



Physicochemical and Bacteriological Analyses of Eniong River Water and Sediment in Southern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The diversity of bacteria in freshwater is influenced by some important physicochemical factors and autotrophic nutrients. Their diversity can be employed as a sensitive pollution indicator in the environment and change because bacteria respond quickly to changes in the ecosystem. Analysis of physicochemical and bacteriological quality of humic freshwater and sediment was carried out on samples from three (3) geo-referenced sections of Eniong River, Akwalbom State designated ST3 (Upstream), ST2 (Midstream) and ST1 (Downstream) using standard analytical methods. The results obtained were compared with World Health Organization (WHO) and Federal Ministry of

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Environment (FMENV) standards. The study revealed that the samples had a mean heterotrophic bacterial count of $5.95 \pm 0.41 \log_{10}$ CFU/g and heavy pollution with faecal bacteria such as ($4.31 \pm 2.51 \log_{10}$ CFU/g TCC and $5.19 \pm 0.33 \log_{10}$ CFU/g FCC), including mean counts of *Staphylococcus spp* ($5.90 \pm 1.16 \log_{10}$ CFU/g), *Vibrio spp* ($.21 \pm 1.04 \log_{10}$ CFU/g), *Salmonella-Shigella* ($3.15 \pm 0.33 \log_{10}$ CFU/g), and *Actinomyces* ($3.29 \pm 2.49 \log_{10}$ CFU/g) that considerably exceeded the recommended range of WHO/FMENV for portability. The most abundance bacteria in were *Bacillus subtilis*, *S. aureus* and *Shigella spp* (55.6%) followed by *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella*, *Nocardia sp*, *Proteus sp.*, *Staphylococcus spp*, *Burkholderia pseudomallei* and *Enterococcus faecalis* with a prevalence rate of 44.4%. The results showed the mean DO (7.67 ± 0.037 mg/ml), EC ($85.07 \pm 0.52 \mu\text{S/cm}$) and temperature (27.480 ± 0.049) of Eniong river water were within acceptable standard values while TSS (12.5 mg/ml) and TDS (19.0 ± 1.30 mg/ml) values were not. Also, Pb, Cd, Vn, Ni and Cu recorded higher values while the concentrations of Fe²⁺, K⁺, Na⁺, Ca²⁺ and Mg²⁺ were remarkably low in both water and sediment. The mean pH of the river and sediment were 6.48 ± 0.018 and 6.42 ± 0.019 , respectively. Similarly, among the anionic surfactant, the mean values for nitrate, carbonate and sulphate were higher in sediment than in water, while carbonate was not detected in water. The findings imply that the quality of Eniong River water and sediment is heavily compromised. Therefore, the creation of awareness on the importance of water safety and hygiene, regular monitoring of drinking water quality and enforcement of compliance with the standards by regulatory agencies are recommended to eschew possible health hazards.

Keywords: Faecal coliforms; physicochemical; pollution; sediment; freshwater.

1. INTRODUCTION

“Sediments are naturally occurring material that is degraded by processes of weathering and erosion, which are most often transported by water (fluvial processes), wind (aeolian) and ice (glacial process)” [1]. “The most common examples of fluvial transport and deposition are beach sands and river channel deposits, though sediment also often settles out of slow-moving or standing water in lakes and oceans. The desert dunes and loess are examples of Aeolian transport and deposition” [2].

“The urban river systems, including the Eniong River, usually receive various types of contaminants including toxic metals, persistent organic pollutants, pathogenic organisms and pharmaceutical drugs such as antibiotics, which constitute major environmental and human health concerns” [3]. “The impact of water deterioration arising from these pollutants constitutes tremendous risks to human and environmental health, especially in developing countries” [4]. “In the aquatic environment, sediments may constitute a reservoir for these pollutants. According to Poté and colleagues, sediments can accumulate contaminants and pathogenic organisms at a concentration of 10-1000 times higher than the overlying water” [5,6,7]. Therefore, the sediment represents an important compartment for the assessment of the pollution in river reservoir systems.

Several studies have demonstrated that “sediments may constitute an important reservoir of faecal indicator bacteria (FIB) in freshwater environments” [7]. “Since bacteria communities are involved in many sediment processes, they may also be used as a reliable indicator of ecosystem integrity. In sediment, high species richness diversity promotes interspecies relationships and inter-population interactions. More diverse bacteria communities can better cope with disturbance and stress than low diversity in what is known as co-metabolism. Bacteria play an indispensable role in the environment and many organisms are dependent on their relationships with bacteria” [8].

“The accumulation of faecal indicator bacteria and pathogenic microorganisms in sediments has been attributed to the sorption of the microorganisms to particles suspended in water, whereas desorption of the microorganisms from sediment can occur under changing physicochemical conditions, such as pH, oxygen availability and redox conditions. Faecal pollution can originate from a variety of human and nonhuman sources, but FIB contamination from human faecal material is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens” [9,10]. “Additionally, the use of wastewater contaminated by bacteria of faecal origin for irrigation is widely practised in developing countries such as in sub-Saharan Africa,

including Nigeria but little is known in these countries regarding the potential health risk associated with its use" [11]. "A larger population of people living in northern Cross River depend largely on humic freshwater from Eniong River for irrigation, drinking and other domestic activities. Studies have demonstrated that pathogens contained in the wastewater used for irrigation and other activities can be transferred to raw vegetables and fresh produce" [12,13].

This study was carried out to determine the levels and concentration of some physicochemical and bacteriological indices of benthic sediment and humic freshwater samples from Eniong River in the Itu Local Government Area of Akwalbom State, Nigeria.

2. MATERIALS AND METHODS

2.1 Description of Study Site

The study area is a humic freshwater ecosystem of Eniong River, a tributary of the Middle course of the Cross River located on the South-Eastern coast of the Niger Delta, Akwalbom State, Nigeria. Eniong River is located on the South-Eastern coast of Niger Delta between latitude $05^{\circ} 15'$ and $56.0''$ N and longitude $05^{\circ} 12'$ N and $05^{\circ} 054''$ E.

