

Trace Metals in Water, Bottom Sediment, Shrimp and Dependent Human Blood in Ukwuani Local Government Area of Delta State, Nigeria

Patrick Omoregie Isibor^{1*}, Imoobe Tunde Ohiokhioya Thadeus²

(Phone: +2347035790793, E-mail: patrickisibor007@gmail.com)*

¹Department of Biological Sciences, Faculty of Science and Technology, Covenant University, Ota, Ogun State, Nigeria

²Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, P.M.B. 1154, Nigeria

ABSTRACT

Trace metals (Fe, Mn, Zn, Cu, Pb, Cd and Cr) levels were investigated in the water, bottom sediment, shrimp (*Macrobrachium vollenhovenii*) of Okumeshi River using Atomic Absorption Spectrophotometer (ASS); and blood of individuals that feed on the shrimps using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The descriptive statistics such as the mean, range and standard error were for significant differences in the heavy metals, nutrients and total hydrocarbons in water and sediment samples was done using ANOVA ($P < 0.05$). Duncan Multiple Range (DMR) test was used to identify the source of variance using SPSS version 19.1. The order of accumulation in the matrices studied was bottom sediment > shrimp > water > human blood. High level of zinc and copper in human blood can be attributed to their high concentrations in all environmental matrices. It can also be attributed to the high biological accumulation factors of the metals in the shrimp. Copper also has a high essentiality in the respiratory pigment haemocyanin of shrimps. These facts are buttressed by the significant correlations of copper and zinc concentrations in the shrimp with their concentrations in human blood. The observed insalubrity of the shrimps is a prognostic of chronic health hazards to the consumers; which is also a function of the predominant oil exploration activities. Copper had an uninterrupted transit into the blood of the consumers from the aqueous phase. While zinc showed some significant level of biomagnification. The study provided an early signal of likely impending catastrophe; necessitating a proactive regulation of trace metals released into the aquatic system through the prevalent anthropogenic activities; mainly oil exploration.

Keywords: Trace metals; water; sediment; sorption capacity; biological accumulation; *Macrobrachium vollenhovenii*

1. INTRODUCTION

The study was conducted on Okumeshi River; within Uwuani Local Government area of Delta State. The river is a source of drinking water, finfish and shellfish to dwellers in near and far communities. Finfish and shellfish products from Okumeshi River serve as exotic local and foreign export products. However, despite its domestic, nutritional and economic values, the river is faced with incessant perturbations due to anthropogenic activities around it. The activities include oil exploration and exploitation, agricultural practices, discharge of domestic and industrial wastes, amongst others. Sadly, water and sediment matrices are receptors of these anthropogenic by-products; as well as they are habitats to aquatic organisms of high nutritional and economic values.

The indiscriminate dumping of refuse, discharge of industrial effluents, petroleum wastes and crude oil spills replete with heavy metals may culminate in serious health consequences on populace that consume the finfish and shellfish harvested from the river. The mechanism is by incorporation of the toxicants from anthropogenic perturbations such as trace metals into the aquatic food; thereby biomagnifying up the pyramid of biomasses through alimentation [1, 2, 3]. Variability in water and sediment properties is a function of a number of factors which have been enormously reported in previous studies. Generally, these factors can be categorized as autochthonous and allochthonous factors working in tandem. Tukura *et al.* [4] attributed variation of physico-chemical properties of water and sediment of Mada River, Nasarawa State, Nigeria to seasonal variation. The water matrix of an aquatic ecosystem is the first receptor of the contaminants released from anthropogenic activities. The sediment then serves as repository to the contaminants [5]. However, the rate of deposition of these contaminants is a function of their sorption capacities; which vary among contaminants. Ogbeibu *et al.* [6] observed that manganese, zinc, copper, cadmium, lead and total hydrocarbons had very high sorption capacities from water into the sediment of Ikpoba River. Furthermore, the contaminants journey from these environmental matrices into the tissues of biota where they are stored indefinitely. Froese *et al.* [7] reported bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects in Saginaw Bay, Michigan, USA. Davies *et al.* [8] reported accumulation of heavy metals in tissues of *Tympanotomus var radula* from sediment of Elechi Creek, Nigeria. Akan *et al.* [9] attributed accumulation of metals in the gills, liver, stomach, kidney, bones and

flesh tissues of *Tilapia zilli*, *Clarias anguillaris*, *Synodontis budgetti* and *Oreochromis niloticus* from River Benue to the concentrations in the ambience. Isibor *et al.* [5] attributed impermissible levels of trace metals in *Clarias gariepinus* of Osse River, Edo State, Nigeria to high levels observed in the water and sediment.

Palaemonid shrimps; particularly *Macrobrachium vollenhovenii* (Herklots, 1851) are very abundant in Okumeshi River in Ukwuani Local Government of Delta State, Nigeria. The shrimps serve as a cheap source of animal protein to dwellers in the nearby communities. They also serve as source of income for the local fishermen and other buyers from far and near. Their high commercial value is partly due to their relatively large body size [10] and tenacious nature [11]. Sadly, this species is highly notorious for accumulation of metals from polluted environments [10, 12]. This is due to their morphology and niche which maximize accumulation of contaminants from bottom sediments and overlying water [11]. Constant trace metals released into Okumeshi River are bound to remain in the environmental matrices for long periods [13, 14, 15] till they are accumulated in the biota.

The study investigated the transit of trace metals from the environment into *M. vollenhovenii* and ultimately blood of dependent populace, with a view to predicting prognostic hazardous exposures.

2. MATERIAL AND METHODS

2.1. The study area

The Study was conducted on a stretch of Okumeshi River from August, 2016 to January, 2017. The river lies between latitude 5° 28' - 5° 48' N and longitude 5° 31' - 5° 40' E; in Ukwuani Local Government Area of Delta State (Figure 1). The river serves as source of water, affordable animal protein and means of transportation to the surrounding communities. The river incessantly receives municipal wastes and industrial effluents [16, 17]. It is a lotic freshwater with predominant vegetation such as palm trees (*Elaeis guinensis*), shrubs, floating *Salvinia* species, *Lemnas* species and water hyacinth (*Eichhornia crassipes*). The study area is located in the Niger Delta area of Nigeria; with sediment which includes Benin, Agbada and Akata formations; ranging from Eocene to Recent age [18, 19]. The study area is made up of the Benin formation which is of Miocene- recent age. The thickness is about 200 m [20].

For the purpose of the study water, sediment and *M. vollenhovenii* samples were collected from four (4) stations which were chosen along the stretch of the river. Station 1 was chosen as the control station. Stations 2 and 3 witnessed varied oil exploration activities over time, while Station 4 was disturbed with agricultural practices and domestic waste deposition. These activities were very severe as at the period of this study. Blood samples were collected from human volunteers who are residents of Ukwuani Local Government and they rely substantially on the supply of *Macrobrachium vollenhovenii* from the river as cheap source of animal protein. Occupations of the blood donors are basically farming and fishing.

