

Heavy Metals and Petroleum Hydrocarbon Concentration in water and Periwinkles (*Tympanotonus fuscatus* L.) obtained from Calabar River, Cross River State, Nigeria

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Abstract— Concentration of heavy metals; Cd, Cr and Pb along with total petroleum hydrocarbon (TPH) in periwinkles (*Tympanotonus fuscatus*) from Calabar River in Nigeria was assessed to determine their suitability for human consumption. Water and periwinkle samples were collected from five stations and taken to the laboratory for analysis. Heavy metals were analysed using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS-AA240FS) after digestion with concentrated Nitric acid while GC-FID (6890N, Agilent) was used to analyze TPH after liquid-liquid extraction of water and Soxhlet extraction of periwinkle tissues. The results obtained showed Pb as the highest (7.73 ± 2.29 mg/l) occurring metal in water at station four, the lowest (1.88 ± 0.90 mg/l) was Cd in station one while TPH was highest (259.47 ± 45.90 mg/l) in station four and lowest (155.39 ± 32.07 mg/l) in station two. Metal concentrations in water across sampling stations were not significantly different ($p < 0.05$) while both metals and TPH exceeded the WHO standards for drinking water. Metal concentrations in periwinkles did not differ significantly ($p < 0.05$) across sampling stations, they had exceeded the FAO standards with Pb and Cd being the highest (6.15 ± 2.25 mg/kg) and lowest (0.69 ± 0.43 mg/kg) in stations three and four respectively while TPH in periwinkles was highest (130.58 ± 34.82 mg/kg) in station five and lowest (98.37 ± 31.52 mg/kg) in station one which exceeded the FAO limit hence water and periwinkles from Calabar River are considered unsafe for consumption. Negative correlations of -0.03 , -0.20 and -0.37 in TPH, Cr and Cd respectively suggests that other sources of these pollutants in periwinkles exist.

Keywords— Calabar River, Concentration, Heavy metals, Periwinkles, Total petroleum hydrocarbon, Water.

I. INTRODUCTION

Heavy metals are inorganic pollutants of great environmental concern as they are non-biodegradable, toxic and persistent with serious negative ecological complications [1]. They include transition metals, actinides, lanthanides as well as the metalloids arsenic and antimony [2]. Most heavy metals do not undergo microbial or chemical degradation [3], and their total concentration persists for a long time in the environment after introduction [4] but changes in their chemical forms (speciation) and bioavailability are possible. Hydrocarbons and other organic compounds, including some organometallic constituents are the main components of petroleum [5] and so total petroleum hydrocarbon (TPH) is

a commonly used gross parameter for quantifying environmental contamination by various petroleum hydrocarbon products such as fuels, oils, lubricants, waxes and others [6].

Periwinkles (*Tympanotonus fuscatus* L.) are mollusks belonging to the class Gastropoda, order Neotaenioglossa and family Potamididae. They are characterized by turreted, granular and spiny shell and live in lagoons, estuaries and mangrove swamps of West Africa [7]. They are commonly found in Calabar River and are part of the delicacies enjoyed by the people in that region primarily due to their relatively cheap price. They are also reported to be good pollution biomonitors because of their sedentary

and bottom feeding habit which makes them accumulate pollutants of all kinds [8].

Several studies reported signs of pollution from untreated industrial effluents, municipal wastewater, run-off from agricultural chemical fertilizers and pesticides, as well as spillage of petroleum products in Calabar River [9] hence this study was conducted to assess the levels of heavy metals and total petroleum hydrocarbon (TPH) in water and tissues of periwinkles (*Tympanotonus fuscatus* L.) obtained from the River in order to determine their suitability for human consumption.

