one of the major causes of this disease. Most gradual deterioration of water quality is as a result of increase in human population and urbanization (Ho and Hui, 2001). As water pollution gets serious, houses in the urban area started to equip with a treating system. People are concerned with the presence of pollutants such as heavy metals and toxic chemicals in their daily drinking water.

The primary objective of drinking water microbiology is to prevent waterborne diseases and this can be achieved through proper water treatment, control practices and monitoring of their effectiveness. Ideally, specific detection of the various waterborne pathogens which includes various species of bacteria, viruses and protozoa would be the most direct approach in determining portability but this would be too tedious, time consuming and expensive (Simango *et al.*, 1992). Potable water should be examined for microbiological and physiochemical quality. WHO (1993) has recommended that increased emphasis be placed on home water treatment. A number of authors have reported a statistically significant deterioration in the microbiological quality of water between the source and point of use in the home. (Simango *et al.*, 1992; Welch *et al.*, 2000). Drinking water from most communities and municipalities is obtained from surface sources such as streams, rivers and lakes. Such natural water sources are likely to be polluted with domestic waste, agricultural waste and industrial waste.

The efficiency of current techniques in detecting waterborne pathogens is often very low, primarily due to low levels of these organisms in water. However detection does not always translate into risks as some strains of the same specie are more pathogenic than others and current detection methods cannot easily discriminate between pathogenic and non-pathogenic subpopulations. Although culture techniques for isolation is nonselective thus allowing nontarget

organisms to proliferate in numbers that over grow the pathogens. Viral pathogens are fastidious in their growth requirements and grow only in special tissue cultures that are expensive and often difficult to maintain (Moyo *et a*l., 2004). The use of indicator bacteria such as faecal coliforms (FC) and faecal streptococci (FS) for assessment of faecal pollution and possible water quality deterioration in freshwater sources is widely used (APHA, 1995). Currently coliforms and *Escherichia coli* are of great importance among bacterial indicators used in water quality definition and health risk (Schlegel, 2002). This study was aimed at investigating the bacteriological quality of Ebutte river as it is influenced by seasonal variation .

MATERIALS AND METHODS

STUDY AREA

The river under study is located in Ehor, the headquarter of Uhunmwonde local government area. The Ebute River watershed is the principal natural water network irrigating the community. The members of the community are engaged in farming and raising of livestock. The catchment is subject to a wide range of activities, including residential (communal habitations), commercial and open spaces. The River is used by the community´ for multiple activities including: agriculture, laundry, drinking, commercial purpose, car washing, bathing, watering of crops for raw consumption and in certain areas swimming by youth without prior treatment. Therefore, an overview of the quality of the River is a major public health issue.

SAMPLING

Five sampling points were chosen within certain intervals and samples were taken from a current and collected monthly over a period of six months which spanned through the rainy season (August 2010 to October 2010) and dry season (November 2010 to January 2011). A total of 30 Samples were collected during the sampling period with each point sampled six times. The Sampling points were. point 1 (P1) which was the upstream, point 2 (P2) which was between the upstream and midstream, point 3 (P3) which was the midstream, point 4 (P4) which was between the midstream and downstream and point 5 (P5) which was the downstream. Samples for bacteriological analysis were collected into sterile clean glass bottles and bottles were labelled before sample collection. Collected samples were transported immediately to the laboratory for bacteriological examinations.

BACTERIOLOGICAL EXAMINATION

The total viable counts were determined using the pour plate method (APHA, 1995).The number of total and faecal coliforms were determined using the most probable number (MPN) method. From each dilution 1ml was added to each triplicate tube containing 50ml, 10ml and

1ml of double and single strength MacConkey broth and then incubated at 37^0C and examined after 48hrs for total coliform and 44^0C in waterbath for faecal coliform. Production of acid and gas meant presumptive positive. McCrady's Probability Table was used to interpret results to get the MPN of the bacteria. Loop full cultures from tubes showing positive result were inoculated in Eosin Methylene Blue (EMB) agar using sterile loop and incubated at 37^0C for 24 hrs. Lactose fermenting and nonfermenting colonies were isolated for further characterization. Microscopic examination was carried out to ensure gram-negative, non- spore forming rods (APHA, 1995).

