



Assessment of Pollution Levels Using Biomarkers in *Callinectes Sapidus* from Estuaries in Rivers State, Nigeria

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Abstract – The increasing anthropogenic pollution in estuarine ecosystems poses a significant threat to aquatic life and ecosystem health. This study aims to assess the pollution levels in selected estuaries of Rivers State, Nigeria, using biomarkers in *Callinectes sapidus* (blue crab) as an indicator of environmental contamination. In situ measurements for some water quality variables were made at the sampling locations. 48 female crabs (weight 149.20 ± 0.02 g) harvested for the estimation of biomarker levels. Mean concentrations of Total Petroleum Hydrocarbons (TPHs), Polycyclic Aromatic Hydrocarbons (PAHs), Zn and Cr (Sig. values=0.000 each), Cd, Pb, and Fe (Sig. t-values=0.003, 0.019 & 0.009 respectively) were significantly higher at the impacted than reference locations, while that of Monocyclic Aromatic Hydrocarbons (MAHs) and Fe (Sig. t-values=0.032 & 0.014 respectively) differed seasonally at $p < 0.05$. Though there was no significant difference in accumulations of the heavy metals and hydrocarbons in tissues of the organism, numerical accumulations of Zn (5.73 ± 2.60 $\mu\text{g/g}$) and TPHs (1.84 ± 1.08 $\mu\text{g/g}$) were highest in the digestive than the other tissues sampled. Mean levels of Lactate Dehydrogenase (LDH), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Malondialdehyde (MDA) (sig=0.000 each) at the OSD locations, and that of total proteins (Sig. t-value=0.030) in the rainy season were all markedly higher in the organism ($p < 0.05$). Elevated MAHs appeared to induce the production of less ALT ($r = -0.584$) and AST ($r = -0.519$), Cr induced the production of less AST ($r = -0.513$) ($p < 0.05$), while MAHs induced the production of less MDA ($r = -0.634$) ($p < 0.01$). Lead and PAHs recorded very high Pollution indices (240,000 & 790,000) in sediments, while Zn and TPHs recorded high toxicity quotients of 1.59 and 2.83 in the organism. Allochthonous input of pollutants from petroleum sources into the creek caused biological disruptions, including tissue bioaccumulation and other biochemical disruptions in proteins and enzyme activities of *C. sapidus*, and these disruptions could rightly infer pollution. Treatment of oily effluents before discharge into the creek is recommended.

Keywords – Biomarkers, Anthropogenic pollution, Bioaccumulation, Ekerekana Creek, estuary.

I. INTRODUCTION

Pollution of aquatic ecosystems, particularly estuaries, has become a critical environmental concern due to the increasing discharge of industrial, agricultural and domestic waste (Kennish, 2002). Estuaries, where freshwater from rivers meets seawater, are highly productive ecosystems that support a diverse range of species, including economically and ecologically significant organisms such as blue crab, *Callinectes sapidus*. These habitats, however, are vulnerable to contamination for pollutants like heavy metals, pesticide and hydrocarbons, which can accumulate in the organisms that inhabit these areas (Moslen, & Aigberua, 2018), (Kennish, 2002)

Callinectes sapidus, commonly known as the blue crab, serve as a suitable bioindicators for environmental pollution, given its wide distribution and high trophic position in estuarine food webs. (He & Yu, 2017) Biomarkers in *C. sapidus*, such as enzyme activity, oxidation stress markers and accumulation of contaminants in tissues, can provide valuable insights into the levels of environmental pollutant and their potential impacts on aquatic life. These biomarkers not only reflect the health status of the species but also serve as early warning indicators of pollution in estuarine ecosystems (Rupp & Gauthier, 2020)

Biomarkers are biochemical, physiological and/or histological measurements that indicate biochemical or cellular alterations in living organisms as response to toxicants (Borges, Piassão, Paula, Sepp, BezL, Hepp, Mielniczki, Pereira, & Cansian, 2018). Kaviraj (2014) defined biomarkers as alterations in biological responses that span molecular, cellular, physiological, and behavioral changes, all of which can be linked to exposure to or the toxic effects of environmental chemicals. They have been used as complementary tools in environmental monitoring (Borges et al., 2018). The use of biomarkers has its origin in human toxicology where they have proved to be very useful as measures of exposure to chemicals as well as to provide early warning signals for specific diseases (Forbes, Palmqvist, & Bach, 2006). Biomarkers serve as a shortcut, allowing for a significantly reduction in number of monitored parameters by indicating the mode of action of inductors.