2.2 Sample Collection

The humic fresh water and sediment samples were collected from three sample stations designated ST3 (Upstream), ST2 (Midstream) and ST1 (Downstream) of the Eniong River as described by Nweke et al., [14]. A total of 9 benthic sediment samples were aseptically collected using Eckman sediment grab into 95% ethanol-sterilized plastic containers in ice park coolers and transported to the Microbiology laboratory within 24 h of collection for physicochemical and bacteriological analysis. Samples were stored in the laboratory at a refrigeration temperature of 4°C until required for further analysis.

2.3 Bacteriological Analysis

Before analysis, the sediment samples were homogenized. Ten (10) g of each sample was weighed out into 90 ml of sterile deionized water and vigorously shaken for 1 minute using a vortex shaker to dislodge the microbiota. Treated samples were allowed to settle for 10 minutes before the withdrawal of supernatant for serial

dilution. Ten-fold serial dilution was carried out for the enumeration of densities of the different microbial groups.

2.4 Enumeration of Bacterial Densities in Eniong River Sediment

Several bacteriological methods were employed for the enumeration of various bacterial groups. The densities of the following groups of bacteria in Eniong River were determined by standard methods:

- (a) Total heterotrophic bacteria (THB)
- (b) Densities of pollution indicator bacteria such as total coliform count (TCC), faecal coliform count (FCC), Salmonella-Shigella count (SSC), Staphylococcus aureus count (SAC), among others.
- (c) Densities of oil-degrading bacteria
- (d) Densities of nitrogen-fixing bacteria
- (e) Densities of Actinomycetes
- (f) Densities of sulphate-reducing bacteria
- (g) Densities of sulphate solubilizing bacteria
- (h) Densities and isolation of *Pseudomonas* species

2.5 Culture Media

The culture media employed in the course of this research include: Nutrient agar (NA), eosin methylene blue (EMB) agar, MacConkey agar (MA), mannitol salt agar (MSA), thiosulfate citrate-bile salt sucrose agar (TCBS), Salmonella-Shigella agar (SSA), Cetrimide agar (CA), and mineral salt medium (MSM), among others. The media were prepared according to recommendations by the manufacturers (Oxoid, UK).

2.6 Determination of Total Heterotrophic Bacteria (THB)

The counts of total heterotrophic bacteria were determined by the pour plate techniques using nutrient agar (NA) as the analytical media. The NA medium was amended with Griseofulvin ($50 \mu\text{g/ml}$) to prevent the growth of fungal contaminants. Inoculated NA plates were incubated at $35-37^{\circ}\text{C}$ for 24 hours before the enumeration of bacterial colonies [15].

2.7 Determination of Densities of Pollution Indicator Bacteria

The bacteriological culture media that were employed are MacConkey agar (MA), eosin

methylene blue agar (EMB), thiosulphate citrate bile salt sucrose (TCBS) agar, mannitol salt agar (MSA), Re-enforced Clostridial agar and *Salmonella-Shigella* agar (SSA). The media were prepared and used for enumeration and isolation of total coliforms (TCC), faecal coliforms (FCC) (*Escherichia coli*), *Vibrio* (VB), *Staphylococcus aureus*, *Clostridium*, *Salmonella* and *Shigella* (SSC), respectively [16].

2.8 Determination of Densities of Actinomycetes and *Pseudomonas aeruginosa*

The density of *Actinomycetes* was enumerated after 7 days of incubation at 28°C±2°C using acidified Nutrient agar/ Starch nitrate agar, while colonies of *Pseudomonas aeruginosa* were enumerated and isolated using Cetrimide agar plates incubated aerobically at room temperature for 24 - 48 hours. *Pseudomonas aeruginosa* produced blue to blue-green pigmented colonies [16].

2.9 Isolation, Purification and Maintenance of Pure Bacterial Cultures

Distinct and representative colonies from the culture plates were selected for characterization. Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allowed to grow for 24 hours before stocking. Pure isolates of the bacteria were maintained on agar slants as stock in McCartney bottles and were preserved in the refrigerator at 4 °C for further use.

2.10 Characterization and Identification of Bacterial Isolates

Various indices were employed to characterize and identify bacterial isolates. These were colonial appearance on solid media, changes in the surrounding medium, pigment production, Gram reaction, microscopic appearance, sugar fermentation and other biochemical tests such as catalase, coagulase, indole, citrate, urease, methyl red, VogesProskauer, oxidase, motility and spore staining tests. The test results were evaluated using characteristics presented in Bergey's Manual of Determinative Bacteriology [17].

2.11 Physicochemical Analysis of the Eniong River Sediment and Water

Standard methods recommended by APHA [18] were adopted for the analysis of the sediment samples. Unstable parameters such as pH of sediment and temperature were measured *in situ*.

2.12 Determination of pH

Measurement of pH was carried out using a Scott Gerate pH meter, which had been previously calibrated in the laboratory [19].

2.13 Determination of Temperature

Temperature was measured *in situ*, using a thermometer and thermistor using a thermocouple electrode calibrated in 0.2°C units from 0°C to 100°C.

2.14 Determination of Total Dissolved Solids (TDS)

This was determined according to APHA [18] instrumental method using the HACH TDS meter (Mettler Toledo conductivity /TDS meter model MC 126).

2.15 Determination of Salinity

The salinity of water as chloride content was determined titrimetrically by the silver-nitrate method [18].

2.16 Determination of Total Suspended Solids

TSS was determined by filtering a well-mixed aliquot (100 ml) of the water sample through a dried and pre-weighed Millipore filter paper using a vacuum filtration apparatus. The filter paper was dried at 105°C. The difference in weight of the filter paper represents the total suspended solids. This was reported in mg/l after calculation [18].

2.17 Determination of Dissolved Oxygen (DO)

Dissolved oxygen (DO) of water was measured using membrane electrodes (Voltametric and galvanometric).

2.18 Determination of Trace Metals

Trace metals such as lead (Pb), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Zinc

(Zn), Vanadium (Vn) and Nickel (Ni) were determined (after nitric acid digestion) using an atomic absorption spectrophotometer [20].

2.19 Determination of Electrical Conductivity

Conductivity was measured with the aid of a conductivity meter. The conductivity meter was calibrated with KCl solution [19].