II. MATERIALS AND METHODS

2.1 Study Area

Calabar River in Cross River State, Nigeria is located between latitude $04^{\circ} 55' 55''$ to $05^{\circ} 02' 50''$ N and longitude $008^{\circ} 16' 35''$ to $008^{\circ} 18' 13.8''$ E. It flows from the north through the city of Calabar, joining the larger Cross River to the south (Figure 1). Five sampling stations with an approximate distance of 4.5 km from one another were chosen along the river course: Ikot Okon Abasi, Tinapa, Unicem, Marina resort and Nsidung beach which were labeled stations 1, 2, 3, 4 and 5 respectively. Station 1 is upstream with clean water and little human activities going on there which served as a control while stations 2 and 4 are tourist sites with some human activities and stations 3 and 5 receive a lot of effluents and other wastes as a result of industrial and commercial activities there.

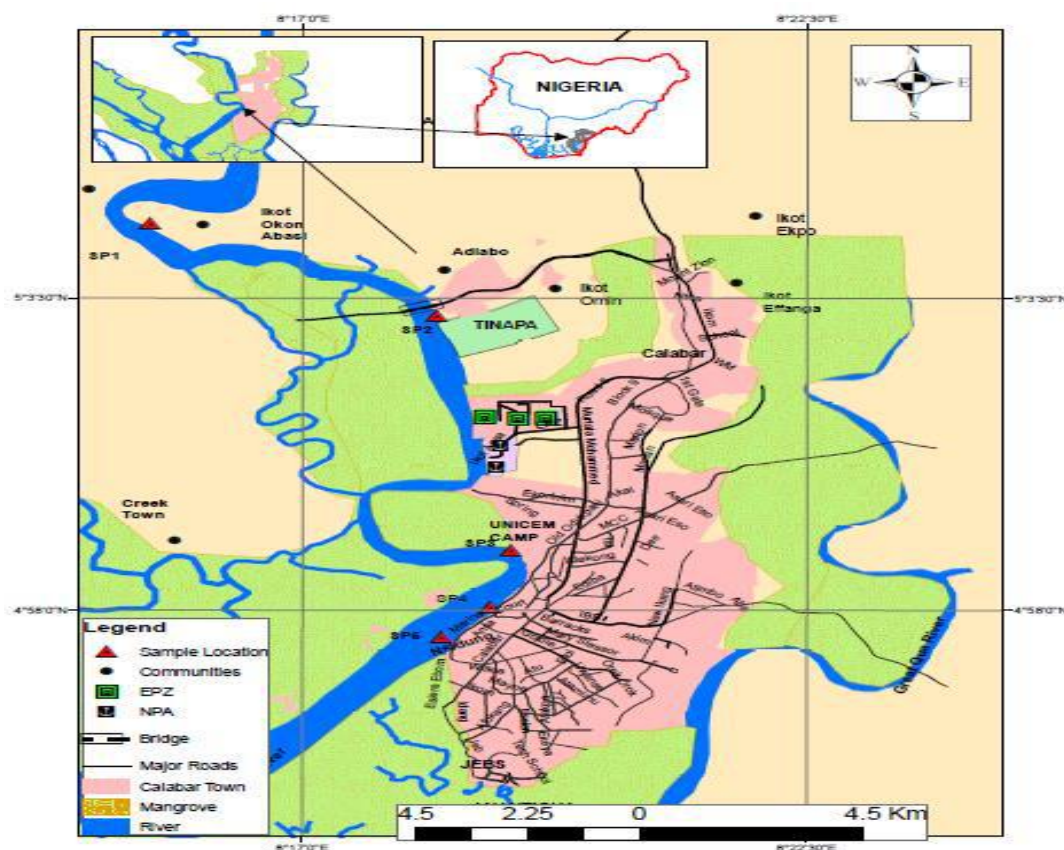


Fig.1: Map of the Study Area showing Calabar River and Sampling Stations.

2.2 Sample Collection

Water samples were collected from the sampling stations using 500 ml plastic bottles that were washed with tap water and detergent, soaked in 10% HNO_3 and rinsed with deionised water. The samples were stored in coolers packed with ice $<4^{\circ}\text{C}$ and transported to the laboratory.