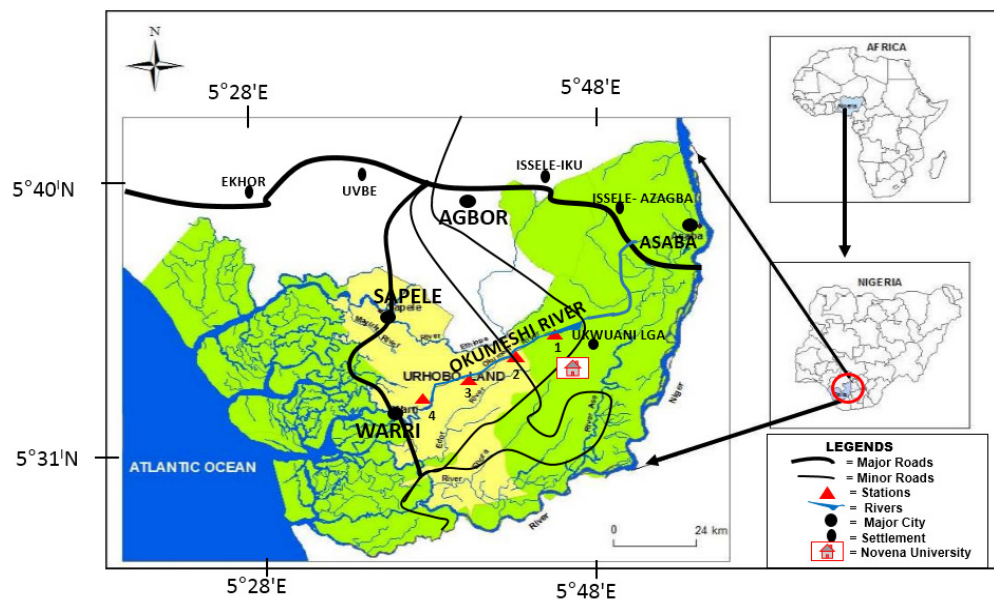


Figure 1. Map of the study area

2.2. Collection and analysis of samples

2.2.1. Blood samples

A total of 150 human volunteers (75 males and 75 females) were recruited into the research with their informed consent; in line with Helsinki Declaration guidelines. Prior to recruitment it was ascertained that they were all non-smokers. Their general health status was also verified through routine inquiries. The health screening procedures were taken due to the fact that health status influences metal content of the blood [21, 22]. 1mL of blood samples was collected from donors in tubes containing lithium heparin anticoagulant; with lid. The tubes and their lids were thoroughly rinsed with de-ionized water and dried

prior to use. The collected samples were taken immediately to the laboratory for analysis. Prior to digestion, all samples were vortexed thoroughly to provide a homogeneous matrix for digestion. Reagent blanks were prepared by addition of deionized water in place of the samples to monitor background concentrations of all analytes. 1 mL of 2M analar grade HNO₃ was added to each blood sample and was allowed to react for 30 minutes. Thereafter, 750 µL of non-stabilized 30% H₂O₂ solution was added to each sample. Once the addition of all reagents was complete, the tubes were sealed with lids and digested by microwave method using the Discovery SPD (CEM, Matthews, NC). After digestion, the samples were analyzed for trace metals using the Thermo (Bremen, Germany) X-Series 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) equipped with a concentric glass nebulizer and peltier-cooled glass spray chamber. At the end of digestion, all samples were removed from the microwave and allowed to cool to room temperature. Samples were stored in a monitored refrigerator at a nominal temperature of 8 °C until analysis. Trace metal calibration standards for ICP-MS analysis were prepared using Fe, Mn, Zn, Cu, Pb, Cd, and Cr standards. Two sets of calibration standards were prepared for metals that were anticipated to be in low concentrations (such as Pb, Cd, Cr, and Mn) and high concentrations (such as Zn, Cu, and Fe). Samples were analyzed without dilution in the original storage containers to minimize the possibility of contamination. For the calibration, standards back-calculated concentrations were ensured to be within ±15% of the nominal concentration (±25% of the nominal concentration for the lowest concentration standard). Quality control checks were done to ascertain that concentrations for all metals were found to be within ±15% of the nominal concentration.

2.2.2. Water samples

Surface water samples were collected at the four stations on a monthly basis using 250 mL sampling bottles; and transported immediately to the laboratory for analysis of the trace metals. Water samples were pretreated and digested using the wet oxidation method described by Martin *et al.* [23]. Samples were fixed with 2 mL nitric acid [23] and then filtered through Whatman filtered paper no 1 and aspirated directly into an Atomic Absorption Spectrometer (Varian Techon Spectr AA – 10; serial number 902 1318) connected to an HP printer (Deskjet 2400 Printer series); for the quantitative determination of all trace metals. The blanks were prepared accordingly. For quality assurance purposes AAS was

calibrated for each metal by dissolving 1 gram analytical grade metal salt in 1 litre of distilled water. Standard and corresponding blanks were run with each set of experimental digest. The detection limits of iron (0.5 mg/L), manganese (0.5 mg/L), zinc (0.5 mg/L), copper (0.05 mg/L), lead (0.03 mg/L), chromium (0.05 mg/L) and cadmium (0.01 mg/L) were carefully observed.

To ascertain quality control and standardization measures, all laboratory procedures were repeated at least 3 times and mean values were compared with standard values supplied by FEPA [24].

2.2.3. Sediment samples

Bottom sediment samples were collected using a Birge-Ekman grab. The sediment samples were collected in foil papers and wrapped with labelled polythene. All samples were preserved in ice coolers and transported immediately to the laboratory for analysis. This routine was conducted repeated at all stations on monthly basis from August, 2016 to January, 2017.

1 gram air-dry sediment sample was placed in a 300 mL calibrated digestion tube. 3 mL concentrated nitric acid (HNO₃) was added, swirled carefully and placed in a rack to settle. The mixture was slowly heat up by gradually increasing temperature to about 145 °C for 1 hour [25]. 4 mL concentrated Perchloric acid (HClO₄) and heat it to 240 °C for another 1 hour. Mixture was allowed to cool to room temperature. It was filtered through Whatman No. 42 filter paper and the volume was made up to 50 mL with de-ionized water. Heavy metals were then determined by Atomic Absorption Spectrophotometer (model-analyst 200 PerkinElmer). For quality control and standardization measures, all laboratory procedures were repeated at least 3 times and mean values were compared with standard values supplied by FEPA [24].

2.2.4. Shrimp samples

A total of 30 representatives [11] of identified *Macrobrachium vollenhovenii* collected from each station on monthly basis were preserved for laboratory analysis. This was done at low tide regime during night periods using gear such as woven cylindrical non-return valve traps, baskets and scoop nets; in conjunction with coconut, cassava and earthworm baits. Some were procured from fishermen and sources of such samples were properly ascertained before procurement. The samples were preserved in four

different coolers with ice; appropriately labeled to indicate the source stations. Whole shrimp was digested according to standard method illustrated by Benhard [26]. 10 grams of wet weight shrimp tissue was placed in silica flasks covered with a glass plate, 20 mL of HNO₃: HClO₄ mixture was added; ratio 5:1 respectively. Digestion process was carried out by heating the mixture at 105°C for about 24 hours according to the illustrations of Turkmen [27]. The extract was made up to 25 mL with HNO₃ (70%) and diluted with deionized water. For quality assurance, reagent blanks were processed simultaneously in triplicates. Each residue was filtered into volumetric flask with the aid of a Whatman filter paper. The solution was tested for metals concentrations using a Perkin Elmer 3110 model Atomic Absorption Spectrophotometer (ASS) and recorded in mg/kg wet weight [11, 28]. The ASS was calibrated for each metal by dissolving 1 gram analar grade metal salt in 1 litre of distilled and deionized water. Standard and corresponding blanks were run with each set of experimental digest. The detection limits of iron (0.05 mg/kg), manganese (0.05 mg/kg), zinc (0.05 mg/kg), copper (0.005 mg/kg), lead (0.003 mg/kg), cadmium (0.001 mg/kg) and chromium (0.05 mg/kg), copper (0.005 mg/kg) were carefully read.