2.20 Determination of Exchangeable Cations in Sediment

The exchangeable cations in the sediment samples were extracted with 1ml ammonium acetate. Potassium (K+) and sodium (Na+) in the extract were determined by flame photometer while calcium (Ca²⁺) and magnesium were determined by an EDTA filtered method [19,21].

2.21 Determination of Total Organic Nitrogen (TON)

This was determined using the Kjeldahl analysis method as described by Association of Official Analytical Chemists (AOAC) [22].

2.22 Determination of Total Organic Carbon (TOC)

The TOC content of the sediment was determined by dichromate wet oxidation as previously described by Ajao and Fagade, [23].

3. RESULTS

3.1 Total Heterotrophic Bacteria (THB)

The humic sediment of Eniong River harbours contains remarkable bacteria load which are presented in (Figs. 1 - 7). Thirteen (13) different bacterial groups were isolated. The results showed that the heterotrophic bacterial counts ranged from 5.92 log₁₀CFU/g downstream to 5.98 log₁₀CFU/g upstream with a mean count of 5.95±0.41 log₁₀CFU/g (Fig. 1).

3.2 Densities of Pollution Indicator Bacteria

The densities of pollution indicator bacteria are presented in Figs. 2 – 6. The results showed that total coliform count ranged from 4.24 log₁₀CFU/g downstream to 4.86 log₁₀CFU/g midstream with a mean count of 4.31±2.51 log₁₀CFU/g (Fig. 2). The results of the faecal coliform counts varied between 5.06 log₁₀cfu/g midstream and 5.28 log₁₀CFU/g downstream with a mean count of 5.19 ±0.33 log₁₀CFU/g

(Fig. 3). *Vibrio* counts were within the range of 5.01 log₁₀CFU/g upstream to 5.09 log₁₀CFU/g downstream with a mean count of 5.21±1.04 log₁₀CFU/g (Fig. 4). *Staphylococcus* count ranged from 5.83 log₁₀CFU/g midstream to 5.97 log₁₀CFU/g upstream with a mean count of 5.90±1.16 log₁₀CFU/g (Fig. 5) while *Salmonella-Shigella* count ranged from 3.0 log₁₀CFU/g upstream to 3.26 Log₁₀CFU/g downstream with a mean count of 3.15±0.33 log₁₀CFU/g (Fig. 6).

3.3 Densities of Actinomycetes

The *Actinomycetes* count is shown in Fig. 7. The result showed that it ranged from 3.0 log₁₀cfu/g downstream to 3.78 log₁₀CFU/g upstream with a mean count of 3.29±2.49 log₁₀CFU/g (Fig. 7). The result also indicates that the most abundant species was heterotrophic bacteria with a mean count of 5.95±0.41 log₁₀CFU/g, followed by *Staphylococcus* with mean counts of 5.90±1.16log₁₀CFU/g. The least abundance was *Actinomycetes* with a mean value of 3.29±2.49 log₁₀CFU/g (Fig. 7). Heterotrophic bacteria showed the highest mean growth in an upstream station (5.97±1.16 log₁₀CFU/g (Fig. 1), followed by *Staphylococcus* 6.0 log₁₀CFU/g in an upstream station (Fig. 5) while the lowest mean count 3.0 log₁₀CFU/g was observed by *Actinomycetes* in downstream station (Fig. 7). Values are means of duplicates ± standard deviation (SD) of the mean. TCC = 10/100 MI and THBC = 100000/mL WHO, [24].

3.4 Distribution and Prevalence of Bacteria in Sediment at Different Locations of Humic Freshwater Ecosystem

The prevalence of bacterial isolates in the humic freshwater sediment ecosystem are presented in Table 1. Amongst the isolates, the most encountered bacteria were *Bacillus subtilis*, *Staphylococcus aureus* and *Shigella* at 55.6% where are the percents of the other two types prevalence in upstream and downstream respectively. This was closely followed by *Pseudomonas aeruginosa* (downstream and midstream), *Bacillus subtilis* (downstream and midstream), *Salmonella* (downstream), *Nocardiasp* (upstream), *Proteus sp.* (midstream), *Staphylococcus* (midstream), *Burkholderiapseudomallei* (upstream) and *Enterococcus faecalis* (midstream) with prevalence rate of 44.4%. In general, the highest incidence of bacteria was found in the downstream station (55.6%) while the least prevalence was in the upstream station (11.1%).

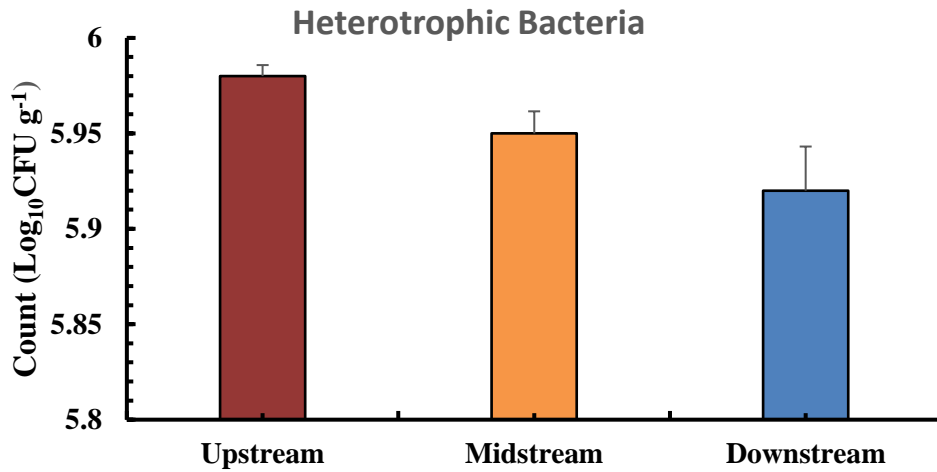


Fig. 1. Heterotrophic bacteria counts in upstream, midstream and downstream of Eniong River

