



Research Article

Quantitative Risk Assessments of Bacteria and Heavy Metals in Ossiomo River, Orhionmwon Local Government Area, Edo State, Nigeria

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ABSTRACT

Water contaminated with heavy metals causes human health risk after prolonged exposure. This study aimed to evaluate the quantitative risk of heavy metals and bacterial diversity of Ossiomo River, Orhionmwon Local Government Area, Edo State, Nigeria. Water samples were collected in triplicates from July 2019 to December 2019, for physiochemical and bacteriological analysis. Heavy metals were determined using Atomic Absorption Spectrophotometer (AAS). The concentrations of metals calculated were chromium (0.003 mg/l), lead (0.009 mg/l) and cadmium (0.005 mg/l) using the USEPA risk assessment guidance. Heterotrophic and coliform bacteria were isolated and enumerated using pour plate and most probable number techniques respectively. Antibiogram test was carried out using Kirby-Bauer antibiotic disc diffusion method. Non-carcinogenic risk (CR) of Pb for adult ranged from 2.91×10^{-5} - 3.72×10^{-5} mg/kg/day, Cd 1.30×10^{-6} - 9.72×10^{-8} mg/kg/day and Cr 1.6×10^{-6} - 9.88×10^{-6} mg/kg/day while Pb for children ranged from 1.42×10^{-5} - 5.65×10^{-5} mg/kg/day, Cd 1.23×10^{-1} - 9.84×10^{-8} mg/kg/day and Cr 1.5×10^{-6} - 3.76×10^{-6} mg/kg/day and the average cancer risk were 11.47×10^{-6} mg/kg/day for children and 11.30×10^{-6} for the adults mg/kg/day. Total heterotrophic bacterial counts ranged between 2.4 ± 1.1 and $17.8 \pm 4.9 \times 10^3$ cfu/ml and mean coliform counts ranged from 23 to 201 MPN/100 ml. Four bacterial isolates were found and identified as *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumoniae*. *Escherichia coli* 37.0 % was found to be the most frequent bacterial isolate while *Pseudomonas aeruginosa* 12.0 % was least. Ciprofloxacin 100 %, ceftriaxone 100 %, azithromycin 90 %, gentamycin 100 %, pefloxacin 95 %, ofloxacin 75 %, cefuroxime 70 % and ceftazidime 60 % were effective drugs against enterobacteriaceae infections whereas, augmentin 0 % and nitrofurantoin 0 % were ineffective. There is the need to improve awareness, monitoring and surveillance of coastal water bodies to curb outbreak of water borne diseases among the dependent communities

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INTRODUCTION

The concentration of heavy metals, its severe toxic effects as well as their persistence in biotic and abiotic environment make it highly toxic for major inhabitants (Chai *et al.*, 2010). The presences of these metallic chemical elements with relatively high density are poisonous at low concentration which could be linked with carcinogenic and non-carcinogenic human risks (Moho and Dhote, 2013). Carcinogenic risks associated with human health could be expressed as the probability that someone could develop cancerous cells after prolonged exposure to contaminated water source ladened with heavy metal deposits while in the non-carcinogenic risks, human could be able to manage exposure in a lifetime (Moraise *et al.*, 2012). Some metals such as arsenic, cadmium, chromium, copper, lead, nickel and zinc as well as other effluents from industries, urban and agricultural runoffs such as insect and disease control agents, among others are major contaminants of surface water (Moraise *et al.*, 2012; Zhang *et al.*, 2015). These toxicants present in the river could flow into neighboring water source and become a potential health threat via ingestion, dermal contact and inhalation (Zhang *et al.*, 2015). The health status of local inhabitants could be assessed and managed through sustainable development (Haque *et al.*, 2018).