In environmental biomonitoring, biomarkers are classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility. Biomarkers of exposure are defined as an early reversible cellular change in the organism. It detects the interaction between a pollutant and target organism and offer an early signal for exposure to micropollutants. They can be specific for single classes of pollutants. Biomarkers of effect assess the physiological impacts on organisms and are directly connected to the risk



of negative health outcomes. According to Gil, and Pla, (2001) that Biomarkers of susceptibility are indicators of an inherent or acquired ability of organisms to respond to the challenges of exposure to specific xenobiotic substances

The Port Harcourt Refining Company Ltd (PHRC), sited on the Okrika Mainland is reported to have been discharging her poorly treated petroleum effluent into the Ekerekana Creek, and the effluent spreads onto the adjoining creeks in the coastal area (Ogbuagu, Idewe, & Nzekwue, 2019; Ogbuagu, Abara & Chiegboka, 2023). An increasing population of inhabitants who are attracted by the presence of this petroleum hydrocarbon industry, especially of the artisanal refiners have contributed more domestic and petroleum effluents in the effluent stream discharges which can lead to significant environmental pollution and long-lasting ecological damages particularly in Estuaries. This creek harbors numerous aquatic organisms including crabs, fishes, periwinkles, crayfish, and prawns which serve as food and a form of livelihood for the inhabitants around and beyond the area. These aquatic foods have many benefits due to their high-quality protein, low saturated fat and omega fatty acid contents (Ogbuagu, Abara & Chiegboka, 2023). pollutants which could threaten their fecundity and survival in the ecosystem.

In recent times, there have been public complaints from inhabitants of the area and fishermen in the creek of tainted oil fish species they usually catch from the creek attributed to the oily discharges (Oluniyi & Augustine, 2018). Also, thick oil scums are seen floating all over the surface of the creek, especially around mangroves and prop roots of aquatic plants. These have brought increasingly concerned to the inhabitants of the area (Oluniyi & Augustine, 2018).

However, whether these petroleum hydrocarbons pollutants in the aquatic environment are being absorbed by the tissue of aquatic organisms, including shellfish, remains unanswered. Therefore, it is crucial and practical to evaluate the pollution level of the creek and determine if these pollutants have been absorbed by organism living there, which are consumed by humans. Furthermore, there's lack of research on the pollution status of the persistent type of pollutants in the creek. For this reason, this work therefore attempted to close the gap in knowledge by assessing the pollution levels, using biomarkers in the tissues *Callinectes sapidus* (blue crab) as an indicator of environmental contamination in selected estuaries of Rivers State, Nigeria

II. MATERIALS AND METHODS

The study was carried out between September, 2022 and November, 2024.

Study Area

Ekerekana in Okrika Local Government Area (LGA) and Iwofe in Obio-Akpor LGA are predominantly low-lying pluvial areas in the eastern part of the Niger Delta on the ocean-ward extension of the Benue Trough. The Niger Delta is one of the seven relief regions of Nigeria, and is characterized by a network of meandering water channels, comprising mainly of creeks and small rivers which drain into short swift coastal rivers (Ojile, 1997).

The Okrika LGA is bounded on the North by Eleme to the East by Ogu/Bolo, on the South by Bonny, on the South-West by Degema and on the North-West by Port Harcourt City L.G.A. It is geographically located between latitudes N04o44'54.24' and longitudes E07o06'10.8' The average elevation of Okrika is 452 meters above sea level. and while the Obio-Akpo LGA is situated between latitudes N4o55'19.2' and E7o02'9.6. (Fig.3.1) it has a general elevation of 30m above the sea level (Oyegun & Adeyemo, 1999). The study area is located in the humid tropical rainforest zone; with annual rainfall range of 2000–4500 mm, annual temperatures of 23 oC (minimum), and 32 oC (maximum) and a high relative humidity amounting to 70 – 90 % (NDES, 2001) The wet season begins in late March and reaches a peak (up to 5000 mm) between July and September. The wettest months are June to October, and dry season begins in late November through February Oil exploration and production activities have been going on for over 65 years in the area and the major economic activities of inhabitants include fishing, farming, trading, artisanal labour and in a few cases civil service. The Ekerekana Creek in Okrika Mainland is impacted by industrial effluent discharges from the proximal Port Harcourt Refining Company (PHRC), as well as from artisanal refining by local operators in its vicinity. The creek also serves for fisheries and as a transportation channel into other neighboring communities and the larger Bonny Estuary.