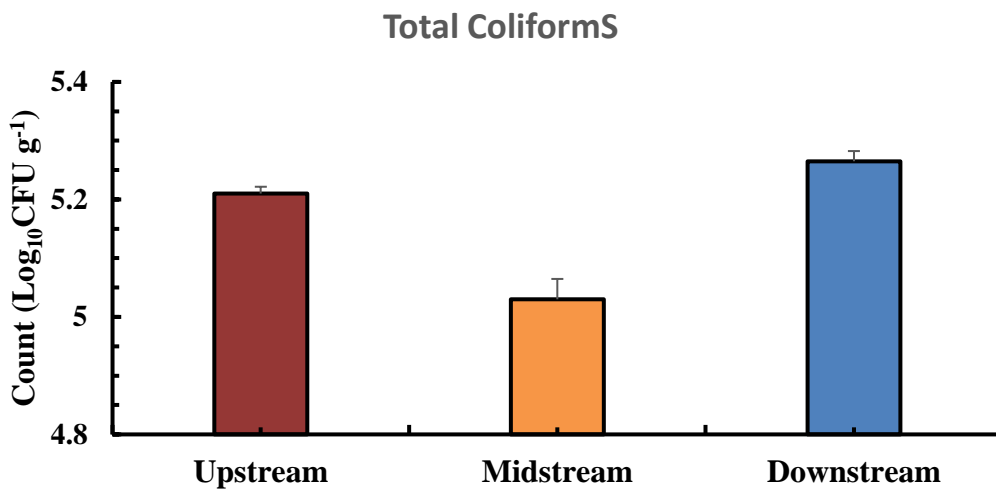


Fig. 2. Total coliform counts in upstream, midstream and downstream of Eniong River

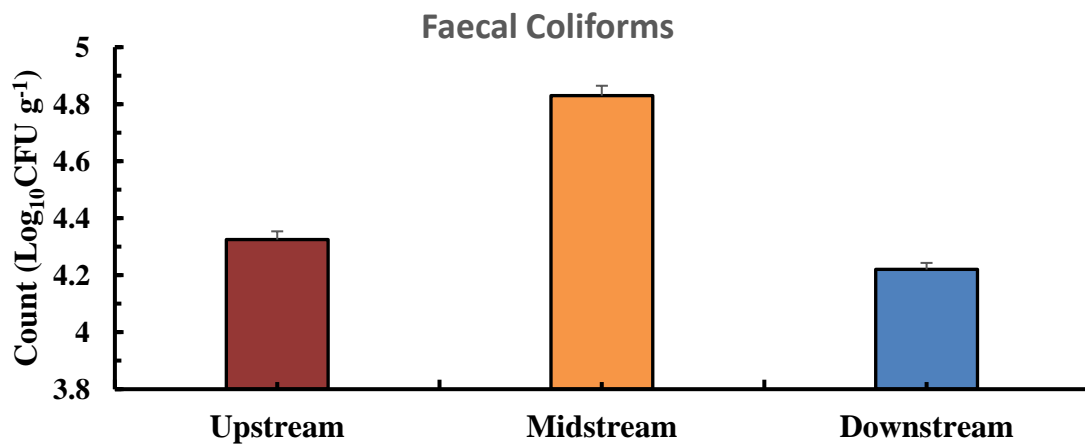


Fig. 3. Faecal coliform bacteria count in upstream, midstream and downstream of Eniong River

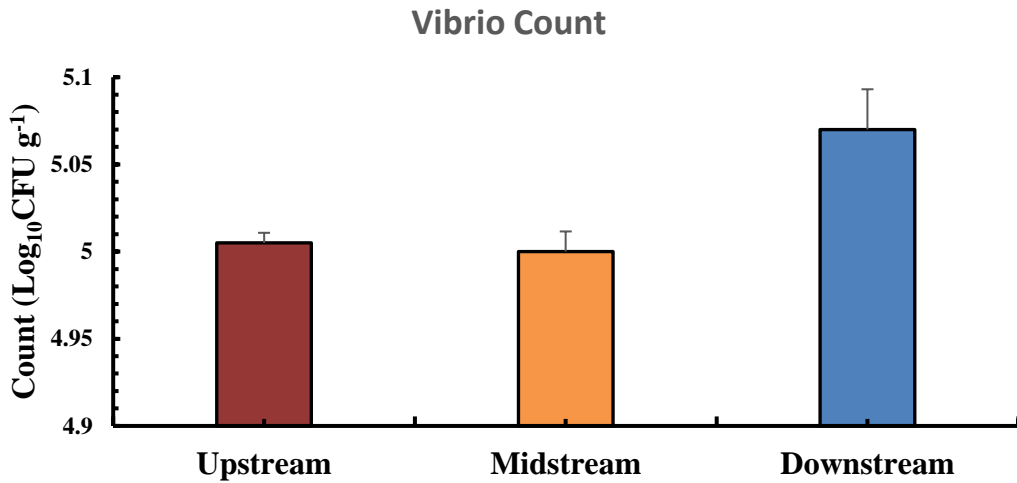


Fig. 4. *Vibrio* counts in upstream, midstream and downstream of Eniong River

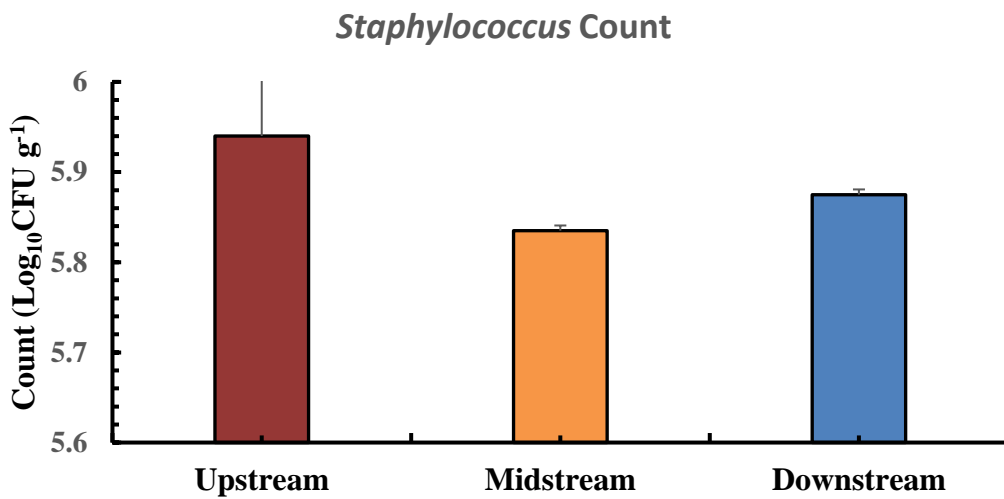


Fig. 5. *Staphylococcus* counts in upstream, midstream and downstream of Eniong River

