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Evaluation of the Physicochemical Properties and Ecological Distribution of Enterobacteriaceae in Sediments of Two Surface Watersheds in Benin City, Nigeria

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ABSTRACT

The assessment of the bottom sediment properties is vital to the health of an aquatic ecosystem, environmental water quality and the related constituents of freshwater environment. This study was aimed at assessing the physicochemical quality of the sediment of two fresh water ecosystems and the ecological distribution of Enterobacteraceae. Sediment samples were collected from Ikpoba and Ogba rivers, in Benin City, Nigeria. Chromocult coliform agar, Heakton agar and m-Endo agar were used for the detection of Escherichia coli, Salmonella isolates and total coliform respectively, as representative of Enterobacteraceae family. The population density of Salmonella, E. coli isolates and coliform ranged between $6.33\times10^{2}\pm0.00$ and $4.36\times10^{3}\pm0.61$ cfu/g, 0.00 ± 0.00 and $2.46\times10^{3}\pm0.09$ cfu/g, and $6.66\times10^{1}\pm0.18$ and $2.13\times10^{3}\pm0.39$ cfu/g respectively. The physicochemical variables of the river sediment range as follows: pH (4.8±0.02 - 5.3±0.01), electrical conductivity (143±1.25 - 210±1.19 µS/cm), organic compound (0.76±0.02 - 1.23±0.10 %), exchangeable acidity (0.7±0.01 - 1.2±0.03 meq/g), chloride (35.3±0.68 - 54.5±0.46 mg/kg), nitrate (3.00±0.21 - 6.11±0.11 mg/kg), sulphate (0.44±0.00 - 0.70±0.03 mg/kg), clay (5.3±0.01 - 8.8±0.01 %), silt (1.6±0.01 - 3.0±0.02 %), sand (89.0±0.01 - 93.5±0.00 %). The study presents baseline data of the physicochemical properties and the distribution of Enterobacteriaceae in sediment which could form a valuable tool for ecological assessment and surveillance of health of the rivers.

1. INTRODUCTION

Sediment of aquatic beds forms a vital part of hydro ecosystems in which diverse pollutants and microorganism can bond and accrue (Yotinov *et al*., 2017). The evaluation and management of contaminated sediments is of higher magnitude when compared to the management of contaminated soils and waters

(Reible, 2014; Yotinov *et al.*, 2017). The indiscriminate disposal of untreated wastewater and the diverse point sources of release of domestic faecal waters into river bodies has made faecal contamination of sediment an immense pollution problem of concern. These pollutants are diverse in origin, which span among organic pollutants from households, organic synthetic compounds from industries and heavy metals from mining related activities (Yotinov *et al.*, 2017).

The survival and persistence of pathogenic microorganism of faecal origin in sediment is vital from ecological, human health and water quality viewpoint. These bacteria are able to attach and form aggregate with particles present in the aquatic environments. This attachment seems firmer in smooth textured sediments which reflect the nature and number of organic matter and clay minerals present. The binding to the surfaces of particle provides chemical and physical protection from abiotic and biotic stimulus thereby enhancing the perseverance of bacteria in the environment (Hassard *et al.*, 2016). Reports of previous research reveals that sediment associated bacteria display higher survival rate in river waters when compared to free living bacteria (Hassard *et al.*, 2016). The dispersal of particle-bound bacteria into water bodies during sediment agitation presents a risk to water quality (Hassard *et al.*, 2016).

Faecal contamination has been identified as a major issue associated with pollution of sediments and surface waters (Tortorello, 2003). In general, a pointer to such pollution is occurrence of large quantities of potential pathogenic microorganisms belonging to the family Enterobacteriaceae. The Enterobacteriaceae have the capability to survive in the environment for a couple of weeks. Their detection and identification are easy to perform; hence they are broadly used as main indicator of the presence of faecal contamination (Tortorello, 2003).

Good quality water resources depend largely on the physicochemical qualities of the sediments and the degree and channel of pollution if any (Tukura *et al.*, 2012). The quality of river sediment impacts on the water quality of the river, which may serve as a source of water supply for potable, agricultural, recreation or industrial use. Chemical conformation of sediment is a function of hydro geochemical progressions in a particular environment and physicochemical assessment of sediment quality provides vital information for water management (Matthieu *et al.*, 2005; Tukura *et al.*, 2012). Therefore, a better understanding of physicochemical properties like pH, conductivity, alkalinity, salinity, hardness, chemical oxygen demand etc., in the water bodies seem to be particularly important issues of present-day research on pollution assessments. Therefore, this study aims to evaluate the physicochemical and microbiological qualities of sediment in two fresh watersheds.