MPN of faecal streptococci was determined using glucose azide broth at 37^0C. Cultures showing positive result were detected by acid production which is seen as a change in colour of the medium from purple to yellow and were sub cultured into MacConkey agar and incubated at 37^0C for 24hrs.Presence of *Streptococcus faecalis* was confirmed with the appearance of pink or red colonies (APHA, 1995). MPN of *Clostridium perfringens was determined using* Differential Reinforced Clostridia Medium (DRCM) in screw capped bottles to prevent oxygen and incubated at 37^0C for 48hrs. Blackening of the medium showed the presence of *Clostridium.* Confirmation for the presence of *Closridium perfringens* was done by sub culturing from positive bottles into cooled litmus milk medium and incubated at 37^0C for 48hrs.Positive bottles showed stormy clot (Bonde, 1963).

STATISTICAL ANALYSIS

SPSS was used to carry out single factor analysis of variance (ANOVA) on the bacteriological counts to test for statistical significance and where significant differences were detected the Duncan's Multiple Range (DMR) test was further used to locate the significantly different means.

RESULTS

Results of the bacteriological quality of Ebutte River water are shown in Tables 1 – 5. Analysis of variance (ANOVA) on total viable counts (TVC) showed that there was a high significant difference in TVC between the two seasons (August to October 2010 and November 2010 to January 2011) with ($P<0.001$) significance. Fig. 1 shows the frequency of distribution of bacterial isolates in the months of sampling. ANOVA on total coliform and faecal coliform counts were significant with ($P<0.05$) level of significance. ANOVA on Faecal Streptococci and *Clostridium perfringens* showed no significant difference in the sampling points and thus level of significance was ($P>0.05$).

TABLE 1: Total viable counts (TVC) (cfu/ml) of Ebutte River water sample from August 2010 to January 2011

Sampling points	August	September	October	November	December	January	WHO Standard
P1	2.02×10^6	1.36×10^6	1.35×10^6	3.40×10^5	3.43×10^5	3.40×10^5	1.0×10^2
P2	2.03×10^6	2.36×10^6	1.70×10^6	1.02×10^6	1.02×10^6	6.77×10^5	1.0×10^2
P3	3.71×10^6	2.70×10^6	2.37×10^6	1.69×10^6	1.35×10^6	1.01×10^6	1.0×10^2
P4	2.03×10^6	2.03×10^6	1.70×10^6	1.02×10^6	6.77×10^5	6.77×10^5	1.0×10^2
P5	1.69×10^6	1.69×10^6	1.68×10^6	1.01×10^6	3.44×10^5	3.40×10^5	1.0×10^2

TABLE 2: Total coliform counts (TCC) of Ebutte River water sample from August 2010 to January 2011

Sampling Points	August	September	October	November	December	January	WHO Standard
P1	110	94	79	70	79	27	Zero/100ml
P2	220	170	170	130	110	110	Zero/100ml
P3	350	280	220	180	140	140	Zero/100ml
P4	220	220	180	110	94	110	Zero/100ml
P5	180	79	110	79	70	33	Zero/100ml

TABLE 3: Faecal coliform counts (FCC) (MPN/100ml) of Ebutte River water sample from August 2010 to January 2011

Sampling points	August	September	October	November	December	January
P1	8	11	11	7	7	5
P2	17	14	14	11	9	7
P3	26	21	26	17	14	9
P4	14	9	17	11	9	9
P5	11	11	11	7	5	5

TABLE 4: Faecal Streptococci counts (FCC) (MPN/100ml) of Ebutte River water sample from August 2010 to January 2011

Sampling points	August	September	October	November	December	January
P1	4	4	4	2	2	2
P2	4	4	4	4	4	2
P3	14	11	6	5	5	5
P4	4	4	4	2	2	2
P5	4	2	2	2	2	<2

TABLE 5: *Clostridium* perfringens counts (MPN/100ml) Ebutte River water sample from August 2010 to January 2011

Sampling points	August	September	October	November	December	January
P1	<2	<2	<2	<2	<2	<2
P2	4	2	<2	<2	<2	<2
P3	6	4	4	2	2	2
P4	2	2	2	<2	<2	<2
P5	<2	<2	<2	<2	<2	<2

