Risk Assessment of Heavy Metals on Human Health and Bacteriological Qualities of Ossiomo River, Edo State, Nigeria

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Abstract
Water contaminated with heavy metals and its health challenges is as a result of human activities in the River. This study evaluates the risk assessment of heavy metals on human health and bacteriological qualities of Ossiomo River, Edo State, Nigeria. Water samples were collected in triplicates from January 2021 to June 2021 for physiochemical and bacteriological analysis. Heavy metals were determined using atomic absorption spectrophotometer (AAS).The concentrations of metals were Cr 0.003, Pb 0.009 and Cd 0.005 mg/L and risk assessment was determined using the USEPA guidance. Heterotrophic and coliforms bacteria were isolated and enumerated using pour plate and most probable number techniques. Antibiogram test was carried out using Kirby-Bauer antibiotic disc diffusion method. The average carcinogenic risks (CR_{ing}) were 11.25×10^{-6} mg/kg/day for children and 10.06×10^{-6} mg/kg/day for the adults. The total heterotrophic bacterial counts ranged from $1.1 \pm 0.0 \times10^{3}$ cfu/mL (Upstream) - $9.1 \pm 2.1 \times10^{3}$ cfu/mL (Midstream) while the mean total coliform counts ranged from 23 MPN/100 mL(Upstream) - 201 MPN/100 mL (Midstream).Six (6) bacteria isolates were isolated and identified as*Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Shigella sonnei, Klebsiella pneumonea*and *Salmonella enterica*.*Proteus mirabilis* had 16 (31.4 %) as the most frequent bacteria isolate while *Escherichia coli* had 3 (5.9%) as the least. Ciprofloxacin 100%, ceftriaxone 100%, azithromycin 90%, gentamycin 100%, pefloxacin 95%, oflaxacin 75%, cefuroxime 70% and ceftazidine 60% were effective against Enterobacteriaceae infections while augmentin 0% and nitrofurantoin 0% were ineffective. Low human activities in the river will help manage adverse health risk on consumers.
Keywords: Ossiomo River, human health, heavy metals, microbial, antibiogram

Introduction

The importance of water to mankind has been linked to multifaceted uses such as hydro electrical power generation, transportation, recreational activities, industrial, consumption, domestic and business purposes (Ibironke *et al.*, 2018). About 3% available freshwater of the earth is insufficient to meet the demands of a fast growing world population (Anita *et al.,* 2011). From 1900 – 1995, there was an observable six fold increase in the world rate of fresh water consumption demand due to population growth. Currently, about one-third of the world's population live in nations with high potable water scarcity associated with low availability of water and increase in population density (Anita *et al.,* 2011).Nations of the world focus on the increasing rate of negative health impacts resulting from the intake of contaminated water are consistently linked to waterborne diseases which are known as leading causes of increased rate of infant mortality and morbidity (Clasen *et al.,* 2007). The consumption of high iron contents in water has been linked with adverse human health risk such as congestion of blood vessels, hypertension and increased respiratory rate (Islam *et al.,*2018).According to Madilonga *et al.* (2021), high levels of lead above 0.01 mg/l have been associated to anaemia, memory loss, anorexia, brain damage, and death. Leachates constitutes different dissolved, suspended organic and inorganic chemical components with high toxic substances from homes, hospitals and pharmaceutical industries leaching into the nearest water during

uncontrolled migration could lead to surface water contamination, responsible for human health disease after consumption (Anita *et al.,* 2011), hence, risk assessment of heavy metals on human health and bacteriological qualities of Ossiomo River, Edo State, Nigeria.