Samples of 30 Periwinkles from each of the five sampling stations were randomly collected monthly from January to June, making a cumulative total of 900 Periwinkles which were cleaned of any sediment, wrapped in hexane-rinsed aluminium foil, labeled and taken to the laboratory for analysis according to [10].



Fig.2: Samples of Periwinkles (*Tympanotonus fuscatus* L.) from the Study Area.

2.3 Analysis of Heavy Metals and Total Petroleum Hydrocarbon (TPH) in Water

Physicochemical parameters: temperature, pH, dissolved oxygen (DO) and Electrical conductivity (EC) were analysed in-situ using a Seabird Scientific Hydrocycle with multi-parameter probe while turbidity, total suspended solids (TSS), and total dissolved solids (TDS) were measured in the laboratory using submersible turbidimeter, gravimetric analysis and TDS meter.

50 ml of water samples meant for heavy metals analysis were digested by addition of 10 ml of concentrated nitric acid and 10 ml of hydrogen peroxide. This was heated on a hot plate to about half the original volume and allowed to cool before its content was filtered into 50 ml standard volumetric flask and filled up to the mark by adding distilled water according to [11]. The analysis was then done using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS-AA240FS).

Analysis of TPH in water samples was done using liquid-liquid extraction by mixing 50 ml of water with 10 ml of hexane and 4g of silica gel which was stirred with a magnetic stirrer for five minutes [12]. The silica gel was filtered and hexane was distilled by drying the residue to

constant weight and TPH was analysed using Borosil glass reactor GC-FID equipment (6890N, Agilent).

2.4 Analysis of Heavy Metals and Total Petroleum Hydrocarbon (TPH) in Periwinkles (*Tympanotonus fuscatus* L.)

Periwinkles to be analyzed for heavy metals were rinsed with distilled water, crushed using mortar and pestle, one gramme was digested using nitric acid and perchloric acid ($\text{HNO}_3\text{--HClO}_4$) 1:1 ratio after which sulphuric acid was added and the mixture was heated at 200°C for 30 minutes according to [13]. It was allowed to cool down and made up to 50 ml with distilled water which was followed by heavy metals analysis using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS-AA240FS).

The Periwinkle samples for TPH analysis were air-dried at room temperature, deshelled and grinded using mortar and pestle after which 10g was transferred into a Soxhlet extractor. 100 ml of dichloromethane and hexane (3:1) was added, the mixture was heated on a hot plate for three hours and then allowed to cool according to [14]. The extract was evaporated to 1 ml at 40°C in a Rotary evaporator, 10 ml of hexane was further added and the TPH analysis was done using the GC-FID machine (6890N, Agilent).

2.5 Statistical Analysis

One way Analysis of variance (ANOVA) was used to test whether significant difference exists among the mean heavy metals and TPH concentrations in water and Periwinkles from the five sampling stations while Student t-test was used to check the difference between the seasonal concentrations and correlation analysis was done to determine if there's a relationship between mean heavy metals and TPH concentrations in water and Periwinkles.

III. RESULTS

3.1 Physico-chemical Parameters of Calabar River

All mean physicochemical parameters of water from Calabar River during the period of this study did not differ significantly among sampling stations and all exceeded the WHO maximum permissible limits [15] except for DO in station four. Mean values of physicochemical parameters of Calabar River during this study are shown in table one.

3.2 Heavy Metals and TPH Concentration in Calabar River

Pb was the highest occurring metal in water from Calabar River with a mean concentration of 7.73 ± 2.29 mg/l in S4 while the lowest was Cd with a mean concentration of 1.88 ± 0.90 mg/l in S1. TPH was highest in S4 with a mean value of 259.47 ± 45.90 mg/l and lowest in S2 with a mean value of 155.39 ± 32.07 mg/l. There was no significant difference among mean metal values from the five sampling stations but significant difference existed among mean TPH values while both metals and TPH exceeded the WHO standards for drinking water [15]. Mean heavy metals and TPH concentrations in water from the five

sampling stations during the study period are presented in table two.