Ossiomo River is a tributary of Benin River, Southern Nigeria, with a supplier from Ikpoba, Okhviahe and Akhianwan Rivers. Ossiomon River drains into the Benin River at Koko, Delta State and empties itself into the Atlantic Ocean (Tawari-Fufeyin *et al.*, 2008). The water source is important for its role in fishery, domestic use, washing, bathing and recreational activities. The rapid development and change of lifestyle in the past few decades such as increased rates of construction (bridge), development of industrial layouts, increased transportation and human population, intensive fertilizer and insecticide application as well as the use of other chemical substances by communities including Ologbo community have inevitably altered the quality of surface water bodies of valleys including Ossiomo River, now poses health risks to consumers (Tawari-Fufeyin *et al.*, 2008), hence, this study evaluates the quantitative risks of heavy metals and bacterial diversity of Ossiomo River, Edo State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out in Ossiomo River (Latitudes 6°30'- 6°32'0''N; Longitudes 5°39'- 5°40'30''E), a tributary of Benin River located in Abudu community, Orhionmwon Local Government Area, Edo State, Southern Nigeria. Ossiomo River (Fig. 1) stretches over a distance of 250 km within Edo and Delta States, Nigeria

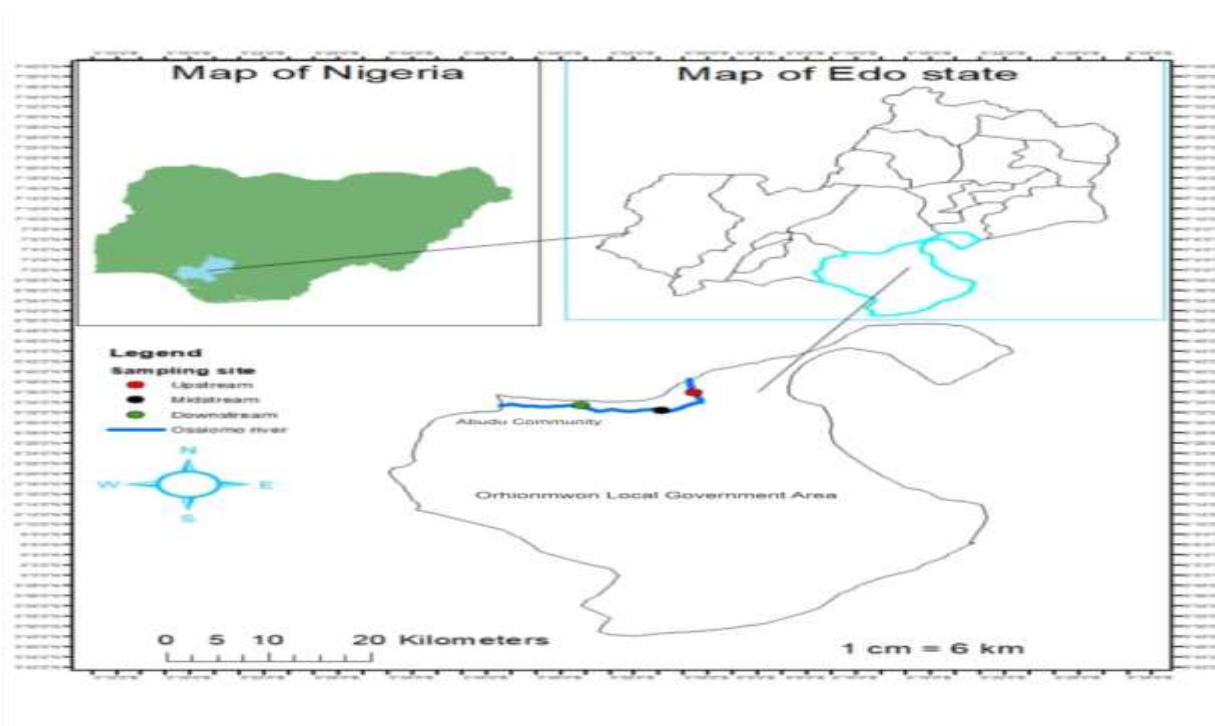


Figure 1: Map of Orhionmwon L.G.A., showing the locations of the sampling sites along Ossiomo River stretch.

Sampling

Water samples were collected in triplicates monthly for a period of Six (6) months from July 2019 – Dec. 2019. The sampling locations were identified for as station 1 (Upstream), station 2 (Midstream) and station 3 (Downstream), samples were collected in sterile 1 L screw cap glass bottle and transported to the laboratory for physicochemical and bacteriological analyses.