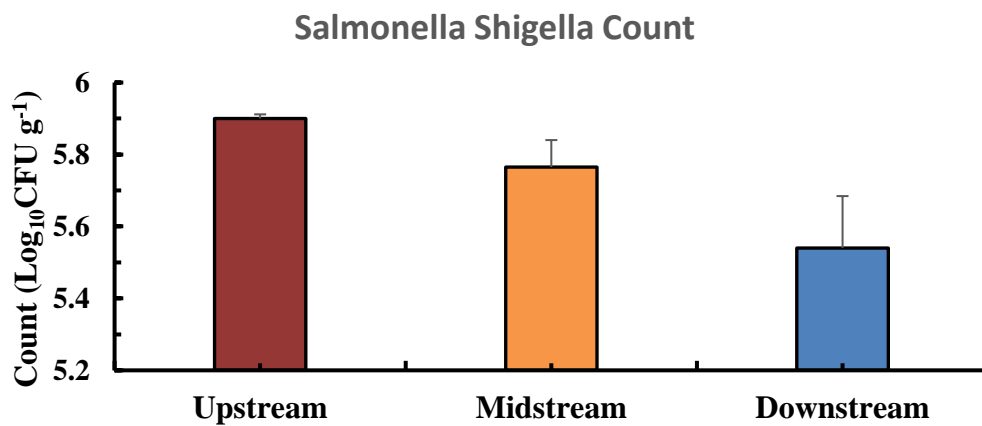


Fig. 6. *Salmonella Shigella* counts in upstream, midstream and downstream of Eniong River

Table 1. Distribution and prevalence of bacteria in Eniong River sediment ecosystem

Isolates	Upstream ST₁	Prevalence Rate(%)	Midstream ST₂	Prevalence Rate(%)	DownstreamST₃	Prevalence Rate (%)
<i>Staphylococcus aureus</i>	+2	22.2	+(4)	44.4	+ (5)	55.6
<i>Bacillus subtilis</i>	+(5)	55.6	+(4)	44.4	+ (4)	44.4
<i>Shigella</i>	+(2)	22.2	+(3)	33.3	+ (5)	55.6
<i>Pseudomonas aeruginosa</i>	+(2)	22.2	+(4)	44.4	+ (4)	44.4
<i>Burkholderiapseudomallei</i>	+(4)	44.4	+(2)	22.2	+(2)	22.2
<i>Enterococcus faecalis</i>	+1	11.1	+(4)	44.4	+ (3)	33.3
<i>Salmonella</i>	+(1)	11.1	+(2)	22.2	+ (4)	44.4
<i>Nocardiasp</i>	+(4)	44.4	+(3)	33.3	+(1)	11.1
<i>Proteus sp</i>	+(3)	33.3	+(4)	44.4	+(2)	22.2
<i>Klebsiella</i>	-	-	+2	22.2	+ (3)	33.3
<i>Escherichia coli</i>	-	-	+(1)	11.1	+ (3)	33.3
<i>Micrococcus</i>	+2	22.2	+(1)	11.1	+(1)	11.1
<i>Enterobacter sp</i>	-	-	-	-	+ (2)	22.2
<i>V. cholera</i>	-	-	-	-	+(1)	11.1
Species Richness	10	288.7	12	377.4	14	455.3

Note: Values in parenthesis are frequencies of occurrence of bacteria

Table 2. Physicochemical properties of Eniong River water and sediment samples of humic freshwater ecosystem

Parameter	water (mean)	sediment (mean)	WHO/FMEnv
pH	6.48±0.018	6.42±0.019	6.5-8.5
Temperature	27.48°C±0.049	27.0°C±1.41	20-30
DO	7.67±0.037 (mg/ml)	-	5-10
TDS	19.0±1.30 (mg/ml)	-	250
TSS	12.5±0.34 (mg/ml)	-	50
Conductivity	85.07±0.52 (µS/cm)	-	50-100
Total organic carbon	4.79±0.13 %	11.17±0.067 %	
Total nitrogen	0.24±0.037%	0.55±0.051%	
Chloride	0.130±0.018 (mg/ml)	0.36±0.013(mg/kg)	250
Flouride	0.01±0.00 (mg/ml)	<0.01± 0.00(mg/kg)	
Sulphate	16.0± 1.31(mg/l)	41.0± 2.24(mg/kg)	250
Phosphate	2.17± 0.033(mg/l)	2.01± 0.0058(mg/kg)	
Nitrate	23.4± 0.27(mg/l)	24.2± 0.24(mg/kg)	10-45
Nitrite	0.070± 0.041(mg/l)	0.012± 0.060(mg/kg)	5
Carbonate	-	45.3mg/kg	
Salinity	0.0002± 0.00(mg/l)	-	
Exchangeable cations:			
K+	0.06±0.022mg/l	0.14±0.0082mg/kg	NS
Na+	0.3±0.014mg/l	10.4±0.14mg/kg	NS
Ca ²⁺	4.39±0.11mg/l	10.9±0.84mg/kg	75mg/kg
Mg ²⁺	2.09±0.54mg/l	2.19±0.11mg/kg	50mg/kg
Total Fe	1.73± 0.014(mg/l)	13.25±0.13(mg/kg)	
Cr	0.11± 0.00(mg/l)	0.21± 0.014(mg/kg)	
Cu	0.41± 0.013(mg/l)	0.56± 0.30(mg/kg)	
Pb	0.232± 0.0013(mg/l)	1.124± 0.0054(mg/kg)	
Zn	0.025± 0.0024(mg/l)	0.008± 0.0013(mg/kg)	
Cd	0.015± 0.0041(mg/l)	0.028± 0.0014(mg/kg)	
Vn	0.003± 0.00(mg/l)	0.001±0.00(mg/kg)	
Ni	0.009± 0.0051(mg/l)	0.002± 0.00082(mg/kg)	
Particle size distribution:			
Sand	-	71 %±1.41%	
Clay	-	11 %±0.14%	
Silt	-	17 %±0.22%	