Materials and Methods

Study area

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

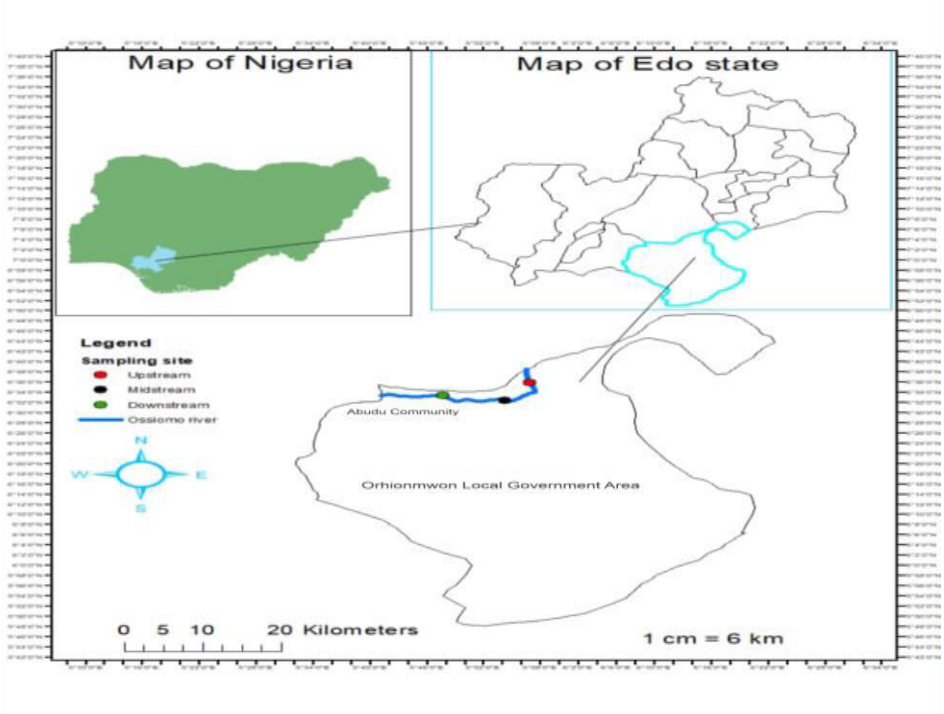

Figure 1: Map of Orhionmwon L.G.A., showing the locations of the sampling sites along Ossiomo River stretch.

Sampling

A total of eighteen (18) water samples were collected in triplicate at monthly interval for a period of Six (6) months from January 2021 – June 2021. The sampling locations were identified for as station 1 (Upstream), station 2 (Midstream) and station 3 (Downstream) respectively. Water samples were collected in sterile 1 L screw cap glass bottle and thereafter transported to the laboratory for physicochemical and bacteriological analyses.

Physicochemical parameters

Physicochemical parameters such as dissolve oxygen (DO), biochemical oxygen demand (BOD_5) 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. Other*in-situ* measurement were conducted on some parameters such surface water temperature, electrical conductivity, pH and total dissolved solids using an ExTech multimeter (EC 400, ExTech instruments, Nashua, NH, USA .

Heavy metals

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(nose and mouth), direct ingestion and dermal (skin) 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 values obtained from midstream samples (where human activities were mostly observed) were used to determine the potential carcinogenic risks (CR_{ing}) for Pb, Cd and Cr across the sampling periods.

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

All data analyzed were focused on midstream due to the high level of anthropogenic activities.

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

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

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.

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

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.

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).

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

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

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

Where CR_{ing} is the Carcinogenic risk via ingestion route and SF_{ing} is the carcinogenic slope factor for Pb is 8.5 x10, Cd is 6.1 x 10^3 and 5.0 x 10^2 mg/kg/day (Ibironke *et al.,* 2021; Ullah *et al;* 2014).

Bacteriological analyses of sampled water

Isolation and enumeration of total viable bacterial isolates were cultured using nutrient agar, MacConkey agar and eosin methylene blue agar as suitable media for isolation of totalheterotrophic bacterial using pour plate methods while total coliforms were determined using Most Probable Number (MPN) according to Cheesebrough (2000). Plates were incubated at 37 ^{0}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 ^{0}C according to methods 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 techniques outlined in Cheesebrough (2000). Suspected colonies were subjected to different sugars which were oxidase, indole, urease, citrate, fructose, galatose, 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 Kirby-Bauer antibiotic disc diffusion method according to Clinical Laboratory Standard Institute (CLSI, 2008). A total of Eleven (11) tested antibiotics disc (Oxiom) where 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 interpretive for susceptibility, intermediate or resistant comparing 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 (Klindworth*et al.*, 2013). The DNA obtained was spectophotometrically 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 DNA 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 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 prims version 6.0 wasbe used as statistical tools Ogbeibu (2005).