The actual concentration of trace metals was calculated thus:

Actual concentration of metal = RD X Dilution factor [28].

Where RD = ASS reading of digest

Dilution factor = $\frac{\text{Volume of digest used}}{\text{Weight of sample digested}}$

2.3 Statistical analysis

The descriptive statistics such as the mean, range and standard error were for significant differences in the heavy metals, nutrients and total hydrocarbons in water and sediment samples was done using ANOVA ($P < 0.05$). Duncan Multiple Range (DMR) test was used to identify the source of variance using SPSS version 19.1.

In order to discern the major parameters of key importance i.e. active components in the matrices, the principal components were analyzed using descriptive statistics such as communalities, total variance, percentage variance and rotated component matrix. Parameters with communality values less than 0.75 were considered insignificant while components with Eigen values less than 1 were also considered

insignificant [29]; hence eliminated so that fewer components were further subjected to the Varimax rotation stage using Keiser normalization method [29, 30, 31].

Distribution coefficient (K_d) was used to determine the sorption capacity of the parameters into the sediment. Values less than 6 mg/L were considered insignificant.

$$\text{Distribution coefficient (Kd)} = \frac{M_{\text{ads}}}{M_{\text{sol}}} \quad [32].$$

Where M_{ads} = metals adsorbed into the soil and M_{sol} = metal concentration in water.

The Biological Accumulation Factors (BAF) and Biota-Sediment Accumulation Factors (BSAF) of the trace metals in the *M. vollehovenii* under study were calculated using the following methods;

$$\text{BAF} = \frac{\text{CF (mg/kg)}}{\text{CW (mg/kg)}}$$

Where BAF is the biological accumulation factor, CF is concentration of metals in the shrimp and CW is concentration of metals in water.

$$\text{BSAF} = \frac{\text{CF (mg/kg)}}{\text{CS (mg/kg)}}$$

Where BSAF is the biota-sediment accumulation factor, CS is the concentration of the metals in sediment and CW is the in water.

All sample replicates were collected on spatial and temporal basis;

Spatial replicates: On monthly basis, the mean concentrations of each metal was calculated thus: T/N

Where T= Total concentration of metal in all samples.

Temporal replicates: This was expressed as the total monthly values divided by number of months. Hence, sample size was expressed as the number of months; which is N= 6 in all cases. This was then expressed as Mean $\pm$ Standard Error.

3. RESULTS

Concentration of iron observed in water at Station 2 was significantly higher than that of other stations ($P < 0.05$); which exhibited no significant difference (Table 1). However, the levels of iron across all stations were below the recommended limit by FEPA. The levels of manganese detected in the water of Stations 3 and 4 were higher than the regulatory limit, which was higher than the levels obtained at Station 2 and control. Copper concentration in water at Station 4 was higher than the regulatory limit and other

227 stations ($P < 0.01$). Generally, despite the spatial heterogeneity in the levels of the trace metals in the
 228 water, levels of most of the metals were within regulatory limits throughout the study period.

229

230 **Table 1:** Summary of heavy metals(mg/L) in water of Okumeshi River

METALS	STATION 1 MEAN±S.E(RANGE)	STATION 2 MEAN±S.E(RANGE)	STATION 3 MEAN±S.E(RANGE)	STATION 4 MEAN±S.E(RANGE)	P VALUE	FEPA [24]
Fe	1.81±0.16^B (0 – 2.4)	4.71±0.25^A (0 – 3.5)	1.50±0.19^B (0 – 2.9)	1.48±0.27^B (0.2 – 5.4)	P<0.05	20
Mn	0.31±0.01^B (0 – 0.1)	0.26±0.06^B (0 – 0.7)	0.97±0.22^A (0 – 2.3)	1.26±0.34^A (0 – 3.7)	P<0.001	0.5
Zn	0.7±0.03^B (0 – 0.4)	0.59±0.15^A (0.1 – 2.6)	0.92±0.3^A (0.2 – 5.2)	0.985±0.25^A (0 – 3.3)	P<0.05	1
Pb	0.1±0.003(0 – 0.1)	0.08±0.01(0 – 0.2)	0.03±0.24(0 – 2.7)	0.33±0.26(0 – 2.7)	P>0.05	< 1
Cu	0.3±0.01^C (0 – 0.1)	0.14±0.03^B (0 – 0.5)	0.13±0.02^B (0 – 0.4)	1.05±0.26^A (0 – 2.76)	P<0.01	< 1
Cd	0.5±0.03^B (0 – 0.4)	0.08±0.02^B (0 – 0.3)	0.23±0.12^B (0 – 1.6)	0.4±0.16^A (0 – 0.7)	P<0.01	<1
Cr	0.02±0.01^C (0 – 0.2)	0.35±0.01^B (0 – 1.1)	0.71±0.18^A (0 – 2.1)	0.73±0.2^A (0 – 2.3)	P<0.01	<1

231 **Note:** Values with similar superscript letters have no significant difference Number of sample replicates (N) = 18.
 232 $P>0.05$ means there is no significant difference, **P<0.05** means there is significant difference, **P<0.01** means there is
 233 much significant difference, and **P<0.001** means there is very much significant difference. ND = Not Detected. FEPA
 234 means Federal Ministry of Environment [24].

235 In the water matrix, only manganese had a significant communality extraction; which was 0.909 (Table 2).

236 Components 1 (4.152) and 2 (1.232) had significant Eigen values and they contributed 51.898 and
 237 15.395 % respectively to the total percentage of variance. Varimax rotated matrix of water (Table 3)
 238 showed that manganese (0.947) > copper (0.883) > zinc (0.817) > chromium (0.759) > Pb (0.790) was the
 239 sequence of trace metal activity in component 1. While Cd (0.782) and Fe (0.812) were the only active
 240 metals in component 2.

241 **Table 2:** Principal components of water

Communality extractions						
Fe	Mn	Zn	Cu	Pb	Cd	Cr
0.680	0.671	0.699	0.909	0.555	0.478	0.544
Eigen values by principal components						
1	2	3	4	5	6	7
4.152	1.232	0.776	0.717	0.429	0.315	0.202
Percentage of total variance (%)						
51.898	15.395	9.698	8.958	5.263	4.932	2.527

242 **Note:** Communality values from 0.75 and above are significant; hence emboldened
 243 Eigen values from 1 and above are significant; hence emboldened

244

245

246 **Table 3:** Varimax rotated component matrix of water

Component	Mn	Cu	Zn	Cr	Pb	Cd	Fe
1	0.947	0.883	0.817	0.759	0.790	0.675	0.144
2	0.112	0.054	0.061	0.318	0.070	0.782	0.812

247 **Note:** Communality values from 0.75 and above are significant; hence emboldened

248

249 The mean concentration of Fe in sediment at Station 2 was significantly higher than Station 3 > Station 4
 250 > Station 1 ($P < 0.05$). Concentrations of all trace metals (except lead) in the sediment at all stations;
 251 except control exceeded the regulatory limit. The trend of manganese was Stations 2 and 3 > Station 4
 252 > Station 1 ($P < 0.001$).