2. MATERIALS AND METHODS

2.1. Description of Study Site

Samples were collected from four different locations. These include Ikpoba river location on Ikpoba slope (6'21'6.504" N; 5'38'43.402"E) and Upper mission (6'21'16" N; 5'38'35.523"E), Ogba river locations on Ekewan road (6'19'32.922"N; 5'35'10.813"E) and Ogba road location (6'17'15.574"N and longitude 5'34'56.148"E.). While the Ikpoba slope and Upper Mission locations are situated in Ikpoba-Okha Local Government Area of Edo State, Ekewan and Ogba locations are situated in Oredo Local Goverenment. Anthropogenic activities around the study area were also investigated by both site assessment and inquiries from the inhabitants.

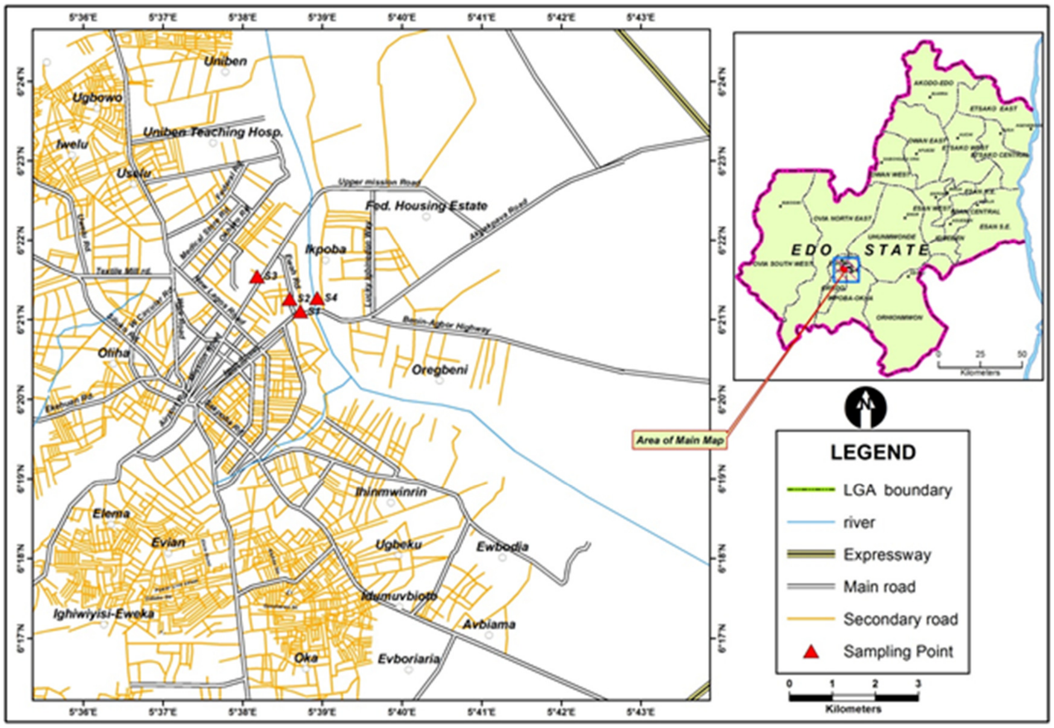

Figure 1: Map of study locations

2.2. Sample Collection

Sediment samples were collected using Smith McIntyre grab (0.2 m^2) from top surface area of 5 cm and carefully transferred to sterile polythene bags, placed on ice and transported to the laboratory for analysis according to the method described by Jacob et al. (2013). Samples were collected from May to July 2017.

2.3. Physicochemical Analysis

Sediment samples were analyzed for pH, electrical conductivity(µS/cm), organic compound (%), exchangeable acidity (meq/g), sodium (meq/g), potassium (meq/g), calcium (meq/g), magnesium (meq/g), chloride (mg/kg), phosphorus (mg/kg), nitrate (mg/kg), sulphate (mg/kg), clay, silt, sand. The analysis was carried out according to standard procedure as described elsewhere (APHA 1998).