3.3 Heavy Metals and TPH Concentration in Periwinkles (*Tympanotonus fuscatus* L.)

The metal with the highest mean concentration in tissues of periwinkles was Pb which was 6.15 ± 2.25 mg/kg in station three while the lowest was Cd with a concentration of 0.69 ± 0.43 mg/kg in station four. The highest and lowest TPH concentrations were 130.58 ± 34.82 mg/kg and 98.37 ± 31.52 mg/kg in stations five and one respectively. Mean metal values in Periwinkles did not differ significantly across sampling stations but mean TPH did and all exceeded the Food and Agricultural Organization (FAO) standards [16]. Mean heavy metals and TPH concentrations in periwinkles from the five sampling stations during the study period are presented in table three.

3.4 Relationship between Heavy Metals and TPH Concentrations in Water and Periwinkles (*Tympanotonus fuscatus* L.)

All mean metal and TPH concentrations differed significantly between water and periwinkle samples from Calabar River during the study with water samples having higher concentrations. TPH, Cr and Cd concentrations in water and periwinkles had very weak negative correlations of -0.03 , -0.20 and -0.37 respectively while Pb concentrations in water and periwinkles had a weak positive correlation of 0.09 . The relationship and comparison of mean metal and TPH concentrations between water and periwinkles from Calabar River are shown in figures 3 and 4 respectively.

Table 1: Table 1: Mean \pm Standard Deviation of Physico-chemical Parameters from the Five Sampling stations in Calabar River, their ANOVA Test and Comparison against WHO Standards

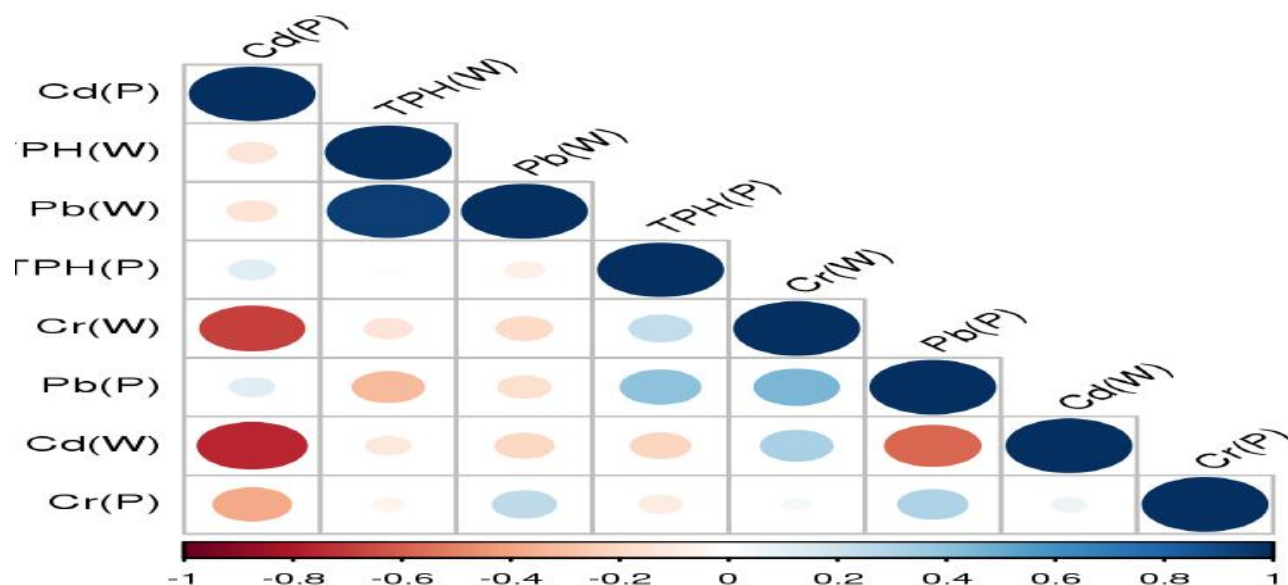
Parameters	S1	S2	S3	S4	S5	F ratio	P. Value	Inference	WHO limit
Temp ($^{\circ}$ C)	29.59 \pm 3.79	29.34 \pm 5.51	29.49 \pm 4.59	30.28 \pm 2.96	30.49 \pm 4.55	0.08	0.99	No Sig. Diff.	25
pH	5.65 \pm 0.95	6.04 \pm 0.47	5.19 \pm 0.78	5.81 \pm 0.65	5.87 \pm 0.65	1.21	0.33	No Sig. Diff.	6.5–8.5
DO (mg/l)	5.84 \pm 0.68	5.49 \pm 1.05	5.44 \pm 0.72	6.19 \pm 0.96	5.74 \pm 0.47	0.86	0.50	No Sig. Diff.	>6.00
BOD ₅	7.05 \pm 1.89	8.29 \pm 2.31	11.61 \pm 1.73	7.89 \pm 1.67	7.22 \pm 2.94	4.43	0.007	Sig. Diff.	5
EC(us/cm)	1247.48 \pm 241.13	1053.02 \pm 163.32	1501.19 \pm 679.76	1029.08 \pm 297.54	1470.38 \pm 402.81	1.82	0.15	No Sig. Diff.	500
Turbidity(NTU)	8.10 \pm 1.56	7.68 \pm 1.74	9.53 \pm 1.30	8.83 \pm 2.93	8.23 \pm 1.39	0.87	0.49	No Sig. Diff.	5
TSS (mg/l)	34.62 \pm 6.09	36.80 \pm 11.62	40.56 \pm 5.33	37.97 \pm 9.06	34.09 \pm 8.81	0.53	0.72	No Sig. Diff.	<30
TDS (mg/l)	2399.19 \pm 422.44	2202.41 \pm 760.04	2682.52 \pm 550.53	1925.86 \pm 326.18	1979.77 \pm 726.79	1.72	0.18	No Sig. Diff.	250–500