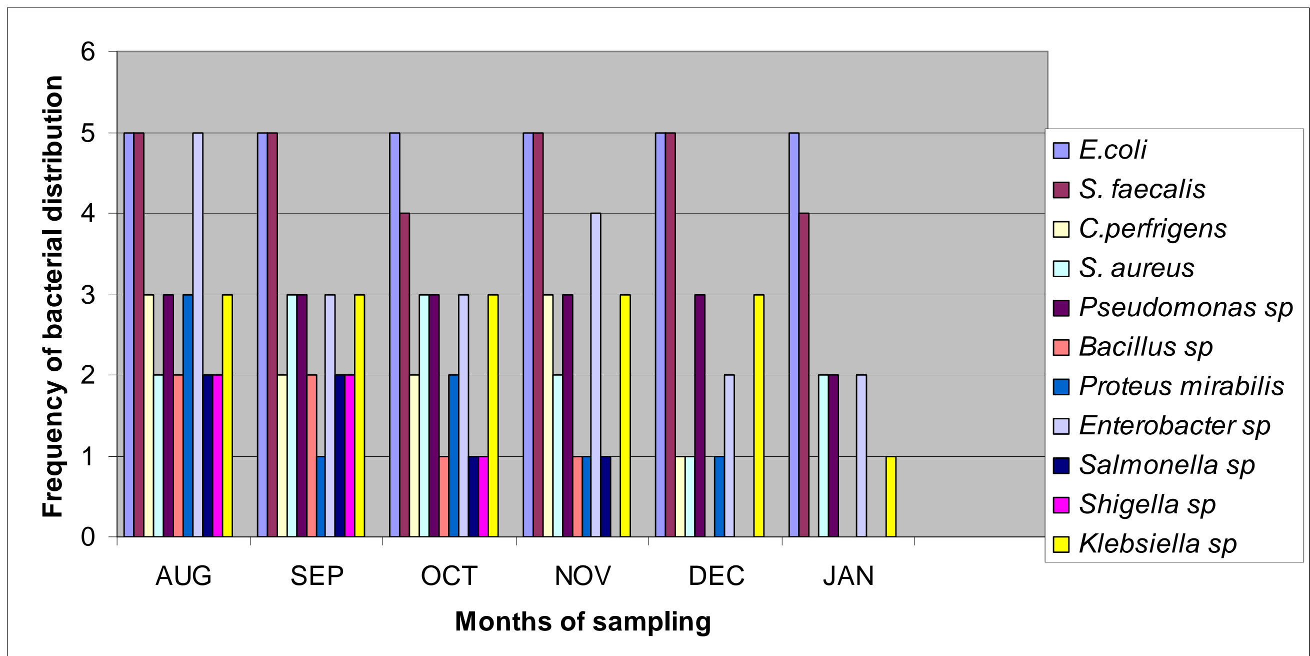

Fig.1: Frequency of distribution of bacterial isolates in the sampling months (August, 2010 to January, 2011)

TABLE 7: DETERMINATION OF SIGNIFICANT DIFFERENCE AMONG THE BACTERIAL COUNTS

PARAMETERS	AUG 2010	SEPT 2010	OCT 2010	NOV 2010	DEC 2010	JAN 2010	P-VALUE
	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	
Total viable count	$2.29^{A} \pm 0.36$	$2.028^{A} \pm 0.237$	$1.76^{A} \pm 0.17$	$1.05^{B} \pm 0.22$	$0.75^{B} \pm 0.19$	$0.61^{B} \pm 0.13$	**P<0.001**
Total coliform	$216^{A} \pm 39.06$	$168.6^{A} \pm 37.85$	$151.8^{B} \pm 25.32$	$113.8^{B} \pm 19.73$	$98.60^{B} \pm 12.38$	$84.0^{B} \pm 22.74$	**P<0.05**
Faecal coliform	$15.80^{A} \pm 3.09$	$15.20^{A} \pm 2.11$	$13.20^{B} \pm 2.78$	$10.60^{B} \pm 1.83$	$8.80^{C} \pm 1.49$	$7.00^{C} \pm 0.89$	**P<0.05**
Faecal Streptococci	5.60±2.14	5.40±1.40	3.60±0.75	3.40±0.60	3.60±0.75	3.20±0.80	P>0.05
Clostridium perfringens	3.20±0.80	2.40±0.40	2.40±0.40	2.00±0.00	2.00±0.00	2.00±0.00	P>0.05

Similar letters indicate no significant difference; P<0.001: Highly significant; P<0.05: Significant; P>0.05: Not significant