2.4. Microbiological Analysis

The pre-treatment of sediment was done using ultrasonic disintegrator UD-20 automatic (Techpan) so as to loosen the attached bacterial cells from the sediment. One gram of sediment was measured into 9 ml of sterile distilled water followed by serial dilution. Each diluent was plated out onto each respective media: Hektoen agar (Lab M Limited, UK) for *Salmonella* detection, Chromocult coliform Agar (CCA) (Lab M, United Kingdom) for *Escherichia coli* detection and m-Endo Agar (Biolab, SA) for total coliform detection. Media were prepared and analyzed according to manufacturer's instructions. An aliquot of 0.1 ml of each sediment serial diluted sample was spread inoculated on CCA and incubated for 24 h at 37 °C. Blue or violet colour colonies on CCA were enumerated as presumptive *Escherichia coli* isolates and expressed as colony forming unit per gram (cfu/g) for the sediment. For *Salmonella* detection, an aliquot of 0.1 ml was spread on Hektoen agar and incubated for 24 h at 37 °C. Blue to dark green colonies with or without black centers were enumerated as presumptive *Salmonella* isolates. Also, m-Endo Agar was inoculated with the respective diluents and incubated at 44 °C for 24 h. Metallic green colonies on m-Endo Agar was enumerated as total coliform. All presumptive *Escherichia coli* and *Salmonella* isolates were subjected to an array of culture-based tests which are biochemical (catalase, oxidase, indole, citrate, and sugar fermentation test), morphological (Gram reaction), and cultural (colony) characterization.

2.5. Phenotypic Identification of *Escherichia coli* and *Salmonella* Isolates

Presumptive *Escherichia coli* isolates (that showed a Gram-negative reaction with 3 % KOH, catalase-positive and oxidase-negative) were characterized further using the Analytical Profile Index 20E (API 20E) (BioMerieux, Marcy-l'Etoile, France). *Salmonella* isolates were characterized by means of culture-based and biochemical procedures using Gram-reaction with 3% KOH test, oxidase, urease reactions, indole and motility tests. Gram-negative rod isolates that appeared negative for urease, oxidase, and indole were designated presumptive *Salmonella* isolates and identified further with the Analytical profile index (API 20E). All strips were examined accordingly, and identification evaluated using an API lab plus software (BioMerieux, Marcy l'Etoile, France).

2.6. Data Analysis

All data were analyzed using the Statistical Package SPSS version 21.0. Descriptive statistics was used to determine the mean and standard deviation. Mean difference was elucidated using One Way Analysis of Variance (ANOVA) and Duncan Multiple Range test. The *p*- values less than 0.05 were statistically significant.

3. RESULTS AND DISCUSSION

The pollution of aquatic habitats by wastewater from municipal and domestic related activities presents a pressing concern to the environment. Such pollution is usually as a result of indiscriminate release of untreated wastewater and domestic fecal matters, via numerous point and non-point sources, into the fresh water habitat. The results of physicochemical analysis of sediment samples is as presented in Table 1.

Table 1: Physicochemical analysis of sediment samples from the different location