Results and Discussion

The hazard quotient (HQ) recorded in this study from the analysis of some selected heavy metals namely Lead, Cadmium and Chromium concentrations in Ossiomo River via ingestion route were computed for adults (70 years) and children (6 years) are shown in Table 1 above. The average levels of non-carcinogenic risk (HQ) of the water were observed in a descending order of Cr > Cd > Pb for adults and Cr > Pb >Cd for children ranging from 1.32×10^{-2} – 8.3×10^{-1} (adult) and 1.8×10^{-1} – 5.2×10^{-2} (children). According to Liang *et al.* (2011), heavy metals pollution can cause severe health impacts when the HQ and HI values of a metal is higher than 1 (HQ>1 and HI>1). In this study, the HQ and HI values through ingestion exposure pathway for both adults and children were less than 1 (HQ<1 and HI<1) in all sampling locations and periods. Similar findings were reported by Madilonga *et al.* 2021 in Mutangwi River, South Africa and Ibironke *et al*. 2018 in Ndawuse River, Abuja, Nigeria.

The carcinogenic risk (CR_{ing}) associated with Lead, Chromium and Cadmium via ingestion route were calculated for both adults and children in this study. The results of the carcinogenic risk (CR_{ing}) of Pb for adult ranged from 3.31×10^{-5}- 3.78×10^{-5}, Cd 1.62×10^{-7} - 8.14×10^{-6} and Cr 1.6×10^{-6} – 2.77×10^{-6} while Pb for children range from 1.42×10^{-5}- 5.95×10^{-5}, Cd 1.72×10^{-2} - 3×10^{-6} and Cr 2.4×10^{-6}- 4.2×10^{-6} mg/kg/day. The average carcinogenic risks (CR_{ing}) were 11.25×10^{-6} mg/kg/day for children and 10.06×10^{-6} mg/kg/day for the adults are shown in Table 1. The above results suggest higher cancer risk for children than adults after a long period of exposure. The calculated cancer risk due to exposure to Cr, Pb and Cd, and the cumulative cancer risk values present in this study were above the acceptable cancer health risk limit of $1.00\times10^{-6}$and 1.00×10^{-4} (ie., 1 case of cancer per every 1,000,000 to 1 case of cancer per every 10,000). Results from this study revealed that a lifetime exposure to the consumption of water contaminated with these heavy metals can pose cancer risk for both children and adults. This findings was similar to the report of Odigie *et al.,*2023 on quantitative risk assessment of bacteria and heavy metals in Ossiomo River, Orhionmwon Local Government Area, Edo State, Nigeria which attributed high load of heavy metals in water to human activities and potential adverse impact on human health according to Li and Zhang (2010).

The results in table 2 reveals the total heterotrophic bacterial counts ranged from $1.1 \pm 0.0 \times10^3$ cfu/mL (Upstream) - $9.1 \pm 2.1 \times10^3$ cfu/mL (Midstream). Midstream samples had highest bacteria count of 3.1 ± 0.1 - $9.1 \pm 2.1 \times10^3$ cfu/mL, followed by downstream samples $3.1\pm0.16.2\pm2.1\times10^3$ cfu/mL while upstream samples had the lowest bacteria count of 1.1 ± 0.0 - $4.6\pm1.0\times10^3$ cfu/mL. The significant difference ($P<0.05$) of bacterial counts observed in upstream, midstream and downstream during rainy season could be linked to increased bacterial activities in the water associated with high levels of water pollution from industrial waste and nutrient runoffs from agricultural lands which are not usually common during dry season. These findings are in accordance with the reports of Fagorite *et al.* (2019) on microbial assay of Otamiri river, Owerri, which attributed high bacterial load in river during rainy season to presence of heavy organic pollutants.

The results in table 3 shows the mean total coliform counts ranged from 23 MPN/100 mL(Upstream) - 201 MPN/100 mL (Midstream). Midstream samples had the highest bacteria counts of 76 – 201 MPN/100 mL, 34 – 152 MPN/100 mL (downstream) and 23 – 126 MPN/100 mL (upstream).The high values recorded from midstream sample were as a result of anthropogenic activities and other forms of contaminants present in the water. This finding is in agreement with the study of Odigie *et al.* (2023) who reported that high counts of bacterial load is a reflection of the levels of water contamination with organic matters present in the water bodies. These unprotected water sources could also become contaminated with microorganisms via rainfall runoffs and agricultural impacts, sewage effluence and feaces from wildlife which makes the water unfit for human consumption and other domestic uses according to Mulamattahil *et al.*(2014).