253 **Table 4:** Summary of heavy metals (mg/kg) in Sediment of Okumeshi River

METALS	STATION 1 MEAN±S.E(RANGE)	STATION 2 MEAN±S.E(RANGE)	STATION 3 MEAN±S.E(RANGE)	STATION 4 MEAN±S.E(RANGE)	P VALUE	FEPA [24]
Fe	0.03±0.06^D (0 – 2.4)	10.9±2.2^A (9 – 63.8)	8.44±0.19^B (0 – 2.9)	2.38±0.27^C (0.2 – 5.4)	P<0.05	1
Mn	0.01±0.003^C (0 – 0.1)	8.24±0.6^A (2 – 10.7)	5.97±0.22^A (1.2 – 8.3)	4.26±0.34^B (0 – 3.7)	P<0.001	0.4
Zn	0.35±0.06^D (0 – 0.4)	7.59±0.15^A (0.1 – 2.6)	4.92±0.3^B (0.2 – 5.2)	1.99±0.25^C (0 – 3.3)	P<0.05	3
Pb	0.01±0.001(0 – 0.1)	0.04±0.01(0 – 0.2)	0.03±0.24(0 – 2.7)	0.03±0.26(0 – 2.7)	P>0.05	0.05
Cu	0.05±0.015^C (0 – 0.1)	14.1±0.3^B (6.3 – 22.2)	21.11±2^A (8.2 – 31.6)	12.01±2^B (3.4 – 22.7)	P<0.001	0.3
Cd	0.01±0.001^C (0 – 0.3)	7.03±0.02^A (1.2 – 10.3)	3.13±0.12^B (0 – 5.6)	4.8±0.16^B (0.3 – 6.7)	P<0.01	0.05
Cr	0.01±0.001^D (0 – 0.2)	0.3±0.01^C (0 – 1.1)	8.33±0.18^A (0 – 2.1)	4.73±0.2^B (0 – 6.3)	P<0.01	2

254 **Note:** Values with similar superscript letters have no significant difference Number of sample replicates (N) = 18.
 255 $P>0.05$ means there is no significant difference, **P<0.05** means there is significant difference, **P<0.01** means there is
 256 much significant difference, and **P<0.001** means there is very much significant difference. ND = Not Detected. FEPA
 257 means Federal Ministry of Environment [24].
 258

259 Iron (0.784), manganese (0.745), copper (0.802), and cadmium (0.778) had significant communality
 260 extractions in the sediment. While only components 1 (3.954), 2 (1.373) and 3 (1.287) were of significant
 261 Eigen values (Table 5), and they constituted 49.426, 19.166 and 15.09 % respectively of the total
 262 variance. After subjecting only the significant components to further rotation to generate the rotated
 263 component matrix; copper (0.896)> cadmium (0.755)> Cr (0.756) was the sequence observed in
 264 component 1 (table 6). The sequence in component 2 was: Mn (0.871) > Pb (0.787) > Zn (0.771). Fe
 265 (0.812) was the only active metal in component 3.

266

267 **Table 5:** Principal components of sediment

Communality extractions						
Fe	Mn	Zn	Cu	Pb	Cd	Cr
0.784	0.745	0.699	0.802	0.555	0.778	0.544
Eigen values by principal components						
1	2	3	4	5	6	7
3.954	1.373	1.287	0.563	0.487	0.320	0.217
Percentage of total variance (%)						
49.426	19.166	15.090	5.037	4.087	2.999	1.710

268 **Note:** Communality values from 0.75 and above are significant; hence emboldened
 269 Eigen values from 1 and above are significant; hence emboldened
 270
 271

Table 6: Varimax rotated component matrix of sediment

Components	Cu	Cd	Cr	Mn	Pb	Zn	Fe
------------	----	----	----	----	----	----	----

1	0.896	0.755	0.756	0.687	0.656	-0.136	0.401
2	0.014	-0.174	0.447	0.871	0.787	0.771	0.064
3	0.022	0.334	0.142	0.456	0.675	0.056	0.812

Note: Community values from 0.75 and above are significant; hence emboldened

Iron (3.28), manganese (6), copper (28.83), cadmium (12.35) and chromium (7.38) had significant sorption into the bottom sediment from the water medium (Table 7) i.e. their sorption capacities are above the significant level of 6 mg/L. Copper had an outstandingly high sorption into the bottom sediment: Cu> Cd> Cr > Mn.

Table 7: Distribution coefficients (Kd) of parameters in sediment and water (mg/L)

Fe	Mn	Zn	Cu	Pb	Cd	Cr
3.28	6	4.65	28.83	0.244	12.35	7.38

Note: Values < 6mg/L are insignificant, = or > 6 mg/L are significant [32]. Significant values are emboldened.

Generally the levels of most metals observed in the *M. vollenhovenii* at the perturbed sections of the river were distinctively higher than the counterparts at the control station (table 8). The concentration of iron observed in *M. vollenhovenii* at Station 2 is significantly higher than that of Station 3 > 4 > Station 1 (P< 0.01). The levels of iron and in the shrimps at all stations exceeded the standard recommendation by FEPA [24]. Most high concentrations of trace metals were detected in shrimps at Stations 2 > 3 > 4. The trend can be attributed to the respective extents of perturbations as observed around the stations. Only manganese, lead and chromium exhibited no significant difference shrimps across the stations. The levels of these trace metals in the shrimps were also below FEPA regulatory limit.

Table 8: Summary of **trace** metals (mg/kg) in *Macrobrachium vollenhovenii* of Okumeshi River

PARAMETERS	STATION 1 MEAN±S.E(RANGE)	STATION 2 MEAN±S.E(RANGE)	STATION 3 MEAN±S.E(RANGE)	STATION 4 MEAN±S.E(RANGE)	P VALUE	FEPA [24]
Fe	0.3±0.06^D (0 – 1.2)	11.2±2.1^A (6.2 – 16.8)	8.44±1.19^B (2.2 – 12.9)	1.78±0.27^C (0.2 – 3.4)	P<0.01	100
Mn	0.001±0.001(0 – 0.1)	0.23±0.01(0 – 0.7)	0.35±0.01(0 – 1.1)	0.32±0.04(0 – 1.7)	P>0.05	1
Zn	0.35±0.06^C (0 – 0.84)	4.99±0.15^A (0.1 – 8.6)	2.92±0.3^B (0.2 – 6.9)	0.99±0.2^C (0 – 1.5)	P<0.05	75
Pb	ND	0.01±0.01(0 – 0.2)	0.02±0.24(0 – 2.7)	0.03±0.26(0 – 2.7)	P>0.05	0.05
Cu	0.05±0.015^C (0 – 0.1)	14.12±0.01^A (0 – 0.5)	12.3±0.02^A (0 – 0.4)	2.05±0.22^B (0 – 2.76)	P<0.001	3
Cd	ND^c	6.08±0.02^A (0 – 0.3)	2.23±0.12^B (0 – 1.6)	2.8±0.16^B (0 – 0.7)	P<0.01	2
Cr	0.003±0.001(0 – 0.12)	0.04±0.01(0 – 0.9)	0.01±0.002(0 – 1.2)	0.03±0.2(0 – 0.7)	P>0.05	-

Note: Values with similar superscript letters have no significant difference Number of sample replicates (N) = 18. P>0.05 means there is no significant difference, **P<0.05** means there is significant difference, **P<0.01** means there is much significant difference, and **P<0.001** means there is very much significant difference. ND = Not Detected. FEPA means Federal Ministry of Environment [24].