Parameters (unit)	Ikpoba	Upper mission	Ekewan	Ogba	*p*-value
pH	$5.3{\pm}0.01^{c}$	$4.8{\pm}0.02^{a}$	$4.9{\pm}0.01^{a}$	$5.1{\pm}0.02^{b}$	0.001
Electrical conductivity (µS/cm)	$143{\pm}1.25^{a}$	$179{\pm}1.16^{c}$	$210{\pm}1.19^{d}$	$174{\pm}1.03^{b}$	0.000
Organic compound (%)	$0.76{\pm}0.02^{a}$	$1.14{\pm}0.01^{c}$	$1.23{\pm}0.10^{d}$	$0.85{\pm}0.02^{b}$	0.000
Exchangeable acidity (meq/g)	$0.7{\pm}0.01^{a}$	$1.2{\pm}0.03^{c}$	$1.1{\pm}0.00^{b}$	$0.8{\pm}0.00^{a}$	0.001
Sodium (meq/g)	$0.35{\pm}0.01^{a}$	$0.69{\pm}0.00^{c}$	$0.71{\pm}0.00^{c}$	$0.55{\pm}0.01^{b}$	0.000
Potassium (meq/g)	$0.8{\pm}0.00^{a}$	$0.12{\pm}0.00^{a}$	$0.13{\pm}0.01^{a}$	$0.10{\pm}0.00^{a}$	0.359
Calcium (meq/g)	$1.78{\pm}0.21^{b}$	$2.23{\pm}0.17^{c}$	$2.27{\pm}0.03^{d}$	$1.64{\pm}0.03^{a}$	0.000
Magnesium (meq/g)	$0.34{\pm}0.16^{a}$	$0.62{\pm}0.00^{c}$	$0.64{\pm}0.01^{d}$	$0.39{\pm}0.00^{b}$	0.000
Chloride (mg/kg)	$35.3{\pm}0.68^{a}$	$50.2{\pm}0.25^{c}$	$54.5{\pm}0.46^{d}$	$38.2{\pm}0.13^{b}$	0.000
Phosphorus (mg/kg)	$2.16{\pm}0.15^{a}$	$5.11{\pm}0.11^{c}$	$5.19{\pm}0.97^{d}$	$2.57{\pm}0.27^{b}$	0.000
Nitrate (mg/kg)	$3.00{\pm}0.21^{a}$	$6.00{\pm}0.13^{c}$	$6.11{\pm}0.11^{c}$	$3.80{\pm}0.12^{b}$	0.000
Sulphate (mg/kg)	$0.44{\pm}0.00^{a}$	$0.63{\pm}0.00^{b}$	$0.70{\pm}0.03^{b}$	$0.51{\pm}0.00^{a}$	0.001
Clay (%)	$8.8{\pm}0.01^{d}$	$5.3{\pm}0.01^{a}$	$8.1{\pm}0.13^{c}$	$6.8{\pm}0.11^{b}$	0.000
Silt (%)	$2.1{\pm}0.00^{b}$	$1.6{\pm}0.01^{a}$	$3.0{\pm}0.02^{c}$	$1.7{\pm}0.00^{a}$	0.000
Sand (%)	$89.0{\pm}0.01^{a}$	$93.5{\pm}0.00^{b}$	$89.0{\pm}1.07^{a}$	$92.5{\pm}0.19^{b}$	0.000

Values are in triplicate of mean ± standard deviation from May to July 2017. Values which carry same alphabets across rows show no significant difference while values with different alphabets show significant difference.

The physicochemical variables from the river sediment in Table 1 range as follows: pH (4.8±0.02 - 5.3±0.01), electrical conductivity (143±1.25 - 210±1.19 µS/cm), organic compound (0.76±0.02 - 1.23±0.10 %), exchangeable acidity (0.7±0.01 - 1.2±0.03 meq/g), sodium (0.35±0.01 - 0.71±0.00 meq/g), potassium (0.10±0.00 - 0.8±0.00 meq/g), calcium (1.64±0.03 - 2.27±0.17 meq/g), magnesium (0.34±0.16 - 0.64±0.01 meq/g), chloride (35.3±0.68 - 54.5±0.46 mg/kg), phosphorus (2.16±0.15 - 5.19±0.97 mg/kg), nitrate (3.00±0.21 - 6.11±0.11 mg/kg), sulphate (0.44±0.00 - 0.70±0.03 mg/kg), clay (5.3±0.01 - 8.8±0.01 %), silt (1.6±0.01 - 3.0±0.02 %), sand (89.0±0.01 - 93.5±0.00 %).

The pH of the fresh water habitat is a vital index of water quality and the degree of pollution in the aquatic environments. The pH of the sediment observed in the study was highly acidic. Although the pH recorded was lower than that of sediments in Mada river (Tukura *et al.,* 2012), however the pH of sediment was generally acidic. The low pH may be due to oxidation of ferrous sulphate to sulphuric acid (FeS to H_2SO_4) (Ramanathan, 1997; Tukura *et al*., 2012). Ferrous sulphate in river results in weathering progressions of naturally occurring iron minerals which include siderite, magnetite, goethite and hematite. At low pH, the adsorbed metal ion in sediments contends with hydrogen ion resulting in the remobilization into the water column. The variation in pH among sampling location may be due to redox changes in the different locations (Tukura *et al*., 2012). Iron do not play a direct role in altering water; however, water agitation brings about aeration of water. The aeration makes oxygen available to form hydrated oxides with iron (iron corrosion) which changes colour to a reddish-brown to a silvery colour.