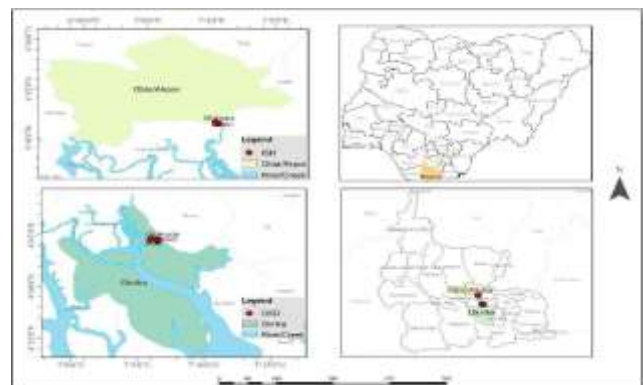


Figure 1. Aerial Map showing the sampling locations at Iwofe Creek (ISD) in Obio-Akpo Local Government Area (LGA) and Ekerekana Creek (OSD) in Okrika LGA, Rivers State, Nigeria



Figure 2. Aerial Photographs of the sampling points at Iwofe Creek (ISD 1, ISD 2 & ISD 3) in Obio Akpor LGA and Ekerekana Creek (OSD 1, OSD 2, OSD 3, OSD 4, OSD 5, OSD 6, OSD 7, OSD 8 & OSD 9) in Okrika LGA

totaling 9 sampling points were established. The sampling locations were designated as OSD A (consisting of sampling points OSD 1, OSD 2, & OSD 3), OSD B (consisting of sampling points OSD 4, OSD 5 & OSD 6) and OSD C (consisting of sampling points OSD 7, OSD 8 & OSD 9) (Fig .2). At the Iwofe creek, a sampling location designated as ISD (consisting of sampling points ISD 1, ISD 2, and ISD 3) were also established. This brought the total number of sampling points to 12 in both the impacted and reference locations. Each sampling point was geo-referenced with a Garmin GPmap 78 instrument.

Field Method: Two sampling Locations– the Ekerekana Creek in Okirika Local Government Area (LGA) (OSD; Impacted), and Iwofe Creek in Obio Akpor LGA (ISD; Reference) (Fig. 2). At Ekerekana creek, three sampling locations, each consisting 3 sampling points,

Table 1: GPS Coordinates of the sampling Locations at Ekerekana and Iwofe Creeks

Sample location	Sample point	Latitude	Longitude	Description
OSD	OSD 1	4.740	7.108	Ekerekana Creek
	OSD 2	4.741	7.109	Ekerekana Creek
	OSD 3	4.744	7.101	Ekerekana Creek
	OSD 4	4.742	7.109	Ekerekana Creek
	OSD 5	4.741	7.099	Ekerekana Creek
	OSD 6	4.743	7.099	Ekerekana Creek
	OSD 7	4.743	7.109	Ekerekana Creek
	OSD 8	4.742	7.108	Ekerekana Creek
	OSD 9	4.742	7.111	Ekerekana Creek
ISD	ISD 1	4.821	7.071	Iwofe Creek
	ISD 2	4.819	7.074	Iwofe Creek
	ISD 3	4.818	7.070	Iwofe Creek

In situ determination of water temperature, pH, Electrical Conductivity (EC), salinity, Total Dissolved Solids (TDS) and Dissolved Oxygen (DO) was made with a pre-calibrated HANNA HI 9828 pH/ORP/EC/DO meter (water) at each of the sampling points.

Water samples collected at 5cm depth in 250mL glass bottles at each location for the determination of hydrocarbon contents were fixed with concentrated H₂SO₄, while those for the determination of heavy metals were fixed with concentrated HNO₃, all in the ratio of 2:500.

Sediment samples collected with 10 x 12cm Eckman Grab from each location in the creek were transported to the laboratory in labelled polythene bags.

A total of 48 female blue crabs (*Callinectes sapidus*) of approximately the same size and weight (149.2 ± 0.02 g) were collected for the study. Of this number, 36 crabs were obtained from the impacted locations and 12 from the reference location. The female crabs were identified with the presence of egg pouch on their ventral region. All 48 crabs were utilized for bioaccumulation studies and biomarkers determination. Crabs were sampled during their inter-moult period to minimize potential influences of moult-related physiological events on biomarker responses.