Fe (0.772), Mn (0.945), Cu (0.784) had significant communality extractions (Table 9), while components 1 (4.054), 2 (2.973), 3 (1.887) and 4 (1.563) only had the significant Eigen values; hence were the only considered components. Varimax rotated component matrix showed that Cu (0.896)>Fe (0.725)> Zn (0.796)> Cd (0.887) were active in component 1. Cr (0.767) was the active metal in component 2, Pb (0.783) in component 3 and Mn (0.772) in component 4.

Table 9: Principal components of *Macrobrachium vollenhovenii*

Communality extractions						
Fe	Mn	Zn	Cu	Pb	Cd	Cr
0.772	0.945	0.223	0.784	0.251	0.278	0.223
Eigen values by principal components						
1	2	3	4	5	6	7
4.054	2.973	1.887	1.563	0.487	0.320	0.217
Percentage of total variance (%)						
39.426	21.166	17.090	14.037	3.087	2.999	2.195

Note: Communality values from 0.75 and above are significant; hence emboldened
Eigen values from 1 and above are significant; hence emboldened

Table 10: Varimax rotated component matrix of *Macrobrachium vollenhovenii*

Components	Cu	Fe	Zn	Cd	Cr	Pd	Mn
1	0.896	0.785	0.796	0.887	0.656	0.636	0.401
2	0.014	-0.174	0.447	0.559	0.767	0.271	0.541
3	0.023	0.561	0.334	0.243	0.012	0.783	0.472
4	0.455	0.056	0.667	0.451	0.042	0.402	0.772

Note: Communality values from 0.75 and above are significant; hence emboldened

As seen in Figure 2, significant Biological Accumulation Factors (BAF) were observed in iron (3.27), zinc (2.89), copper (17.6) and cadmium (9.17). The BAF of copper in the shrimp was outstandingly higher than others, while manganese, lead and chromium exhibited no significant BAFs. The high BAF of copper in *M. vollenhovenii* can be attributed to its threshold of essentiality in the respiratory pigment of the shrimp.

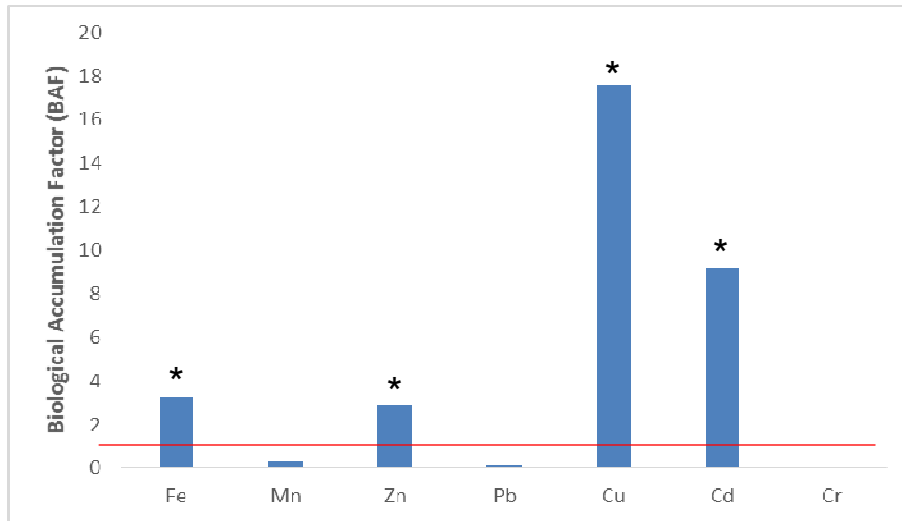


Figure 2- Biological Accumulation factors (BAF) of trace metals in *M. vollenhovenii*
 NOTE: BAF values of 1 and above signify significant values; indicated by the red line. Asterisked bars indicate trace metals of significant BAF.

Significant Biota-Sediment Accumulation Factor (BSAF) was observed only in iron (1). Other trace metals exhibited non-significant BSAF (Figure 3). This implies that the shrimps accumulated more trace metals from the water matrix than from the sediment matrix.

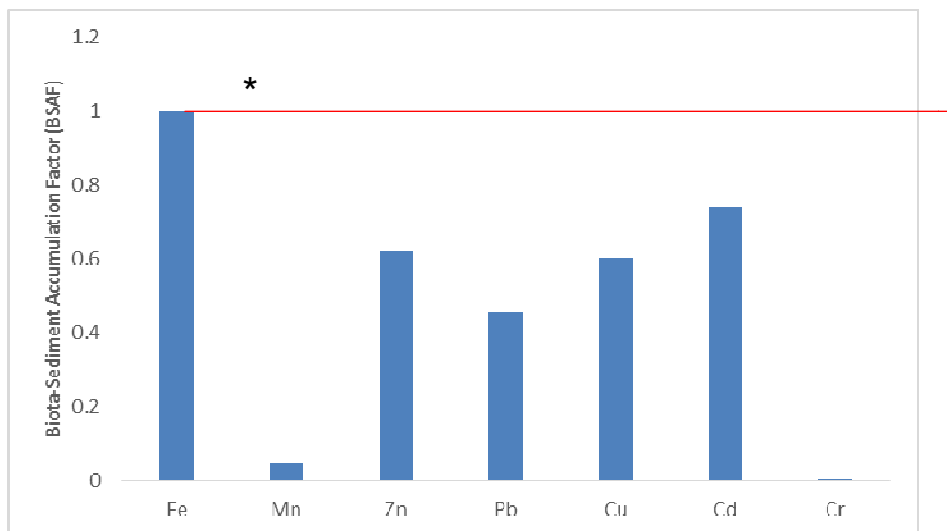


Figure 3- Biota-Sediment Accumulation factors (BSAF) of trace metals in *M. vollenhovenii*
 NOTE: BAF values of 1 and above signify significant values; indicated by the red line. Asterisked bars indicate trace metals of significant BAF.

The levels of zinc (8864.68 ± 12.72) and copper (1387.48 ± 22.21) observed in the blood samples of the individuals who consume *M. vollenhovenii* of the river are higher than the recommended ranges; which are 4400- 8600 and 800- 1300 $\mu\text{g/L}$ respectively (Table 11). The levels of other trace metals in the

blood samples of the subjects were within the acceptable range recommended by Iyengar and Woittiez [33].

Result showed that significant concentrations of metals were accumulated only in shrimp samples obtained from the perturbed sections of the river. This necessitated the need to trace these metals in the consumers. Therefore only *M. vollenhovenii* harvested from perturbed sections of the river were further assessed in the study. Significant correlations were observed between the trace metals in the *M. vollenhovenii* tissues and blood samples of subjects (Table 12). Level of iron in the blood samples highly correlated positively (0.81) with that in the *M. vollenhovenii* tissues. High correlations were also observed between the manganese (0.72) in the blood and that in the shrimp samples; zinc was 0.95, copper was 0.87 and cadmium was 0.67. Only chromium in the blood and the shrimp showed no significant correlation. The non-essential trace metals in the shrimps correlated negatively with the essential trace metals in the blood samples.