Table 4 shows the cultural, morphological, biochemical and physiological characteristics of bacteria isolates. Phenotypically, Six (6) bacterial isolates were isolated and they include*Pseudomonas* sp*, Escherichia* sp*, Proteus* sp*, Shigella* sp*, Klebsiella* spand *Salmonella* sp. Plate 1 reveals the molecular characterization of the presence of *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Shigella sonnei, Klebsiella pneumonea*and *Salmonella enterica.*

The results shown in table 5 revealed the percentage frequencies of occurrence of bacterial isolates. *Proteus mirabilis* 16 (31.4 %) was the most prevalence bacteria isolate while *Escherichia coli* 3 (5.9%) had the least frequency of occurrence.
Table 6 shows the antibiotic susceptibility results which revealed that levefloxacin 100%, ciprofloxacin 100%, ceftriaxone 100%, azytrhromycin 90%, gentamycin 100%, pefloxacin 95%, oflaxacin 75%, cefuroxin 70% andceftazidine 60% were effective drugs against Enterobacteriaceae infections while augmentin 0% and nitrofuranton 50% were ineffective.

Conclusion

The presence and consumption of pathogens contaminated surface water especially in the rural areas has been of public health concern and the need for developing countries to be more focus on improved standards for drinking water for safety of health cannot be overemphasized. The carcinogenic risk effects of heavy metals and pathogens present in water have potential public health consequence for children and adults over a period of exposure, hence, attention must be given to the water treatment prior to consumption.

Table 1: Potential Carcinogenic Risk Assessment Values (CR_{ing}) of Pb, Cd and Cr for Adult and Children in Consuming Ossiomo River Through Ingestion Pathway during the Study Period.

		Adult				Children			
Metals 2021	**RfD_{ing}**	**Exp_{ing}**	**HQ**	**HI**	**CR_{ing}**	**Exp_{ing}**	**HQ**	**HI**	**CR_{ing}**
Jan									
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}	5.59×10^{-5}
Cd	0.5	1.09×10^{-3}	2.17×10^{-2}	1.30×10^{1}	1.78×10^{-6}	1.5×10^{-3}	3×10^{-2}	1.8×10^{-1}	2.46×10^{-7}
Cr	3	1.24×10^{-3}	4.12×10^{1}	2.47×10^{-2}	2.47×10^{-6}	1.88×10^{-3}	6.25×10^{-1}	3.75×10^{-2}	3.75×10^{-6}
Feb									
Pb	1.4	3.21×10^{-3}	2.30×10^{-2}	1.38×10^{-1}	3.78×10^{-5}	4.88×10^{-3}	3.48×10^{-2}	2.1×10^{-1}	5.74×10^{-5}
Cd	0.5	1.04×10^{-3}	2.08×10^{-2}	$1.25\times10^{-}$	1.70×10^{-6}	1.68×10^{-3}	3.15×10^{-2}	1.89×10^{-1}	2.58×10^{-7}
Cr	3	1.33×10^{-3}	4.43×10^{-1}	2.66×10^{-2}	2.70×10^{-6}	2.03×10^{-3}	6.75×10^{-1}	4.05×10^{-2}	4.10×10^{-6}
Mar									
Pb	1.4	3.16×10^{-3}	2.28×10^{-2}	1.37×10^{-1}	3.72×10^{-5}	4.8×10^{-3}	3.43×10^{-2}	2.06×10^{-1}	5.65×10^{-5}
Cd	0.5	1.09×10^{-3}	2.17×10^{-2}	1.30×10^{-1}	1.78×10^{-6}	1.65×10^{-3}	3.3×10^{-2}	1.98×10^{-1}	2.71×10^{-7}
Cr	3	1.38×10^{-3}	9.88×10^{-1}	5.93×10^{-2}	2.77×10^{-6}	2.1×10^{-3}	7.0×10^{-1}	4.2×10^{-2}	4.2×10^{-6}
Apr									
Pb	1.4	3.1×10^{-3}	2.19×10^{-2}	1.32×10^{-1}	3.60×10^{-5}	4.65×10^{-3}	3.32×10^{-2}	2.0×10^{-2}	5.47×10^{-5}
Cd	0.5	1.04×10^{-3}	2.10×10^{-2}	1.26×10^{-1}	1.7×10^{-7}	1.58×10^{-3}	3.2×10^{-2}	1.92×10^{-1}	2.60×10^{-7}
Cr	3	1.29×10^{-3}	4.28×10^{-1}	2.57×10^{-1}	2.6×10^{-6}	1.95×10^{-3}	6.5×10^{-1}	4×10^{-2}	4.0×10^{-6}
May									
Pb	1.4	3.0×10^{-3}	2.15×10^{-2}	1.29×10^{-1}	3.55×10^{-5}	4.58×10^{-3}	3.27×10^{-2}	1.96×10^{-1}	5.4×10^{-6}
Cd	0.5	9.88×10^{-4}	2×10^{-2}	1.2×10^{-1}	1.62×10^{-7}	1.5×10^{-3}	3.0×10^{-2}	1.8×10^{-1}	3×10^{-6}
Cr	3	1.24×10^{-3}	4.13×10^{-1}	2.48×10^{-2}	2.5×10^{-6}	1.88×10^{-3}	6.24×10^{-1}	3.8×10^{-2}	3.76×10^{-6}
June									
Pb	1.4	2.48×10^{-3}	2×10^{-2}	1.21×10^{-1}	3.31×10^{-5}	1.21×10^{-3}	8.62×10^{-1}	5.2×10^{-2}	1.42×10^{-5}
Cd	0.5	7.0×10^{-4}	1.28×10^{-2}	8.3×10^{-2}	8.14×10^{-6}	1.05×10^{-3}	2.1×10^{-1}	1.31×10^{-1}	1.72×10^{-7}
Cr	3	7.91×10^{-4}	2.64×10^{-1}	1.58×10^{-2}	1.6×10^{-6}	1.2×10^{-3}	4.0×10^{-1}	2.4×10^{-2}	2.4×10^{-6}
Average					$\sum$**10.06**$\times10^{-6}$				$\sum$**11.25**$\times10^{-6}$
USEPA Limit							**1.0×10^{-4} - 1.0×10^{-6}**		