Physicochemical parameters

Physicochemical parameters such as Dissolve Oxygen (DO), Biochemical Oxygen Demand (BOD₅) were estimated using Winkler's methods. While nitrate, sodium, potassium, calcium, magnesium, chloride, phosphorus and sulphate were determined using UV-Spectrophotometer following the methods (APHA, 2005). Temperature, electrical conductivity, pH and total dissolved solids were measured using an ExTech multimeter (EC 400, ExTech instruments, Nashua, NH, USA).

Heavy metals analysis: Heavy metals such as iron, lead, zinc, cadmium, copper, and chromium were analyzed using Atomic Absorption Spectrophotometer (900H, Perkin Elmer, Akron, OH, USA) according the methods APHA(2005).

Quantitative risk assessment analysis of heavy metals

The human exposure risk to trace metals could be classified into three main routes including inhalation, ingestion and dermal adsorption exposure (USEPA, 1989). However, the most common exposure routes to water are direct ingestion and dermal adsorption (Asare – Donkor *et al.*, 2016). The estimated human risk index through ingestion pathway was calculated for adults and children showing Exp_{ing} , HQ_{ing} , HI and CR_{ing} . The data from midstream were human activities were mostly observed were used to determine the potential carcinogenic risks (CR_{ing}) for lead, chromium and cadmium across the sampling periods.

In this study, direct ingestion was adopted for the estimation of quantitative risk assessment of heavy metals (Exp_{ing}) for adults (70years) and children (6 years), using the equations and some constant values. Average concentration (C_w) mg/l, Ingestion Rate (IR) 3L/day (adult), 1.5

$$Exp_{ing} = \frac{C_{water} \times IR \times EF \times ED}{BW \times AT} \quad (1)$$

Where Exp_{ing} : exposure through ingestion of water (mg/kg/day).

Potential non-carcinogenic risks due to exposure to heavy metals were determined by comparing the calculated contaminant exposure with the

$$HQ_{ing} = \frac{Exp_{ing}}{RFD_{ing}} \quad (2)$$

Where RFD_{ing} is ingestion toxicity reference dose (mg/kg/day) and the value for the selected metals were derived from the literature (USEPA, 1989; WHO, 2011; Hasan *et al.*, 2021).

An $HQ < 1$ is assumed to be safe and accepted as significant non-carcinogenic but when $HQ > 1$ may be a major concern for potential health risk in association with over exposure of humans to the contaminants.

$$H1 = \sum_{ii=1}^n HQ_{ing} \quad (3)$$

Carcinogenic risk (CR) via ingestion route was estimated using equation

$$CR_{ing} = \frac{EXP_{ing}}{RFD_{ing}} \quad (4)$$

Where CR_{ing} is the Carcinogenic risk via ingestion route and SF_{ing} is the Carcinogenic slope factor for Pb is 8.5×10^3 and 5.0×10^2 mg/kg/day (Ibironke *et al.*, 2021; Ullah *et al.*; 2014). The CR_{ing} values for other metals were not calculated due to unavailability of the SF_{ing} values.

(children), Exposure Frequency (EF) 365 day/year, Exposure Duration (70 years) adult, (6 years children), Average Body weight (BW, kg) 60.7 (adult), 20 (children), Average Time (AT) (365×70) adult (365×6) children USEPA (1989).

reference dose (RFD) (USEPA, 1989; Bironke *et al.*, 2018).

Equation 2 was used to estimate the hazard quotient (HQ) toxicity in order to determine the non-carcinogenic potential risks of an individual through the ingestion route.

To access the overall potential for non-carcinogenic effects posed by more than one metal through the pathway, the sum of the computed HQ across metal, is expressed as a hazard index (H1) using equation 3 below (USEPA, 1989).

$H1 > 1$ showed that regular exposure to the surface water could have a potential adverse impact on human health (Li *et al.*, 2010; Ullah *et al.*, 2014).

Bacteriological analyses of sampled water

Isolation and enumeration of total heterotrophic bacterial isolates were cultured using nutrient agar, MacConkey agar using pour plate methods while eosin methylene blue agar as suitable media for isolation of total fecal and total coliforms were determined using Most Probable Number (MPN) according to Cheesebrough

(2000). Plates were incubated at 37° C for 24 hours and discrete colonies were further subcultured onto Nutrient agar to obtain pure isolates. Pure culture isolates were stored in Nutrient agar slant at 4° C according to Cheesebrough (2006).