Electrical conductivity may predict the position of inorganic pollution which is a degree of ionized particles and total dissolved solid in the water (Tukura *et al*., 2012). The range of values recorded for electrical conductivity recorded was relatively narrow (143 to 210 µS/cm) compared to the wide range (68 to 1,029 µS/cm) reported elsewhere (Chigor *et al*., 2012). The sediments accumulate precipitated and suspended particles onto the base of the water thereby are a reservoir of diverse contaminants of certain substances that have low degree of degradability and solubility (Biney *et al*., 1994; Singare *et al*., 2014). Pollutants are preserved in sediments for very long-time duration depending on the biochemical and physical-chemical properties of the substrata (Singare *et al*., 2014).

Nitrate was found in all sampled locations with Ekewan axis location and upper mission location appreciably higher than the Ikpoba and Ogba locations. Nitrate could be found in diverse area in the environment, however the sources of the nitrate detected in the study could have been as a result of decomposed animal waste as animal activities was highly predominant around the sampled location and through municipal and sewage wastes as seen in Ogba location. Also, runoff of fertilizer from farm land may have also contributed to the nitrate. The release and periodic sedimentation of sewages waste released into the river maybe source of phosphoric content in sediment. The report in this study is comparable with those documented by (Ekeanyanwu *et al*., 2010; Tukura *et al*., 2012).

Table 2: Distribution of some members of Enterobacteriaceae in sediments from the study locations

Location	*Salmonella* (cfu/g)	*Escherichia coli* (cfu/g)	Coliform (cfu/g)
Ikpoba	$1.00\times10^3\pm0.04^a$	$2.46\times10^3\pm0.09^c$	$1.13\times10^3\pm0.73^c$
Upper mission	$6.33\times10^2\pm0.00^a$	$1.50\times10^3\pm0.85^c$	$6.66\times10^1\pm0.18^a$
Ekewan	$4.36\times10^3\pm0.61^b$	$1.14\times10^1\pm0.12^a$	$2.13\times10^3\pm0.39^c$
Ogba	$9.66\times10^2\pm0.00^a$	$3.33\times10^2\pm0.60^b$	$4.00\times10^2\pm0.68^b$
p-value	0.001	0.000	0.000

The microbiological analysis of the sediments samples reveals high pollution of organic and fecal matters. This seems to be highly of anthropogenic influence as observed during the on-site assessment. At Ikpoba location, abattoir effluents were discharged into the river. Bacteria from abattoir waste discharged into water columns can subsequently be adsorbed onto sediments, and when the bottom stream is disturbed, the sediment releases the bacteria back into the water columns thus presenting long-term health hazards (Nafarnda *et al*., 2012). At upper mission location, a shrine is located near the river where its worshippers come and render sacrifices thereby leading to the pollution of the river. At Ogba location, the inhabitants of the area lack proper municipal waste disposal, hence their domestic effluents and sanitary waste including those from bathing and sewage are channeled into the river. Also, at Ekewan river location, cattle activities were found along the bank of the river. These anthropogenic activities seen around the river has without doubt contributed to the microbial contamination of the river which includes *Salmonella* isolates, coliforms and *E. coli* detected in the sediments. Coliforms generally attach to tiny constituents (<2 µm), thereby enhancing easiness of transport and dispersal into the environment (Muirhead *et al*., 2006; Goldscheider *et al*., 2010). When water base flow occurs and there seems to be no turbulence, bacteria bound to sediment scarcely contribute to microbial pathogens in water. However, when turbulence is initiated, this results in mixing, an elevation in oxygen distribution, bubble generation, surface stress which increases the rate of dispassion of bacteria from sediment which could be influenced by strain and shape of bacteria and the binding strength within bacteria biofilm (Gomez-Suarez *et al*., 2001;). In fresh water environment, the dispersal rate of *E. coli* during sequential events is linked to the multiplication of the bacteria between events (Shelton *et al*., 2014) and the depth of sediment which is subject to scour (Harvey *et al*., 2012). The major targets to prevent sediment pollution are preventing the modification and deformation of water basins, reducing the basic pollution of hydro ecosystems, and enhancing the natural self-purification processes in sediments (Bridges *et al*., 2011; Yotinov *et al*., 2017).

4. CONCLUSION

Due to rapid urbanization, environmental pollution by municipal waste has tremendously increased. The release of domestic and anthropogenic related waste pollutes aquatic habitat. This further accumulates in the sediment and persist for a long time period. This long term persistence of these contaminant in sediments impacts on the health of the environment. Hence there is need for continual monitoring of environmental waters and also the deployment of preventive measures to alleviate the contamination of the environment.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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