Key: WHO -World health organization; FMENV-Federal ministry of environment

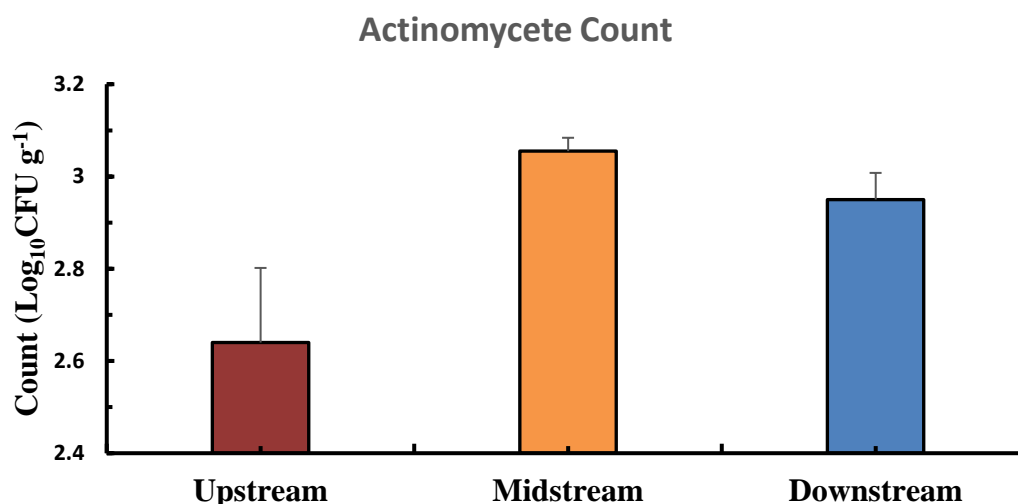


Fig. 7. Actinomycetes counts in upstream, midstream and downstream of Eniong River

3.5 Physicochemical Properties of Eniong River Water and Sediment Samples

The physicochemical properties of humic water and sediment samples are presented in Table 2. The results showed that the mean pH of the surface water and sediment samples were 6.48 ± 0.018 and 6.42 ± 0.019 respectively. The temperature levels were ambient with mean values of $27.48 \text{ }^\circ\text{C} \pm 0.049$ and $27.0 \text{ }^\circ\text{C} \pm 0.41$ recorded for humic water and sediment, while the DO, TDS, TSS and conductivity recorded for the surface water samples were 7.67 ± 0.037 mg/l, 19.0 ± 1.30 mg/l, 12.5 ± 0.34 mg/l and 85.0 ± 0.052 $\mu\text{S/cm}$, respectively. The total organic carbon content of sediment was 11.17 ± 0.067 % whereas 4.79 ± 0.13 % was recorded for the surface water sample. The percentage of total nitrogen in both sediment and surface water was 0.55 ± 0.051 % and 0.24 ± 0.037 %. The exchangeable cation level in sediment was low at K^+ (0.14 ± 0.0082 mg/kg); Na^+ (10.4 ± 0.14 mg/kg); Ca^{2+} (10.9 ± 0.84 mg/kg) and Mg^+ (2.19 ± 0.11 mg/Kg) while the water column recorded lower values of 0.06 ± 0.022 mg/l; 0.3 ± 0.014 mg/l; 4.39 ± 0.11 mg/l and 2.09 ± 0.54 mg/l for K^+ , Na^+ , Ca^{2+} and Mg^{2+} respectively. The concentration of nutritive salts also varied between the surface water and sediment samples but was generally higher in humic sediment samples. For example, mean concentrations of 0.36 ± 0.018 mg/kg Cl^- , $<0.01 \pm 0$ mg/kg F^- , 41.0 ± 1.31 mg/kg SO_4^{2-} , 2.01 ± 0.033 mg/kg PO_4^{2-} , 24.2 ± 0.27 mg/kg NO_3^- and 0.012 ± 0.041 mg/kg NO_2^- were respectively observed in sediment samples, while 0.13 ± 0.013

mg/l Cl^- , 0.01 ± 0.00 mg/ml F^- , 16.0 ± 2.24 mg/l SO_4^{2-} , 2.17 ± 0.0058 mg/l PO_4^{2-} , 23.4 ± 0.24 mg/l NO_3^- and 0.040 ± 0.06 mg/l NO_2^- were obtained from the surface water samples analyzed. The mean salinity of the surface water was 0.0002 ± 0.00 mg/l. In humic sediment, the trace metal levels were 0.002 ± 0.0082 mg/kg Ni, 0.001 ± 0.00 mg/kg Vn, 0.028 ± 0.0014 mg/kg Cd, 0.008 ± 0.0013 mg/kg Zn, 1.124 ± 0.0054 mg/kg Pb, 0.56 ± 0.30 mg/kg Cu, 0.21 ± 0.014 kg/kg Cr and 13.25 ± 0.13 mg/kg Fe. On the other hand, trace metals in surface water were 0.009 ± 0.0051 mg/l Ni, 0.003 ± 0.00 mg/l Vn, 0.015 ± 0.0041 mg/l Cd, 0.025 ± 0.0024 mg/l Zn, 0.232 ± 0.0013 mg/l Pb, 0.41 ± 0.013 mg/l Cu, 0.11 ± 0.00 mg/l Cr and 1.73 ± 0.014 mg/l Fe. The result of particle size distribution (PSD) for the humic sediment showed a composition of sand fractions (71 ± 1.41 %) followed by silt (17 ± 0.22 %) and clay (11 ± 0.14 %) fractions.