Phenotypic and biochemical identification of isolates

Phenotypic identification of bacterial isolates was carried out with focus on Gram staining and biochemical reactions using standard bacteriological techniques outlined in Cheesebrough (2000). Suspected colonies were subjected to oxidase, indole, urease, citrate, fructose, galactose, lactose, maltose, mannitol, mannose, nitrate reduction, triple sugar iron (TSI) and sucrose.

Antibiotic susceptibility test of bacterial isolates

The antibiotic susceptibility test for each isolate was performed on freshly prepared, dry surfaced Mueller - Hinton agar (Oxoid) using the agar-disk diffusion method according to Clinical Laboratory Standard Institute (CLSI, 2008). A total of Eleven (11) tested antibiotics disc (Oxiom) were employed and they were augmentin (30 µg), ceftriaxone (30 µg), nitrofurantoin (300 µg), ofloxacin (5 µg), azithromycin (15 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg) and ceftazidime (30 µg). The ranges of the diameter were measured in millimeter (mm) of each antibiotic disc which is interpretive for susceptibility, intermediate or resistant compared with the performance standards of CLSI (2008).

Bacterial DNA extraction

Bacterial DNA was extracted according to the lysozyme-based cell lysis and DNA precipitation procedure for bacteria. The DNA obtained was spectrophotometrically quantified using the NanoDrop® Technologies (USA) at 260nm and the purity level of DNA samples was determined at a ratio of absorbance (A260/A280). The

purity ratio ranging from 1.8 and 2.2 was used as DNA template for Polymerase Chain Reaction (PCR) (Lee *et al.*, 2003; Fietto *et al.*, 2004).

Amplification of the 16S rRNA genes

The 16S rRNA gene from total bacterial isolates and genomic DNR respectively were amplified by Polymerase Chain Reaction (PCR) using bacteria universal primers (27F-AGAGTTGATCCTGGCTCAG and 1492R-GGTTACCTTGTTACGACTT). The PCR amplification was carried out in a Techne TC-412 Thermal Cycler (Model FTC41H2D, Bibby Scientific Ltd, UK) in a 50 µl reactions containing 25 µl of 2 × PCR Master Mix (Norgen Biotek, Canada), 1.5 µl of template DNA (0.5 µg), 1 µl of both forward and reverse primers (2.5µM of each) and 21.5µl of nuclease free water in a PCR tube added in that order. PCR was carried out at an initial denaturation step at 94 °C for 2 min, followed by 30 cycles at 94 °C for 30 sec, 52 °C for 30 sec and 72 °C for 2 min, and a final extension step at 72 °C for 5 min. PCR products (amplicons) were separated by electrophoresis on a 1% agarose TAE gel containing ethidium bromide and visualized by UV trans illumination (Foto/UV 15, Model3-3017, Fotodyne, USA) according to methods Klindworth, *et al.*, (2013).

Statistical analysis

Data generated were analyzed by one-way analysis of variance (ANOVA) using F-test and T-test to determine the significance differences in group results. SPSS version 21 and graph primis version 6.0 was used as statistical tools Ogbeibu (2005).

RESULTS AND DISCUSSION

The results of Hazard Quotient (HQ) presented in Table 1 ranged from 1.28×10^{-2} - 9.88×10^{-1} (adult) and 2.1×10^{-1} - 8.62×10^{-2} (children). According to Liang *et al.* (2011), heavy metals pollution have been known to cause severe health impacts when the HQ and Hazard Index (HI) values of a metal is higher than 1 (HQ>1 and HI>1). In this study, the average (HQ) and (HI) for adults were 9.26×10^{-1} and 9.53×10^{-1} ,

and 10.09×10^{-1} and 8.48×10^{-1} (children) were less than 1 ($HQ < 1$ and $HI < 1$) in all sampling locations and periods. This finding proved that there were no indications for non-carcinogenic risk linked with heavy metals such as lead, chromium and cadmium in Ossiomu River. Similar findings were reported by Madilonga *et al.* (2021) in Mutangwi River, South Africa and Ibronke *et al.* (2018) in Ndawuse River, Abuja, Nigeria.