Table 11: Trace metals in blood samples of Ukwuani residents (August, 2016 to January, 2017); against control and standard ranges

Metals	Ukwuani residents	Umelu residents (control)	Certified Range [33]
Fe	421.5 ± 12 (372.4- 493.2)	311.2 ± 4.3 (172.4- 293.2)	309- 521
Mn	11.42 ± 0.04 (10.78- 12.76)	10.2 ± 0.02 (9.8- 10.6)	8- 18.7
Zn	8864.68 ± 122.72* (2542.3- 14012.2)	6064.8 ± 12.2 (5842.8- 6282.7)	4400- 8600
Pb	0.031 ± 0.001 (0.01- 0.05)	4.8 ± 0.01 (3.01- 5.05)	8- 18.7
Cu	2387.48 ± 322.21* (252- 4402)	987.8 ± 14.22 (952- 122.6)	800- 1300
Cd	0.9 ± 0.08 (0.76- 1.42)	0.59 ± 0.2 (0.23- 0.82)	0.3- 7
Cr	4.21 ± 0.004 (4.14- 4.34)	6.2 ± 0.8 (3.04- 8.98)	2.8- 45

NOTE: Values are expressed in µg/L and sample size = 6. Emboldened and asterisked values signify significant values; higher than certified range

The concentrations of zinc (Figure 4) and copper (Figure 5) in the blood of Ukwuani residents were higher than that of control. Zinc and copper concentrations increased with increasing age brackets i.e. (20- 28 years) < (29- 37 years) < (38- 46 years) < (47- 55 years). This is an indication that the residents of

Ukwuani have been exposed to zinc and copper. This also implies that older people are more susceptible to zinc and copper exposure than younger ones. This might be due to dwindling metabolic capacity with age, coupled with higher exposure duration in older individuals.

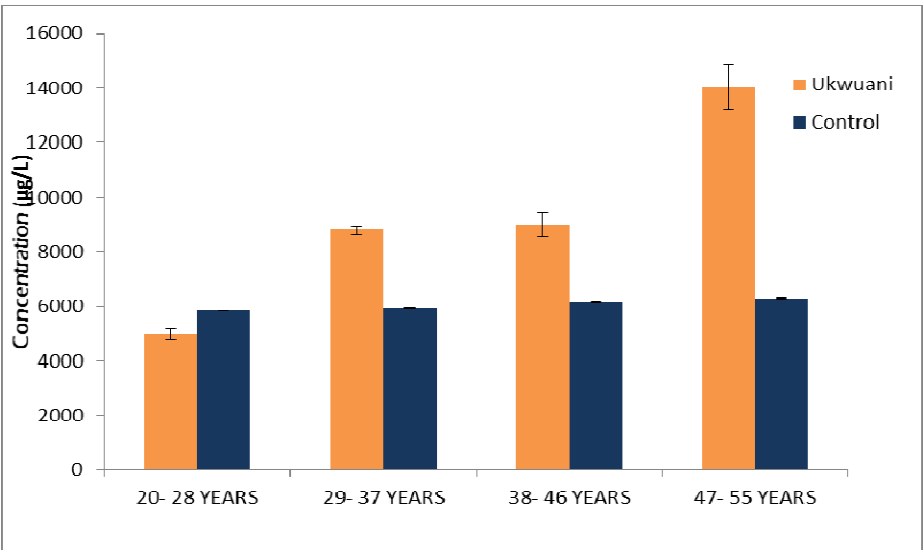


Figure 4- Concentrations of zinc in different age groups of study area versus control

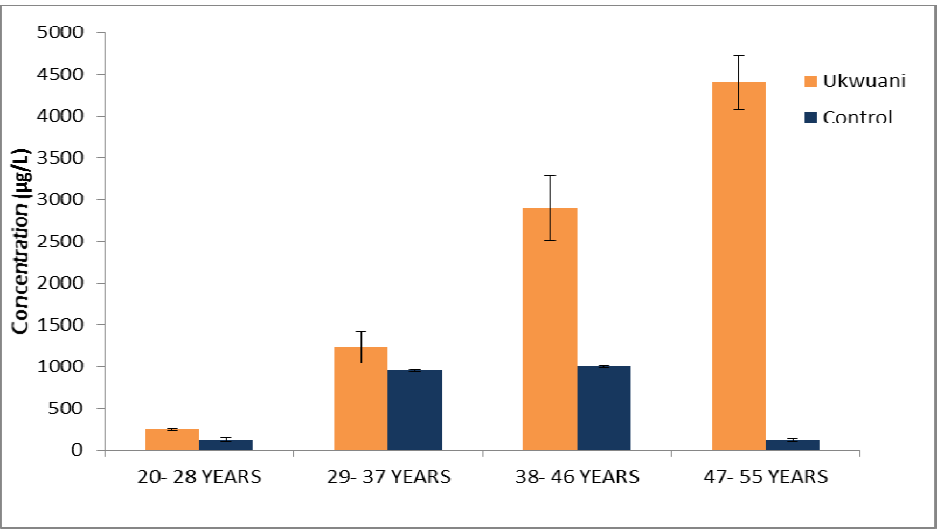


Figure 5- Concentrations of copper in different age groups of study area versus control

370
371

Table 12: Correlation of trace metals in *M. vollenhovenii* with the concentrations in human blood samples

S- Fe	S- Fe	S- Mn	S- Zn	S- Pb	S- Cu	S- Cd	S- Cr	H- Fe	H- Mn	H- Zn	H- Pb	H- Cu	H- Cd	H- Cr
	1													
S- Mn	0.42	1												
S- Zn	0.53	0.55	1											
S- Pb	0.02	-0.02	0.01	1										
S- Cu	0.52	0.43	0.82	0.61	1									
S- Cd	0.17	0.01	-0.03	0.02	0.22	1								
S- Cr	0.31	0.34	0.58	0.04	0.44	-0.67	1							
H- Fe	0.81	0.52	0.04	0.22	0.47	0.41	0.33	1						
H- Mn	0.42	0.72	0.51	0.33	0.62	0.21	0.44	0.24	1					
H- Zn	0.02	0.29	0.95	0.44	0.02	0.34	0.71	0.47	0.76	1				
H- Pb	0.33	0.21	-0.75	0.29	0.02	0.07	0.09	0.02	-0.78	0.44	1			
H- Cu	0.03	-0.09	0.04	-0.06	0.87	-0.82	0.05	0.41	0.06	-0.22	0.36	1		
H- Cd	0.45	0.27	0.07	0.41	0.06	0.67	0.22	0.12	0.04	-0.01	-0.27	0.46	1	
H- Cr	0.04	0.08	0.49	0.06	0.02	0.33	0.35	0.24	0.17	0.02	-0.71	-0.66	0.19	1

NOTE: Values ≥ 0.5 signify significant correlations; hence emboldened, < 0.5 are insignificant. Prefix S= shrimps samples, while Prefix H= Human blood samples

372
373

4. DISCUSSION

Spatially heterogeneous patterns of the trace metals in the river were apparent. The studied environmental matrices also accumulated the metals at different rates i.e. sediment > *M. vollenhovenii* > water > human blood. Concentrations of most of the metals analyzed in water of the river were higher at the perturbed stations than the control; although most of them were lower than the regulatory limit, except manganese and copper (Table 1) i.e. concentration of manganese at Stations 3 and 4 were significantly higher than the concentration at the control station and the regulatory standard limit ($P < 0.001$). While concentration of copper in water at Station 4 was higher than other stations and control ($P < 0.01$). Manganese and copper were also the active components in the water medium (Table 3). Slightly high concentrations of copper and manganese in water at these stations is attributable to oil exploration activities, use of combustion engine boats and saw machines at the saw mill. Although manganese is essential for healthy bones [13]; a constituent of mucopolysaccharides needed for healthy joint formation. Manganese is also essential for regulation of red blood cells and the reproductive cycle in vertebrates generally. It is a constituent of a number of metalloenzymes that occupy key roles in metabolism [34, 35]. Due to the high essentiality of manganese, there is little risk of exposure unless at extremely high concentrations; which is not the case in the current study area. High concentration of manganese in water at Gelegele section of Osse River is attributable to oil exploration activities [36, 37].