4. DISCUSSION

The findings of this research revealed that the humic sediment of Eniong River harbours remarkable bacterial loads. A total of 13 different bacterial groups were isolated, identified and characterized. The best growth was observed for heterotrophic bacteria. The densities of heterotrophic bacteria from the sediment were slightly lower than values reported in Australia where the numbers ranged from 8.30 Log₁₀CFU/g cells to 10.56 Log₁₀CFU/g cells dry weight of sediment [23]. The increase in population levels of heterotrophic

microorganisms may be associated with inputs from stormwater [25].

Bacteria isolated from Eniong River include *V. cholera*, *V. parahaemolyticus*, *E. coli*, *S. typhi*, *Shigella sonnei*, *Enterobacter agglomerans*, *Staphylococcus aureus*, *S. epidermidis*, *Proteus vulgaris*, *B. cereus*, *P. aeruginosa*, *Desulfovibrio vulgaris*, *Desulfuromonas* sp., *Desulfobacter* sp., *Micrococcus* sp., *Burkholderia* sp., *Lactobacillus casei*, *Sphingomonas* sp., *Serratiamarcescens*, *B. subtilis*, *C. botulinum*, *C. perferingens*, *Mycobacterium* sp., *Flavobacteriumaquatile*, *Brevibacterium* sp., *Achromobacter* sp., *Nitrosomonas* sp., *Desulfovibrio*hydrophilus, *Citrobacterfreundii*, *P. fluorescens*, *Pseudomonas putida*, *Acetobacteracetii*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Yersinia pestis*, *Streptococcus* sp, and *Aeromonashydrophila* while. The actinomycetes include *Streptomyces griseus* and *Nocardia* species. Previous studies have reported similar results [26,27]. Some of these species are opportunistic pathogens of human origin while others are indicators of pollution of water bodies. These pathogens are associated with infections such as catheter-associated bacteraemia, urinary, gastrointestinal, and respiratory tract infections as well as wound infections [28].

“The occurrence of pollution indicator groups of bacteria varied with the sampling station, with high densities of total coliform mean count of 4.31 ± 2.51 log₁₀CFU/g obtained from the sediment. The high incidence of coliforms observed from the humic freshwater sediment sample may be attributed to human impact and a pointer to the inherent risk of disease outbreak if the contaminated water is deliberately or accidentally consumed. This assertion is confirmed by the equally high densities of *Escherichia coli*, *Vibrio*, *Salmonella* and *Shigella* in the freshwater sediment. This finding is in agreement with the report that reduction in faecal coliforms often correlates with reduction in *Salmonella* species and other pathogenic microorganisms” [28]. “Humans and animals could be exposed to the pathogens directly by coming in contact with contaminated sediments and water or indirectly by consuming or drinking water or seafood contaminated by the pathogens. World Health Organization recommended one *E. coli* per 100 ml of water to be normal” [29]. However, the HBC were within statutory criteria but TCC and FCC exceeded acceptable limits [24]. “Such high *E. coli* and coliform loads may be linked to open defecation,

soil erosion, discharge of industrial and domestic wastes, bathing/washing and recreational activities, thereby resulting in the proliferation of waterborne pathogens as earlier reported in different rivers” [30]. “The elevated faecal coliform counts further validate obvious waterborne faecal pollution as commonly reported in developing countries” [31]. The *Vibrio* counts recorded in the sediment were within a range of 5.01 Log₁₀cfu/g upstream to 5.09 Log₁₀cfu/g downstream with a mean count of 5.21 ± 1.04 Log₁₀cfu/g. In the Eniong River sediment, the presence of *Pseudomonas* and *Bacillus* was detected in considerable densities. This could be attributed to their widespread in water and soil ecosystems as reported by Rogers et al. [32].

The densities of *Clostridium* sp, *Actinomycetes* and *Lactobacillus* were relatively low in the sediment. Bacteria belonging to the genus *Lactobacillus* are members of the lactic acid bacteria (LAB), a broadly defined group characterized by the formation of lactic acid as the sole or main end product of carbohydrate metabolism. The diversity of microbial groups observed in the sampled points may have been favoured by the interplay of the various ecological factors and anthropogenic activities. Such a plethora of microorganisms has also been reported by other workers [33]. The low count in the aquatic sediment system of *Actinomycetes* may be because *Actinomycetes* live predominantly aerobically, meaning they need oxygen for their metabolism [34].

In this study, hydrocarbon-utilizing bacteria from humic freshwater sediment were isolated, and characterized and their ability to utilize crude oil is determined. Among the isolates, *Bacillus subtilis*, *Micrococcus* sp, *Bacillus cereus* and *Pseudomonas aeruginosa* exhibited very strong crude-oil degrading potentials. Previous reports by Chikere and Okpokwasili [35], Essiene et al. [36], and more recently by Ferrer [31] corroborate the findings of this study. The results of the present study showed that many humic sediment bacteria could degrade crude oil and can also survive in a filter-sterilized humic water medium.

Physicochemical and microbiological characteristics of freshwater ecosystems are influenced by several environmental and human activities. Analytical results of the surface water and sediment chemistry revealed that the humic ecosystem is a typically freshwater ecosystem.

The pH exhibited a narrow amplitude of variation typical of freshwater ecosystems. The pH levels recorded were slightly lower than the WHO [29] recommended range of 6.5 – 8.50 for fishing. The consumption of low-pH water could lead to acidosis, which results in peptic ulcers [37]. This is slightly different from the work carried out by Duru et al. [38] where they observed pH was 5.80 -7.01 and that of Usoro et al. [34] with pH 6.62 - 6.69. On the contrary, pH ranges of 6.2 - 7.5 and 6.0 - 8.5 have previously been reported in the Cross River [39] and Andoni River [40] respectively, all within the Niger Delta area of Nigeria. "The slightly low pH observed could be a result of human activities. These activities may have caused the death of some aquatic life forms leading to the release of proteins including ammonia upon death and decay. The released ammonia dissolved in water hence causing a drastic change which manifests as low pH" [38].