The carcinogenic risk (CR_{ing}) associated with lead, chromium and cadmium via ingestion route as estimated for both adults and children revealed the average values of 11.45×10^{-6} (children) and 11.30×10^{-6} (adults) are shown in Table 1. The above results suggest higher cancer risk for children than adults through ingestion pathway after a long period of exposure. The calculated cancer risk due to exposure to chromium, lead and cadmium, and the average cancer risk values recorded in this study were above the acceptable cancer health risk limit of 1.00×10^{-6} and 1.00×10^{-4} (i.e, 1 case of cancer per every 1,000,000 to 1 case of cancer per every 10,000). Pawelczyk (2003) asserted that a risk of 1.00×10^{-3} level will require protective measure of the river. Results from this study revealed that a lifetime exposure to these heavy metals concentration can pose cancer risk for both children and adults. These findings were similar to the report of Madilonga *et al.* (2021) and Ibronke *et al.* (2018).

Table 2 showed results of total heterotrophic bacterial counts ranging from 2.4 ± 1.1 to $17.8 \pm 4.9 \times 10^3$ cfu/ml across the sampling locations and periods. Midstream samples had the highest bacterial counts of $15.9 \pm 7.0 - 17.8 \pm 4.9 \times 10^3$ cfu/ml followed by downstream samples $8.2 \pm 6.3 - 13.6 \pm 2.4 \times 10^3$ cfu/ml, while the least counts of $7.4 \pm 1.0 \times 10^3$ cfu/ml was recorded in upstream samples. The significant difference ($P < 0.05$) in bacterial load observed in the studied locations could be attributed to the levels of contamination resulting from unhygienic human activities in the different sampling

locations of the river. The results in Table 3 revealed the total coliform counts ranged from 23 to 201 MPN/100ml. Upstream samples had the lowest count of 126 MPN/100ml followed by downstream 152 MPN/100ml, while midstream samples 201 MPN/100ml recorded the highest bacterial count. The highest bacterial load recorded in this study could be linked to increased anthropogenic activities and the presence of other forms of contaminants in and around the water body, specifically in the various sampling locations. These findings were in accordance with the report of Adieze *et al.* (2016) who reported that high bacterial load is attributable to the presence of organic matters in the water bodies. Table 4 shows the cultural, morphological, biochemical and physiological characteristics of bacterial isolates. Phenotypically, Four (4) bacterial isolates were isolated and they include *Klebsiella* sp., *Pseudomonas* sp., *Escherichia* sp. and *Salmonella* sp. Molecular characterization revealed the presence of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella enterica* as shown in Plate 1. The results shown in Table 5 revealed the percentage occurrence of bacterial isolates. *Escherichia coli* (37.0 %) was reported to be the most prevalent bacteria isolate while *Pseudomonas aeruginosa* (12.0 %) had the least frequency of occurrence.

Table 6 showed the results of antibiogram assay of bacteria isolates. *Escherichia coli* exhibited high level of susceptibility to gentamicin (100 %) and ciprofloxacin (100 %), followed by ceftriaxone (75 %), ofloxacin (75 %) and levofloxacin (75 %) while azithromycin (50 %) and ceftazidime (50 %) were found to be resistant. But augmentin (0 %) and nitrofurantoin (0 %) showed no effect on the isolate. *Pseudomonas aeruginosa* showed high susceptibility to ceftriaxone (100 %) and ciprofloxacin (100 %), followed by pefloxacin (95 %), cefuroxime (65 %, ofloxacin (62 %) and levofloxacin (57 %) while azithromycin (50 %)

and ceftazidime (50 %) were found to be ineffective. Augmentin (0 %) and nitrofurantoin (0%) showed no resistance. cefuroxime (70 %), levofloxacin (68 %), pefloxacin (67 %), ceftazidime (62 %), ciprofloxacin (61 %) and ofloxacin (60 %) were effective against *Klebsiella pneumonia* while azithromycin (50 %) ceftriaxon (0 %), gentamicin (0 %), augmentin (0 %) and nitrofurantoin (0 %) were ineffective. *Salmonella enterica* was sensitive to ceftriaxon (100 %), ciprofloxacin (100 %), cefuroxime (94 %), levofloxacin (87 %), azithromycin (80 %), ofloxacin (76 %) and ceftaxidine (60 %) while pefloxacin (50 %), gentamicin (0 %), augmentin (0 %) and nitrofurantoin (0 %) were resistant.