Concentrations of trace metals were higher in the sediment (Table 2) than the water medium (Table 1) due to the repository nature of the sediment. Through continuous biomagnification, concentrations of copper could rise to attendant levels in organisms at the higher trophic levels and could ultimately culminate in tissue injury in vertebrates; due to its high oxidation potential i.e. excess copper may cause oxidative damage to biological systems, including peroxidation of lipids and other macromolecules.

The journey of the trace metals through all the matrices studied showed that movements of the metals are interactive. The sequence of active components in the water medium was $Mn > Cu > Zn > Cr > Pb > Cd > Fe$ (Table 3), while that of sediment was $Cu > Cd > Cr > Mn > Pb > Zn > Fe$. This change in trend resulted from the complex interactions among the metals and with the matrices. This is quite evident in their respective sorption capacities which was: $Cu (28.8) > Cd (12.35) > Cr (7.38) > Mn (6)$.

There was a very high correlation between the sorption capacities and the component sequence in the sediment. Very high sorption capacities of copper (141.76) and cadmium (400) were earlier observed at Ikpoba River, Benin City by Ogbeibu *et al.* [38]. Only sorption capacities of manganese, zinc and lead in the current study area were relatively low compared to the values obtained at Ikpoba River; there is somewhat correlation in the sorption capacities of the rest trace metals. The slight variability between the results obtained from the current study area and that of Ikpoba River can be attributed to the distinguished anthropogenic activities i.e. Ikpoba River was perturbed by effluents from a nearby brewery [38].

The levels of zinc and copper observed in the human blood were higher than the standard limits. This can be attributed to the sequence of components in the shrimps, which was: Cu (0.896) > Fe (0.785) > Zn (0.796) > Cd (0.887) > Cr (0.767) > Pb (0.783), Mn (0.772). The outstanding occurrence of copper in the sampled *M. vollenhovenii* can be attributed to bioavailability of copper in the matrices, particularly water; coupled with the fact that shrimps are notorious accumulators of copper due to the high essentiality of copper in their respiratory pigment haemocyanin [39]. Result indices show that only shrimps at the oil exposed Stations 2 and 3 accumulated copper beyond regulatory limit. This is due to the high bioavailability and affinity for copper (Table 8). Besides copper the shrimp exhibited affinity in the following order Cu > Fe > Zn > Cd > Cr > Pb > Mn (Table 10). High affinity of *M. vollenhovenii* for these metals necessitates stringent mitigation of the perturbing anthropogenic activities around the river. High bioavailability of the metals to the shrimps is enhanced by its feeding habit and niche i.e. they are detritivorous animals which feed on epibenthic organisms such as polychaetes, molluscs and other crustaceans [40]. Also morphologically, their abdomen not being flexed beneath the thorax maximizes bioaccumulation of contaminants from the sediments and overlying water [10]. However Figures 2 and 3 showed that most of the trace metals were accumulated more from the water than the sediment matrix. Significant bioaccumulation of some trace metals were earlier reported in the *M. vollenhovenii* of Ovia River, Nigeria [37] and Egbokodo River, Nigeria [11].

Zinc and copper were slightly above recommended limits in the human blood (Table 11). Excess zinc in consumers of the *M. vollenhovenii* may cause anaemia, damage to the pancreas and decrease levels of high density lipoprotein cholesterol [41]. While impressive levels of copper observed in the blood

of the consumers of *M. vollenhovenii* may elicit liver cirrhosis [42]. These health consequences are liable to ensue if the trend in the aquatic environment is left unmitigated over an appreciable period of time. The high levels of the trace metals in the blood can be traced to their substantial availability in the shrimps. This is also buttressed by the fact very high positive correlations occurred between zinc (0.95) and copper (0.87) in the shrimp and blood samples of the dependent populace (Table 12).

5.CONCLUSION

Results showed that Okumeshi River was impacted by the anthropogenic activities which may have some health impacts on the consumers of the *Macrobrachium vollenhovenii* of the river. High level of zinc and copper in human blood necessitates a prompt bioremediation in the aquatic environment. The observed insalubrity of the shrimps is a prognostic of chronic health hazards to the consumers; which is also a function of the predominant oil exploration activities. Copper had an uninterrupted transit into the blood of the consumers from the aqueous phase. While zinc showed some significant level of biomagnification. The study provided an early signal of likely impending catastrophe; necessitating a proactive regulation of trace metals released into the aquatic system through the prevalent anthropogenic activities; mainly oil exploration.

COMPETING INTERESTS

The study neither contains samples obtained from clinical studies nor personally identifiable patient data. There is stringent anonymity of the volunteers who donated blood in the interest of the benefits the research promises to offer to safeguard public health. The authors hereby declare that no competing interests exist.

REFERENCES

1. Mansour SA, Sidky MM. Ecotoxicological studies. 3: Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. Food Chem., 2002; 78: 15-22.
2. Jezierska B, Witeska M. The Metal Uptake and Accumulation in Fish Living in Polluted Waters. Twardowska, I. et al. (eds.). Soil and Water Pollution Monitoring, Protection and Remediation. 2006; 3(69):107-114.
3. Akintujoye JF, Anumudu CI, Awobode HO. Assessment of heavy metal residues in water, fish tissue and human blood from Ubeji, Warri, Delta State, Nigeria. J. Appl. SC. Environ. Manage. 2013; 17(2): 291- 297.