Table 2: Mean \pm Standard Deviation (mg/l) of Heavy Metals and TPH in Water from the Five Sampling stations in Calabar River, their ANOVA Test and Comparison against WHO Standards

Metals	S1	S2	S3	S4	S5	F ratio	P. Value	Inference	WHO limit
Cd	1.88 \pm 0.90	2.68 \pm 1.10	2.94 \pm 1.50	2.91 \pm 1.77	3.91 \pm 1.81	1.48	0.24	No Sig. Diff.	0.003
Cr	3.88 \pm 1.45	3.81 \pm 1.62	4.14 \pm 1.39	4.75 \pm 1.53	4.68 \pm 1.25	0.59	0.67	No Sig. Diff.	0.05
Pb	7.68 \pm 1.87	7.13 \pm 1.59	7.73 \pm 1.88	7.76 \pm 1.59	7.73 \pm 2.29	0.12	0.97	No Sig. Diff.	0.01
TPH	207.77 \pm 48.62	155.39 \pm 32.07	177.05 \pm 75.82	259.47 \pm 45.90	164.68 \pm 42.62	4.08	0.01	Sig. Diff.	300

Table 3: Mean \pm Standard Deviation (mg/kg) of Heavy Metals and TPH in Periwinkles from the Five Sampling stations in Calabar River, their ANOVA Test and Comparison against WHO/FAO Standards

Metals	S1	S2	S3	S4	S5	F ratio	P. Value	Inference	FAO limit
Cd	0.79 \pm 0.85	0.89 \pm 0.79	1.25 \pm 0.82	1.09 \pm 0.87	1.37 \pm 0.69	0.56	0.69	No Sig. Diff.	0.10
Cr	1.69 \pm 1.04	1.85 \pm 1.02	1.69 \pm 0.79	1.55 \pm 0.87	1.68 \pm 1.36	0.06	0.99	No Sig. Diff.	1.00
Pb	5.77 \pm 0.43	5.82 \pm 0.81	6.19 \pm 1.02	5.83 \pm 0.87	5.56 \pm 1.39	0.35	0.85	No Sig. Diff.	0.10
TPH	118.91 \pm 18.08	120.29 \pm 26.90	165.48 \pm 32.18	97.00 \pm 33.86	135.04 \pm 34.21	4.76	0.005	Sig. Diff.	2.00



Positive correlations are displayed in blue and **negative correlations** in red color. Color intensity and the size of the circle are proportional to the **correlation coefficients**.