CONCLUSION

Recently, more concern is raised on the impacts of heavy metals in environmental studies especially in water bodies. Drinking water which contains a significant amount of heavy metal might cause adverse health impacts on human and could results in severs cancer disease. In this study, the Hazard Quotient (HQ) values of selected heavy metals contents in Ossiomo River were less than 1 which indicates no health risk effects. However, precaution needs to be taken due to human activities and potential carcinogenic exposure risk (CR_{ing}) over a period of time especially for young children using this water. Ossiomo River contaminated with *E. coli* and other Enterobacteriaceae is not suitable for irrigation, recreation and domestic use, hence, water treatment is recommended before consumption.

Table 1: Potential carcinogenic risk assessments values (CR_{ing}) of Pb, Cd and Cr for adult and children in consuming Ossiomo River source through injection pathways between July 2019 and December 2019

Metals	RfD_{ing}	Adult				Children			
		Exp_{ing}	HQ	HI (mg/kg/day)	CR_{ing}	Exp_{ing}	HQ	HI (mg/kg/day)	CR_{ing}
July									
Pb	1.4	2.74×10^{-3}	1.77×10^2	1.06×10^{-3}	2.91×10^{-5}	3.75×10^{-3}	2.68×10^{-2}	1.6×10^{-1}	4.41×10^{-5}
Cd	0.5	2.97×10^{-4}	5.94×10^1	3.56×10^1	4.86×10^{-7}	4.5×10^{-2}	9×10^{-1}	5.4×10^{-2}	7.28×10^{-8}
Cr	3	4.94×10^{-3}	1.65×10^1	9.88×10^{-1}	9.88×10^{-6}	7.5×10^{-4}	2.5×10^{-1}	1.5×10^{-2}	1.5×10^{-6}
Aug									
Pb	1.4	2.65×10^{-3}	1.89×10^{-2}	1.13×10^{-1}	3.12×10^{-5}	4.05×10^{-3}	2.89×10^{-2}	1.74×10^{-1}	4.7×10^{-5}
Cd	0.5	3.79×10^{-4}	7.58×10^{-1}	4.55×10^{-3}	4.46×10^{-6}	6×10^{-4}	1.2×10^{-2}	7.2×10^{-2}	9.84×10^{-8}
Cr	3	6.43×10^{-4}	2.14×10^{-1}	1.29×10^{-2}	1.29×10^{-6}	9.75×10^{-4}	3.25×10^{-1}	1.95×10^{-1}	1.95×10^{-6}
Sep									
Pb	1.4	2.67×10^{-3}	1.91×10^{-2}	1.14×10^{-1}	3.14×10^{-4}	4.05×10^{-3}	2.89×10^{-2}	1.73×10^{-1}	4.76×10^{-5}
Cd	0.5	4.94×10^{-4}	9.88×10^{-1}	5.93×10^{-2}	8.1×10^{-7}	7.05×10^{-4}	1.5×10^{-1}	9×10^{-2}	1.23×10^{-1}
Cr	3	6.92×10^{-4}	2.3×10^{-1}	1.38×10^{-3}	1.38×10^{-6}	1.1×10^{-3}	3.5×10^{-1}	2.10×10^{-2}	2.1×10^{-6}
Oct									
Pb	1.4	2.77×10^{-3}	1.98×10^{-2}	1.2×10^{-1}	3.25×10^{-5}	4.2×10^{-3}	3×10^{-2}	1.8×10^{-2}	4.94×10^{-5}
Cd	0.5	5.93×10^{-4}	1.19×10^{-2}	7.12×10^{-2}	9.72×10^{-8}	9.0×10^{-4}	1.8×10^{-2}	1.08×10^{-1}	1.48×10^{-6}
Cr	3	8.40×10^{-4}	2.8×10^{-1}	1.68×10^{-2}	1.68×10^{-6}	1.28×10^{-3}	4.25×10^{-1}	2.52×10^{-2}	2.55×10^{-6}
Nov									
Pb	1.4	2.94×10^{-3}	2.1×10^{-2}	1.26×10^{-1}	3.46×10^{-6}	4.50×10^{-3}	3.21×10^{-2}	1.93×10^{-1}	5.29×10^{-5}
Cd	0.5	7.91×10^{-4}	1.58×10^{-1}	9.48×10^{-2}	1.30×10^{-6}	1.2×10^{-3}	2.4×10^{-2}	1.44×10^{-1}	2.40×10^{-6}
Cr	3	9.39×10^{-4}	3.13×10^{-1}	1.88×10^{-1}	1.88×10^{-6}	1.43×10^{-3}	4.75×10^{-1}	2.85×10^{-2}	2.88×10^{-6}
Dec									
Pb	1.4	3.11×10^{-3}	2.22×10^{-2}	1.33×10^{-1}	3.66×10^{-5}	4.73×10^{-3}	3.38×10^{-2}	2.03×10^{-1}	5.56×10^{-5}
Cd	0.5	9.39×10^{-4}	1.88×10^{-1}	1.13×10^{-1}	1.54×10^{-7}	1.43×10^{-3}	2.85×10^{-2}	1.71×10^{-1}	2.34×10^{-7}
Cr	3	1.09×10^{-3}	3.62×10^{-1}	2.17×10^{-1}	2.18×10^{-6}	1.65×10^{-3}	5.5×10^{-1}	3.3×10^{-1}	3.30×10^{-6}
Average					$\Sigma 11.30 \times 10^{-6}$				$\Sigma 11.41 \times 10^{-6}$
USEPA Limit									$1.0 \times 10^{-4} - 1.0 \times 10^{-6}$