4. Tukura BW, Gimba, CE, Ndukwe IG, Kim BC. Physicochemical Characteristics of Water and Sediment in Mada River, Nasarawa State, Nigeria. *International Journal of Environment and Bioenergy*. 2012; 1(3):170-178.
5. Isibor PO, Imoobe TOT, Izegaegbe JI, Oluowo EF. Assessment of some heavy metals and total hydrocarbons in *Clarias gariepinus* fish of Osse River, Edo State, Nigeria. *J. Environ. Sci., Toxicol. Food Technol*. 2016; 10(9):144-151.
6. Ogbeibu AE, Oriabure PA, Oboh IP, Edogun IS. The effects of Brewery effluent discharge on the water quality and sediment of Ikpoba River, Benin City, Nigeria. *Journal of Aquatic Sciences* 2014; 29 (1A): 43- 58.
7. Froese KL, Verbrugge DA, Ankley GT, Niemi GJ, Larsen CP, Giesy, J.P. Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. *Environmental Toxicology and Chemistry* 1998; 17(3):484-492.
8. Davies OA, Allison M.E, Uyi HS. Bioaccumulation of heavy metals in water, sediment and periwinkle (*Tympanotonus fuscatus* var *radula*) from the Elechi Creek, Niger Delta. *African Journal of Biotechnology*. 2006; 5 (10), pp. 968-973.
9. Akan JC, Salwa M, Yikala BS, Chellube ZM. Study on the Distribution of Heavy Metals in Different Tissues of Fishes from River Benue in Vinikilang, Adamawa State. *British Journal of Applied Science & Technology* 2012; 2(4): 311-333.
10. Omoigberale MO, Ikponmwosa-Eweka O. Evaluation of heavy metals of the Palaemonid shrimps (*Macrobrachium vollenhovenii*) in Osse River, Nigeria. *Bioscience Research Communications*. 2010; 22(5): 247-254.
11. Isibor PO, Oluowo FE. Evaluation of Some Heavy Metals and Total Petroleum Hydrocarbon in Water and Palaemonid Shrimps (*Macrobrachium vollenhovenii*) of Egbokodo River, Warri, Delta State, Nigeria. *J. Appl. Life Sci. Int*. 2016; 4(6):1-12.
12. Edema U, Egborge ABM. Heavy metal content of *Macrobrachium* species (Crustacea: Palaemonidae) from Warri River, Nigeria. *Tropical Journal of Environment, Science and Health*. 1999; 2 (1):65–70.
13. Li P, Qian H, Howard KWF, Wu J, Lyu X. Anthropogenic pollution and variability of manganese in alluvial sediments of the Yellow River, Ningxia, northwest China. *Environmental Monitoring and Assessment*, 2014; 186(3): 1385-1398.
14. Li P, Qian H, Howard KWF, Wu J. Heavy metal contamination of Yellow River alluvial sediments, northwest China. *Environmental Earth Sciences*, 2015; 73(7):3403-3415.
15. Li P, Wu J, Qian H, Zhou W. Distribution, enrichment and sources of trace metals in the top soil in the vicinity of a steel wire plant along the Silk Road economic belt, northwest China. *Environ Earth Sci*, 2016; 75(10):909.
16. Ekeanyanwu CR, Ogbuinyi CA, Etienajirhevwe OF. Trace Metals Distribution in Fish Tissues, Bottom Sediments and Water from Okumeshi River in Delta State, Nigeria. *Ethiopian Journal of Environmental Studies and Management*. 2010; 3(3):12-17.
17. Ekeanyanwu CR, Ogbuinyi CA, Etienajirhevwe OF. Trace metals distribution in fish tissues, bottom sediments and water of Okumeshi River in Delta State, Nigeria. *Environmental Research Journal* 2011; 5(1): 6- 10.
18. Short KC, Stauble AJ. Outline of Geology of Niger Delta. *AAPG. Bull.*, 1967; 51: 761 – 779
19. Asseez LO. Review of the stratigraphy, sedimentation and structure of the Niger Delta. In *Geology of Nigeria* (C.A. Kogbe, ed.) Elizabethan Publishing Company Lagos, Niger. 1976; Pp 428.

20. Kogbe CA, Mehes K, Salam MB. Biostratigraphy of Upper Cretaceous and Tertiary Sediment penetrated by Gbekebo "B" well, Niger Delta, Nigeria. In: Geology of Nigeria (C.A. Kogbe, ed.) Elizaberthan Publishing Company, Lagos. 1976; Pp 352.
21. Centers for Disease Control and Prevention (CDC). Second nutritional report on biochemical indicators of diet and nutrition in the U.S. population. National Center for Environmental Health. 2012; pp 483.
22. Harrington JM, Young DJ, Essader AS, Summer SJ, Levine KE. Analysis of human serum and whole blood for mineral content by ICP-MS and ICP-OES: Development of a mineralomics method. Biol. Trace Elem. Res. 2014; 160 (1): 132- 142.
23. Martin GR, Smoot JL, White KD. A comparison of surface-grab and crosssectionally integrated stream-water –quality sampling methods. Water Environ. Res. 1992; 64(7):866-876.
24. Federal Environmental Protection Agency (FEPA). Guidelines and Standards for Environmental pollution control in Nigeria. 2003; pp 420.
25. Estafan J, Somar R, Ryan J. Methods of Soil, Plant and Water Analysis: A manual for the West Asia and North Africa Region. International Centre for Agriculture in Dry Areas. 2013; Pp 243.
26. Benhard M. Manual of methods in the aquatic environment research. FAO. Fisheries technical paper FIRI/T. Number 158, Food and Agricultural Organization, Rome; 1976.
27. Turkmen G. Seasonal variation in heavy metals in shrimps *Penaeus kerathurus* (Forskall, 1775) from Izmir Bay. J. Amin. Vet. Adv. 2012; 11(15):2839.
28. Olaifa FE, Olaifa AK, Adelaja AA, Owolabi AG. Heavy metal contamination of *Clarias gariepinus* from lake and fish farm in Ibadan, Nigeria. Afr. J. Biomed. Res. 2004; 7:145–148.
29. Chatfield C, Collin AJ. Introduction to Multivariate Analysis. Biometrical Journal 1980; 24(5): 436. DOI: 10.1002/bimj.4710240504.
30. Hope K. Methods of Multivariate Analysis. University of London Press Ltd. St. Paul's House, Warwick Lane, London EC4, SBN 340 07 331 4 Unibook.1968.
31. Mazlum S. Variable Selection in Water Quality Monitoring Networks". Master of Science Thesis Submitted in Partial Fulllment of the Requirements for the Degree of Master of Science in Environmental Engineering. DokuzEylul University, Graduate School of Natural and Applied Sciences. 1992.
32. Soares MR, Alleoni LRF. Distribution coefficient (KD) of heavy metals in Brazillian soils. 18th World Congress of Soil Sciences, Philadelphia, USA. 2006; pp 134.
33. Iyengar V, Woittiez J. Trace Elements in Human Clinical Specimens: Evaluation of Literature Data to Identify Reference Values. Clin Chem. 1988; 34 (3):474–481.
34. Woolhouse HW. Toxicity and tolerance in the responses of plants to metals. In: Lange OC, Nobel PS, Osmond CB, Ziegler H, eds. Encyclopedia of plant physiology II. New York, NY, Springer-Verlag. 1983. Pp300.
35. Burnell, JN. 1988. The biochemistry of manganese in plants. In: Graham RD, Hannam RJ, Uren NC, eds. Manganese in soils and plants. Dordrecht, Kluwer Publishers. 1988. Pp 408.
36. Omoigberale MO, Ogbeibu AE. Environmental impacts of oil Exploration and Production on the Macrobenthic Invertebrate Fauna of Osse River, Southern Nigeria. Res. J. Environ. Sci. 2010; 4: 101- 114.
37. Oguzie FA, Ehigiator FAR. Concentration of heavy metals in three African prawn (Crustacea: Palaemonidae) from Osse River in Edo State, Nigeria. J. Agric. Sc. Env. 2011; 11 (1):104-113.
38. Ogbeibu AE, Oriabure PA, Oboh IP, Edogun IS. The effects of Brewery effluent discharge on the water quality and sediment of Ikpoba River, Benin City, Nigeria. Journal of Aquatic Sciences 2014; 29 (1A): 43- 58.
39. Van de Broek WLF. Seasonal levels of chlorinated hydrocarbons and heavy metals in fish and brown shrimps from the Medway Estuary Kent. Environmental Pollution. 1979; 19:21-28.

- 559 40. Isibor PO. The Abiotic Ecology and Prevalence of Palaemonid Shrimps (Crustacea:
560 Palaemonidae) of Osse River, Edo State, Nigeria. Journal of Life Sciences International. 2016;
561 9(3):1-11. DOI: 10.9734/JALSI/2016/29497.
- 562 41. Agency for Toxic Substances and Disease Registry (ATSDR). Public Health Statement for Zinc:
563 Public Service. Department of Health and Human Services. 2005; Pp 42.
- 564 42. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for
565 polyaromatic hydrocarbons (PAH) in Goldstein, B.D. et al., (2011). The Gulf Oil Spill. New
566 England Journal of Medicine. 2010; 364 (14): 1334-1348.
- 567