Fig. 3: A Correlogram Showing the Relationship between Heavy Metals and TPH in Water and Periwinkles from Calabar River

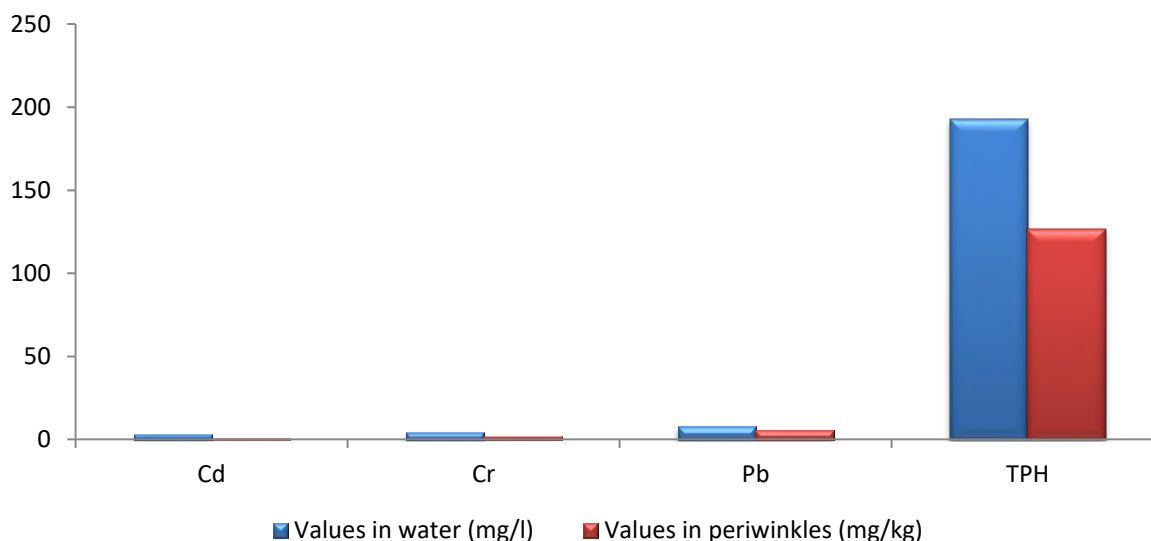


Fig. 4: Comparison of Heavy Metals and TPH in Water and Periwinkles from Calabar River

IV. DISCUSSION

Physicochemical parameters of water from all the five sampling stations in Calabar River during the period of this study have exceeded the WHO permissible limits and did not differ significantly except for BOD₅ which implies that the water is from a common source and is influenced by similar contaminants at all sampling stations. The biological oxygen demand (BOD) indicates high organic content in the water [17] and high BOD recorded in this study may be attributed to the discharge of pollutants into the water through effluent discharge, sewage contamination and washing at the coast which could be the cause of low dissolved oxygen (DO) observed in this study. The low pH may change the concentrations of other substances present in water to more toxic ones, cause irritation to the eyes, skin and mucous membranes as well as increase in corrosivity of water [18]. Electrical conductivity (EC) is a measure of water capacity to conduct an electric charge and is a function of the mineral ions present in the water usually measured as total dissolved solids (TDS) [19]. Both EC and TDS were significantly high in this study and they were reported to influence the incidence of cancer, coronary heart disease, arteriosclerotic heart disease and cardiovascular disease [20]. Turbidity and total suspended solids (TSS) are other positively related parameters and they were very high in this study. They are the most visible indicators of water quality and can inhibit photosynthesis by blocking sunlight [21]. Looking at the physicochemical parameters measured, the water is generally

not safe for consumption as these parameters indicate so many alterations in the overall water quality. These findings on the physicochemical parameters of Calabar River are similar to the results obtained by Ukenye and Taiwo [22] in their studies on the physicochemical status and biological characteristics of some rivers in Nigerian coastal states.