Key: USEPA- United States Environmental Protection Agency, Pb-lead, Cd-Cadmium, Cr-Chromium

Table 2: Total Heterotrophic Bacterial Counts Isolated from July, 2019 to Dec., 2019

Sampling stations	July 2019	Aug	Sept	Oct	Nov	Dec 2019
	($\times 10^3 \pm \text{SD CfU/ml}$)					
Upstream	7.4 \pm 1.7	10.7 \pm 1.0	9.1 \pm 1.7	2.4 \pm 1.1	3.2 \pm 0.2	4.5 \pm 3.1
Midstream	15.9 \pm 7.0	17.8 \pm 4.9	16.0 \pm 1.8	9.2 \pm 6.7	5.4 \pm 2.1	4.7 \pm 2.4
Downstream	8.2 \pm 6.3	13.6 \pm 2.4	12.4 \pm 2.6	6.8 \pm 4.3	3.6 \pm 1.2	4.7 \pm 2.8

Values expressed as mean triplicates, World Health Organization Permissible Limits for portable water = 0, World Health Organization (2011).

Table 3: Total Coliform Counts Isolated from July to December, 2019 (MPN/100 ml)

Sampling Locations	July 2019	Aug	Sept	Oct	Nov	Dec 2019
Upstream	53	75	126	65	23	27
Midstream	83	201	189	121	76	79
Downstream	65	97	152	78	34	35

Values expressed as mean triplicates, World Health Organization permissible limits for portable water =0, World Health Organization (2011).

Table 4: Cultural, morphological, biochemical and physiological characteristics of bacterial isolates

Characteristics	B1	B2	B3	B4
Cell morphology	Rod	Rod	Rod	Rod
Cell arrangement	Cluster	Single	Single	Single
Gram reaction	Negative	Negative	Negative	Negative
Motility	+	+	-	+
Test for enzymes				
Catalase production	-	+	+	+
Spore formation	+	-	-	-
Oxidase	-	+	-	-
Coagulase	-	-	-	+
Citrate utilization	-	+	+	+
Indole	+	-	-	-
Nitrate reduction	+	-	-	+
Urease	-	-	+	-
Acid test	-	-	+	+
Sugar fermentation				
Lactose	+	+	+	-
Glucose	+	-	+	+
Galatose	+	-	+	+
Maltose	+	+	+	+
Mannitol	-	-	+	+
Probable Identity	<i>Escherichia</i> sp	<i>Pseudomonas</i> sp	<i>Klebsiella</i> sp	<i>Samonella</i> sp