Key: Values are expressed in mg/kg/day.

Table 2: Total Heterotrophic Bacterial Counts of Ossiomo River during the Study Period

	2021					
Sampling stations	**January**	**February**	**March**	**April**	**May**	**June**
Upstream	4.6±1.0	3.1±1.2	3.0±1.1	2.0±0.0	1.1±0.0	4.0±1.2
Midstream	5.0±2.2	4.2±1.2	4.0±1.2	3.1±0.1	3.1±0.1	9.1±2.1
Downstream	4.9±1.8	3.2±1.3	3.1±1.1	3.0±1.1	4.1±2.9	6.2±2.1

Key: Values expressed as mean of triplicates determination ±SD. Values are $x10^3$ cfu/mL. World Health Organization Permissible Limits for portable water = 0.

Table 3: Mean Total Coliform Counts of Ossiomo River during the Study Period

	2021					
Sampling stations	**January**	**February**	**March**	**April**	**May**	**June**
Upstream	53	75	126	65	23	27
Midstream	83	201	189	121	76	79
Downstream	65	97	152	78	34	35

Key: Values expressed as mean of triplicate determinations. Values areMPN/100 mL.World Health Organization permissible limits for portable water =0

Table 4: Cultural, Morphological, Biochemical and Physiological Characteristics of Bacteria Isolates

Characteristics	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Cluster	Single	Single	Single	Single	Single
Gram reaction	Negative	Negative	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	*Escherichia* sp	*Pseudomonas* sp	*Klebsiella* sp	*Samonella* Sp	*Shigella* Sp	*Proteus* sp

Table 5: Percentage Frequency of Occurrence of Bacterial Isolates.

	2021					
Bacteria Isolates	**January**	**February**	**March**	**April**	**May**	**June**
*Klebsiella*sp	10 (22.2)	9 (17.6)	8 (16.6)	9 (20.9)	9 (22.5)	10 (17.2)
*Pseudomonas*sp	7 (15.5)	7 (13.7)	14(29.2)	11 (25.6)	4 (10)	7 (12.1)
Shigella sp	8 (17.7)	7 (13.7)	8 (16.6)	7 (16.3)	7 (17.5)	10 (17.2)
Eschericha sp	3 (6.7)	3 (5.9)	4 (8.3)	5 (11.6)	4 (10)	10 (17.2)
Proteus sp	8 (17.7)	16(31.4)	7 (14.6)	6 (13.9)	3 (7.5)	8 (13.7)
Salmonella sp	9 (20)	9 (17.6)	7 (14.6)	5 (11.6)	13 (32.5)	13 (22.4)
Total	**45**	**51**	**48**	**43**	**40**	**58**