Dissolved oxygen in water samples was low when compared to WHO standard of about 10.00 mg/l. DO, indicating low oxygenation. However, the mean DO values were within the threshold limits for drinking water as recommended by WHO [9], but higher concentrations are reportedly used for agricultural purposes in freshwater aquaculture [41]. Limited studies have tried to investigate the relationship between DO and zooplankton abundance [42]. It has been established that dissolved oxygen (DO) is a basic factor for metabolism of the aerobic aquatic organisms, and determines the natural depuration capacity or freshness of a river [43]. The mean temperature results ranged ($27.00\text{C}\pm 1.41$ - $27.48\text{C}\pm 0.049$) which is within the statutory permissible limit. In a similar study, Alabaster and Lloyd (1980) reported a temperature range of 26oC and 30oC, attributing it to the insulating effect of increased nutrient load resulting from industrial discharge into the aquatic ecosystem. This slight variation may be a result of the insulating effects of increased nutrient input, evaporation, freshwater influx and anthropogenic inputs which are in a relationship with those reported by other investigators [44].

Solids found in a water body exist as total suspended or dissolved [45]. In Eniong River, some of the observed solids existed as undissolved suspended solids, TSS (12.5 mg/ml) while others existed as dissolved solids, TDS (19.0 ± 1.30 mg/ml). The occurrence of high levels of TSS and TDS in recent times has been reported in literature [46,14] with evidence of survival of aquatic biota. According to

FAO/EIFAC (1992), TSS of 25-80 mg/l in European waters is not harmful for the fishery, but waters with TSS of 80-100mg/l are unlikely to support a good freshwater fishery in the tropics. Consumption of water with high solids could lead to gastrointestinal upset, which may pave the way for other gastrointestinal diseases (APHA, 2005).

Electrical conductivity levels were generally high in the water samples (85.07 ± 0.52 $\mu\text{S}/\text{cm}$) indicating high ionic concentration. The conductivity recorded here is lower than the WHO of 100 mg/ml. The total organic carbon of the sediment ($11.17\pm 0.06\%$) is higher than that of surface water ($4.79\pm 0.13\%$). The high conductivity value observed in Eniong River could be attributed to the presence of high dissolved solids. The same trend applies to total nitrogen where it was found that the sediment ($0.55\pm 0.051\%$) harbours higher total nitrogen than surface water ($0.24\pm 0.037\%$). Although the concentrations of K⁺, Na⁺, Ca²⁺ and Mg²⁺ obtained in the present study were remarkably low, the humic freshwater ecosystem was halomorphic (salts rich) and laden with nutritive salts such as SO₄²⁻. Exchangeable cations are generally low in the surface water but higher in the sediment. The low concentration of K⁺, Na⁺, Ca²⁺ and Mg²⁺ is in contrast with Unimke et al. [47] who reported high natural occurrence for these cations in tropical waters. The current status may be in response to changes in geomorphological processes and poor adsorption by electrostatic or columbic attractions unto watersuspended solids or surface water colloids. The acidic nature of the sediment is responsible for the low base saturation resulting in the deficiency of these basic cations Ca²⁺, Mg²⁺ and K⁺ in the sediment of Eniong River.

The concentration of nutrients such as chloride, nitrate, sulphate, fluoride and phosphate were relatively high but varied slightly between the humic sediment and water samples. These obtained levels appeared adequate to maintain a varied microbiota but were below the limit of (WHO) standard. Sulphate, nitrate and chloride in water are indicators of agrochemical usage on lands surrounding the river. These may have entered the river as runoff during rainfall. Consumption of sulphate, nitrate and chloridepolluted water could lead to gastrointestinal irritation, infantile methaemoglobin anaemia, etc., in the system [20,45]. "Phosphate pollution levels may be

attributed to geologic weathering, phosphate detergent, fertilizer application and animal wastes, rainfall patterns as well as anthropogenic impacts" [48]. "Since Nigeria has no regulation criterion for phosphates, concentrations tend to always exceed maximum contaminant levels (MCLs) of foreign regulation agencies such as Swaziland Water Service Corporation (SWSC) - i.e., 1.0 mg/L for drinking water, 2.0 mg/L for rivers and industrial effluents; South African criterion of 1.0 mg/L PO₄-P for sewage effluents and Zimbabwe Water (Waste and Effluent Disposal) Regulation" [49]. So, emergency control measures are required to avoid hyper-eutrophication.

Increasing loads of nitrogen (N) and phosphorus (P) in water bodies has become one of the major environmental problems facing the world. Nitrates indicate the presence of fully oxidized organic matter. The mean nitrate values were within the permissible limit earlier reported [9,16] which implies that the analyzed water samples contained ambient levels of oxidized organic matter. "Although nitrates are relatively non-toxic at the concentration recorded, in excess, they may result in eutrophication impede overall survival and growth of plants and cause methemoglobinemia in infants, i.e., blue baby syndrome" [50].

In this study, salinity values were within the range recorded for growth and best sustainability of freshwater aquaculture, while high heavy metal concentrations were recorded. This is in consonant with reports from previous studies [51]. "The enhanced concentration of heavy metals in the sediment may mainly be attributed to increased absorption, sedimentation and flocculation dynamics that take place in the aquatic ecosystem. Heavy metals in aquatic environments are principally associated with geochemical cycles and biological processes and could be greatly influenced by man-mediated activities such as industrial activities, agricultural practices, and waste disposal" [52].

5. CONCLUSION

The findings of this study have revealed the bacteriological and physicochemical qualities of Eniong river and sediment. The results showed the presence of some indicator bacteria in both river water and sediment, suggesting the possibility of faecal contamination from human sources. Similarly, heavy metal concentrations (Pb, Cd, Ni, Vn, Cu), electrical conductivity, loads of nitrogen and phosphate had values higher

than WHO recommended quality standards for drinking water. These show that the water quality of Eniongriver and sediment has deteriorated over time. The concentrations of exchangeable bases and micronutrients (Ca, Mg, K, and Na) analysed in sediments at different locations showed that they are abundant elements that are important in ensuring optimal primary and secondary productivity of the humid marine ecosystem. The results of this study have provided an insight into the microbiological and physicochemical quality of the river and future guide on municipality decisions that will help improve the river quality. It is therefore recommended that severe effort in limiting human activities aimed at deteriorating the quality of Eniong river water and sediment should be implemented.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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