Key: + Positive, - Negative

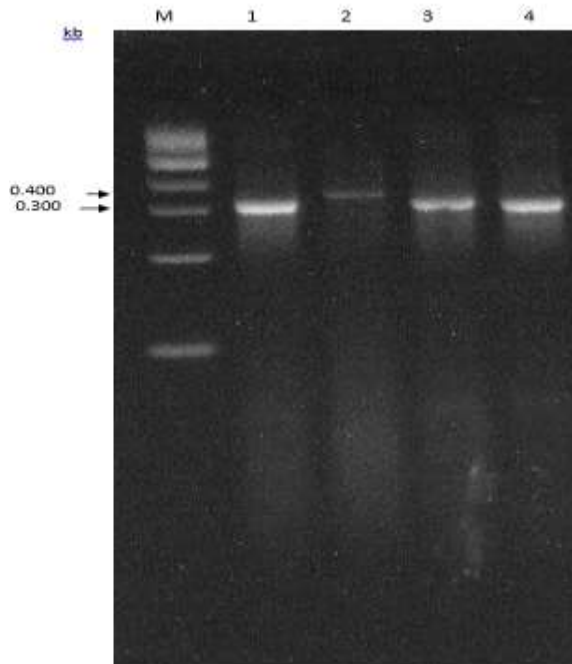


Plate 1: Agarose gel electrophoresis of bacterial isolates and ail-specific amplification by PCR. Lane M- molecular marker (0.300-bp ladder): lane 1-*Klebsiella pneumoniae*, lane 2-*Pseudomonas aeruginosa*, lane 3-*Escherichia coli*, lane 4-*Salmonella enterica*

Table 5: Percentage frequency of occurrence of bacterial isolates of water samples at different sampling locations in Ossiomo River from July to December, 2019

Bacterial Isolates	July	Aug	Sept	Oct	Nov	Dec	Mean
<i>Klebsiella pneumoniae</i>	12(24)	20(22)	17(22)	14(32)	7(22)	6(16)	13(25)
<i>Pseudomonas aeruginosa</i>	10(20)	16(18)	12(16)	7(16)	6(19)	17(45)	6(12)
<i>Escherichia coli</i>	15(29)	32(36)	37(48)	14(32)	7(22)	6(16)	19(37)
<i>Salmonella enterica</i>	14(27)	21(24)	11(14)	9(20)	15(47)	9(24)	13(25)
Total	51	89	77	44	32	38	51

Table 6: Antibiotic susceptibility pattern of bacterial isolates from Ossiomu River

Bacterial Isolates	No. of Isolates	LEV	PEF	CRO	GEN	CIP	CXM	AZM	OFL	CAZ	AUG	NIT
<i>E. coli</i>	2	2 (73)	1 (50)	2 (75)	2(100)	2(100)	1(50)	1(50)	2 (75)	1(50)	0(0)	0(0)
<i>P. aeruginosa</i>	2	1(57)	2 (95)	2(100)	2(100)	1(100)	1(65)	1(50)	2 (62)	1(50)	0(0)	0(0)
<i>K. pneumoniae</i>	2	2 (68)	1(67)	0 (0)	0 (0)	2 (61)	2(70)	1 (50)	2 (60)	1(62)	0(0)	0(0)
<i>S. enterica</i>	1	2(87)	1(50)	2(50)	0(0)	2(100)	1(94)	1(80)	2(76)	1(60)	0(0)	0(0)

Key: *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*.

LEV=Levofloxacin, PEF=Pefloxacin, CRO=Ceftriaxon, GEN=Gentamicin, CIP=Ciprofloxacin, CXM=Cefuroxime, OFL=Ofloxacin, CAZ=Ceftazidine, AUG=Augmentin, NIT=Nitrofurantoin and AZM= Azythromycin. Susceptibility= 91-100 %, Intermediate=51-90 %, Resistance=1-50 %.

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