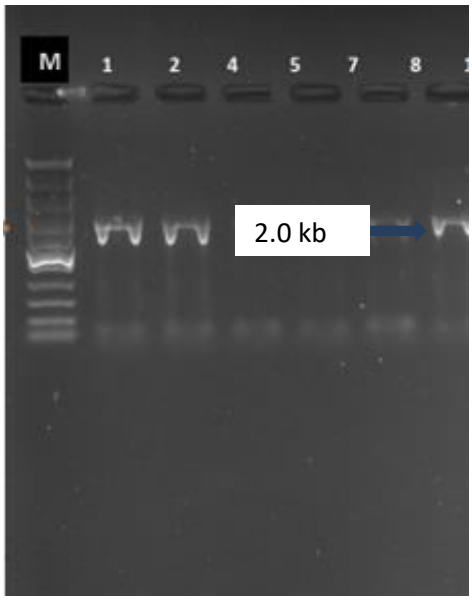

Plate 1: Agarose gel electrophoresis image of bacterial isolates from Ossiomo River. Lane M – molecular maker; Lane 1 - *Klebsiella pneumonea;* Lane 2 - *Pseudomonas aeruginosa;* Lane 4 - *Shigella sonnie;* Lane 5 - *Escherichia coli;* Lane 7- *Salmonella enterica;;* Lane 8 - *Proteus mirabilis*

Table 6: Antibiotic Susceptibility Pattern of Bacterial Isolates

Bacterial Isolates	No. of isolates	LEV 5 µg	PEF 5 µg	CRO 30 µg	GEN 10 µg	CIP 5 µg	CXM 30 µg	AZM 5 µg	OFL 5 µg	CAZ 30 µg	AUG 30 µg	NIT 300 µg
P. mirabilis	2	2(75)	1(60)	2(89)	2(59)	2(100)	1(10)	1(70)	2(70)	1(100)	0(0)	1(50)
E. coli	2	2 (73)	1 (50)	2 (75)	2(100)	2(100)	1(50)	1(50)	2 (75)	1(50)	0(0)	0(0)
S. sonnie	2	2 (85)	1(100)	2(100)	2(65)	2(100)	1(93)	1 (65)	2 (65)	1(100)	0 (0)	0 (0)
P. aeroginosa	2	1(57)	2 (95)	2(100)	2(100)	1(100)	1(65)	1(50)	2 (62)	1(50)	0(0)	0(0)
K. pneumonea	2	2 (68)	1(67)	0 (0)	0 (0)	2 (61)	2(70)	1 (50)	2 (60)	1(62)	0(0)	0(0)
S. enterica	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 aeroginosa, Klebsiella pneumonea, Proteus mirabilis, Shigella sonnie,*
LEV=Levofloxacin, PEF=Pefloxacin, CRO=Ceftriaxon, GEN=Gentamicin, CIP=Ciprofloxacin, CXM=Cefuroxime, OFL=Ofloxacin, CAZ=Ceftazidine, AUG=Augmentin,NIT=Nitrofurantoin and AZM= Azythromycin. Sensitivity= 91-100 %, Intermediate=51-90 %, Resistance 1-50%.