DISCUSSION

The total bacterial counts (TVC) for all the water samples were generally high exceeding the WHO limit of $1.0X10^2$ cfu/ml which is the standard limit of TVC for drinking water (EU, 1998). TVC is indicative of the presence of high organic and dissolved salts in the water. The primary sources of these bacteria in water are animal and human activities. The TVC in colony forming unit (cfu/ml) ranged from 3.40×10^5 cfu/ml in November 2010 and January 2011 (Points1 and P5) to 3.71×10^6 cfu/ml in August 2010 (Point 3). Sampling point 3 showed the highest TVC value (Table 1) which is the point with heavy human and animal activity. TVC values were higher during the rainy seasons (Table 1) and longitudinal profile shows that bacterial population increased from upstream (P1) to midstream (P3) and decreased from midstream to downstream (P4 and P5) (Table1). All samples were found to have TVC higher than the WHO standard acceptable limit and TVC recorded by Shittu *et al.,* (2008) which was 1.0×10^6 cfu/ml.. The TVC was lower in the dry season as compared to rainy season; this was in accordance with Olayemi, (1994). The total coliform count (TCC) for all samples were exceedingly higher than the WHO standard for coliform bacteria in water which is zero total coliform per 100ml of water (Table 2). Table 2 shows the total coliform counts (TCC) for the various points of sampling. The least TCC was observed in January 2011 and it was 27MPN/100ml while the highest TCC was 350MPN/100ml and it was recorded in August 2010. This is contrary to Olayemi, (1994) were higher TC was recorded in the dry season. The high coliform counts obtained in the samples may be an indication that the water sources received faecal contamination (Martin *et al.,*1982*)*. None of the sampling points of the water sources complied with WHO standard for coliform in water and this could be supported by evidence advanced by Shittu *et al*., (2008). According to WHO standard, every water sample that has coliform must be analyzed for faecal coliforms (*E. coli)* (EU, 1998) with a view to ascertaining contamination with human or animal waste and possibly pathogenic bacteria.

Faecal coliform (FC) counts for the various sampling points as shown in Table 3 ranged from 5MPN/100ml in December 2010 and January 2011 (points 5 and 1) to 26MPN/100ml in August (point 3) respectively(Table 3). FC counts were higher in the rainy season and this is contrary to the report by Olayemi,(1994) where he recorded higher TC and FC in the dry season.Faecal Streptococci (FS) counts for the different points as shown in Table 4 ranged from <2MPN/100ml in January 2011 (point 5) to 14MPN/100ml in August 2010 (point 3) (Table 4). FS counts did not vary that much as most points had 2MPN/100 or 4MPN/100ml. The higher faecal coliform counts when compared with faecal Streptococci counts shows that the river was highly contaminated with human excrement than animal excrement.*Clostridium perfringens* MPN/100ml as shown in Table 5 were not detected in most sampling points (Table 5). During the

rainy season, it was detected only in points 2, 3 and 4 while only point 3 had count of 2MPN/100ml in the dry season. *Clostridium perfringens* MPN/100ml ranged from <2 to 6 MPN/100ml (Table 5).

Eleven bacterial genera were routinely isolated in the river which includes pathogens and opportunistic pathogens. Members of the Enterobacteriaceae predominated in the number of isolates and the genera were *Escherichia, Bacillus, Pseudomonas, Klebsiella, Proteus, Staphylococcus, Streptococcus, Clostridium, Salmonella, Shigella* and *Enterobacter* .Figure 1 shows the frequency of bacterial distribution in the sampling points and it showed that the most prevalent isolates were *Escherichia coli, Streptococcus faecalis* and *Enterobacter* sp while the least prevalent were *Shigella* sp., *Bacillus* sp., *Salmonella* sp. and *Clostridium* and they were not isolated in the months of January. This confirms faecal contamination which is more significant in the rainy season.

CONCLUSION

In this study, it was discovered that Ebutte river water had high coliform counts which requires continuous monitoring and treatment process if the water is to be used for drinking purposes. The higher faecal coliform counts when compared with faecal Streptococci counts show that the river was more contaminated with human excrement than animal excrement. In conclusion it is evident that water borne diseases are due to improper disposal of refuse and contamination of water by sewage and surface runoff. Control of human activities to prevent faeces and refuse from entering water body is the key to avoiding bacterial contamination of the river water.

This study recommends that the government and other stakeholders should provide sanitary facilities especially in the rural areas to control river pollution. Also appropriate water treatments or safe potable water sources should be provided in the area to improve the welfare of the rivarine dwellers. There is also the need to educate the villagers on how to handle and locally treat water for domestic use. The government should evolve sanitation programmes and propagate these through environmental education throughout the communities in the river catchments to prevent pollution of water bodies and consequent transmission of water-related diseases. Farmers should also be educated on proper farming practices.

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