All metals analysed in water and periwinkles from the five sampling stations in this study were found to be above the WHO and FAO limits for drinking water and showed no significant difference among sampling stations which is a cause for concern as the metals are toxic and can accumulate in living tissues. Cadmium is efficiently retained in the human body and it accumulates throughout life where it is primarily toxic to the kidney especially the proximal convoluted tubules which are the main sites of accumulation [23]. It can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. Chromium is one of eight metals in the top 50 toxic substances in the world according to the Agency for Toxic Substances and Disease Registry (ATSDR), and it has been classified as carcinogenic to humans by WHO [24, 25]. Lead affects children's brain development leading to mental retardation and behavioural disorders while it causes anaemia, renal impairment, reproductive malfunction and immunotoxicity in adults [26]. These metals could induce toxic effects disrupting aquatic organisms' metabolism, growth or reproduction affecting all trophic levels including humans. A contrasting result was obtained in 2014 by Uwem

and Bassey [27] where metals, Zn, Fe, Cu, and Cd were below the permissible limits in both the water and seafood (crayfish, shrimp, periwinkles and snails) harvested from Itu River which is linked to Calabar River while George and Abowei [28] in 2018 found Zn, Cr, Pb and Fe above the permissible limits in water and sediments of upper new Calabar River. They stated that the source of heavy metals in the aquatic environment could be industrial effluent, domestic waste, dumping of scrap, vessel in the water way and runoff from agricultural land.

TPH in both water and periwinkles from the five sampling stations have exceeded the permissible limits and differed significantly across the stations with stations three and four having the highest TPH concentrations in water and periwinkles respectively. This could be as a result of oil exploration and drilling in other areas washed down the drain through tributaries that feed Calabar River and some industrial activities happening in the vicinity of the River. With this result, periwinkles from Calabar River are considered unsafe for consumption. Effects of TPH on human health include neurological disorders, stress and potential toxicity to genetic, immune, and endocrine systems [29]. Aquatic organisms and ecosystems are immensely affected by individual components of TPH depending on their molecular weight, concentration, exposure indices, environmental conditions and sensitivity of the affected species [30]. Imaobong and Prince [31] in 2016 found the level of total petroleum hydrocarbon in surface water of Cross River estuary to be very high relative to Nigerian permissible limit which poses a serious risk to the survival of aquatic organisms and also affects the quality of water used for various purposes.

Heavy metals and TPH levels are significantly higher in water than in periwinkles and there was a negative or weak positive correlation between values in water and periwinkles. This suggests that there could be other sources of metals and TPH in periwinkles apart from water in which they live and studies on other environmental components such as sediment and soil around the shores is encouraged.

V. CONCLUSION

This study investigated and established a background data for heavy metals and TPH in periwinkles from Calabar River. The highest occurring metal in water was Pb with a concentration of 7.73 ± 2.29 mg/l while the lowest was Cd with a concentration of 1.88 ± 0.90 mg/l. The highest and

lowest TPH concentrations in water were 259.47 ± 45.90 mg/l and 155.39 ± 32.07 mg/l respectively, and all metals and TPH concentrations were above the WHO maximum permissible limits which made the water unsafe for consumption. Pb and Cd were still the highest and lowest occurring metals in periwinkle tissues with values 6.15 ± 2.25 mg/kg and 0.69 ± 0.43 mg/kg respectively while highest and lowest TPH levels in periwinkles were 130.58 ± 34.82 mg/kg and 98.37 ± 31.52 mg/kg respectively. Periwinkles from Calabar River are as well considered unsafe for consumption as metals and TPH values were above the FAO limits.

Further studies on soil from the shores and sediments from Calabar River are suggested and the water should be treated well before use particularly in the area of human consumption.

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