References

1. American Public Health Association (APHA) (2005). *Standards Method for the Examination of Water and Wastewater, 21st Edition*.American Public Health Association, Washington DC.308 pp.
2. Asarr-Donkor, N.K., Boadu, T.A. and Adimado, A.A. (2016). Evaluation of groundwater and surfacewater quality and human risk assessment for treatment of human settelementa around the Bosomtive Crater Lake in Ghana. *Springer Plus* **5:**18-25
3. Cheesbrough, M. (2000). *Medical Laboratory Mannual for Tropical Countries. Volume 11, 2nd ed.*,University Press, Cambridge, Great Britain. 377 pp.
4. Cheesbrough, M. (2006).*District Laboratory Practics in Tropical Countries Part 2*. Cambridge University Press, New York, USA. 434 pp.
5. Clasen, T., Schmidt, W., Rabie, T., Roberts, I. and Caircross, S. (2007). Interventions to improve water quality for preventing diarrhea: systematic review and meta-analysis. *British Medical Journal* 1-10.
6. Clinical and Laboratory Standards Institutes (CLSI). (2008). *Performance Standards for Antimicrobial Susceptibility Testing. Eighteenth Informational Supplement (Document M100-S18). Vol. 4.,* The Clinical Laboratory Standards Institute, Wayne, PA, USA. 354 pp.
7. Fagorite, V. I., Ahiarakwem, C. A., Ibeneme, S. I., Ekeoma, S. C., Ukwajiunor, J. I., Abiahu, C. M. and Poopola, J. O. (2019). Microbial assay of Otamiri River and its sediments in parts of Owerri. *Journal of Geoscience and Environmental Protection***7:**155-166.
8. Fietto, J.L., Aranyo, R.S., Valadao, F. C., Fietto, L.G., Brandao, R.L., Neves, M.J., Gomes, F.C., Nicoli, R.R. and Castro, M.I. (2004).Molecular and physiological comparisons between*Saccharomyces cerevisiae* and*Saccharomycess bounlardii*.*Canadian Journal of Microbiology***50:** 615 – 621.
9. Ibironke, T. E., Ambimbola, M. E., Odiyo, O. and Muhammed, M.A. (2018). Human health risk assessment of trace metals in surface water due to leachate form the municipal dumpsite by pollution ondex: A case study from Ndawuse River , Abuja, Nigeria. *Journal of Open Chemistry* **16:** 214 – 229.
10. Islam, M. S., Mohanta, S.C., Siddique, M. A.B. Abdullah-Al-Mamum, M., Hossain, N., Bith, U. H. (2018).Physicochemical assessment of water quality parameters in Rupsha River of Khuhan region, Bangladesh.*International Journal of Engineering and Sciences***7:** 73 – 78.
11. Klindworth, A., Pruese, E., Schweer, T., Peplies, J., Quest, G., Horn, M. and Glockner, F.O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primer for classic and next-generation sequence diversity studies. *Journal of Nucleic Acid Research***41(1):** 1- 11.
12. Lee, Y.K., Kim, H.W., Liu, C.L. and Lee, H.K. (2003).A simple method for DNA extraction from marine bacteria that produce extracellular materials.*Journal of Microbiological Methods***52:** 245-250.
13. Li, S. and Zhang, Q. (2010). Spatial characterization of dissolved trace elements and heavy metals in the Upper Han River (China) using multivariate Statistical techniques. *Journal of Hazard Matter* **176:** 579 – 588.
14. Madilonga, R., Edokpayi, J. N., Volenzo, E. T., Durowoju, O. S. and Odiyo, J.O. (2021). Water quality assessemnet and evaluation of human health risk in Mutaangwi River, Limpopo Province, South Africa. *International Journal of Environmental research and public health* **18:** 1-16.
15. Mulamattathil, S.G., Bezuidenhout, C., Mbewe, M. and Ateba, C. N. (2014). Isolation of environmental bacterial from surface and drinking water in Mafikeng, South Africa and characterization using their antibiotic resistance profiles. *Journal of Pathogens***21:** 1- 11.
16. Odigie, A. B., Imarhiagbe, E. E. and Ekhaise, F. O. (2023). Quantitative risk assessment of bacteria and heavy metals in Ossiomo River, Orhionmwon Local Government Area, Edo State, Nigeria. *Arid Zone Journal of Basic and Applied Research***2(3):** 34-47.

17. Ogbeibu, A. E. (2005). *Biostatistics; A Practical Approach to Research and Data Handling*. Mindex Press Limited, Benin City, Nigeria.262 pp.
18. Ullah, N., Hashmi, M.Z., Yu, C., Shen, H. and Duan, D. (2015). Concentration of human health risk assessment of selected heavy metals in surface water of the siling reservoir watershed in Zhejiang Province, China. *Polish Journal of Environmental Studies* **23(3):** 801 – 811.
19. United States Environmental Protection (USEPA). (1989). *Risk Assessment Guidance for Superfound, Vol. 1.Human Health Evaluation Mannual (Part A),* Washington DC.540 pp.
20. World Health Organization (WHO). (2011). *Guidelines for drinking water quality* 4h edition, World Health Organization, Geneva, Switzerland. 155 pp.