The crabs were collected with the help of local fisher men using a 15 mm-mesh size fishing net at a depth less than 5 m. The specimens were immediately placed in a plastic container with the habitat sea water and rapidly transported alive to the laboratory for identification,



confirmation Department of Fisheries and Aquaculture Technology, Federal University of Technology, Owerri, Nigeria.

III. LABORATORY ANALYSIS

Analysis of hydrocarbons in sediment and water: The analytical procedure was in keeping with standard methods of APHA (1998) and Fetzer (2000). In sediments, sample extraction procedure involved weighing out 5 g each of sediment samples in a beaker and adding 10 mL of analytical grade hexane to the samples. The mixture was shaken for 5 min and filtered; and filtrates preserved for further analysis.

For water samples, 50 mL of a sample was measured into 1-liter separating funnel, a drop of concentrated H₂SO₄ was added to the sample in the separating funnel to release the hydrocarbon components and 5 mL of analytical grade N-hexane (as solvent) was subsequently added. Samples were vigorously shaken for 5 mins and allowed to stand for another 20 mins. Layers were formed that separated the extract (the top layer) from the lower layer which was discarded and the extract collected in a glass vial for analysis. A column chromatography was set up using silica gel and a glass wool. Extracts were passed through the column to clean and remove biogenic, and then collected for further analysis.

Exactly 250 mL of water sample were filtered and digested with 10 mL concentrated analytical grade HNO₃. The solution was evaporated in a crucible to approximately 5 mL, then filtered into 20 mL standard flask and made up to the mark with distilled water.

Sediment sample were extracted with concentrated HNO₃ in the ratio/proportion of 2 g of sediment sample to 5 mL of acid. The mixture was gently heat in a water bath at a temperature of 150 °C until the sediment became bleached. The mixture diluted to 20 mL with distilled water, decanted and filtered for analysis.

The extracts from water and sediment samples were analyzed for heavy metals with the Perkin Elmer (Analyst 2000 Version 6.0) Atomic Absorption Spectrophotometer. For the hydrocarbons, GC was calibrated using commercially prepared external standards having 16 components of PAHs with concentration of 1000 ppm per component. The GC parameters used are include helium as carrier gas, air and hydrogen as fuel gases, nitrogen as back-up gas, detector temperature of 35 °C, in-let temperature of 25 °C, initial oven temperature of 5 °C, final oven temperature of 300 °C, hydrogen flow rate of 30 mL/min., air flow rate of 300 mL/min., nitrogen flow rate of 30 mL/min, and helium flow rate of 30 mL/min. The GC parameters were set and a PAH extract loaded using a micro-syringe to prompt the GC interphases with Flame Ionization Detector (GC-FID) to run for a period of about 41 min.

Analysis of Heavy metal contents in Water and Sediments

Exactly 250ml of water sample was filtered and digested with 10ml concentrated analytical grade HNO₃. The solution was evaporated in a crucible to approximately 5ml, then filtered into 20ml standard flask and made up to the mark with distilled water. Sediment sample was extracted with concentrated HNO₃ in the ratio/proportion of 2g of sediment sample to 5ml of acid. The mixture was gently heated in a water bath at a temperature of 150°C until the sediment became bleached. The mixture was diluted to 20ml with distilled water, decanted and filtered for analysis. The extracts from water and sediment samples were analyzed for heavy metals (Zn, Cd, Cr, Pb and Fe) with the Perkin Elmer Atomic Absorption Spectrophotometer (AAS) (Analyst 2000 Version 6.0). Tissue analysis for Heavy metals accumulations

The method of Wangboje and Ikhuae (2015) were employed. The crab samples were dissected and their muscle, digestive and ovary tissues were removed. Tissues were oven-dried at 70 °C for 48 h, milled separately with a porcelain mortar and pestle, and kept in foils. Two grams, each of the tissue samples were weighed into 250 mL conical flask, into which 5 mL of HClO₄ and 15 mL HNO₃ were added. The mixture was heated until a clear solution was formed. Five milliliters of 20% HCl were added, the mixture filtered into a 100 mL volumetric flask through a No. 42 Whatman filter paper, and the filtrate made up to mark with distilled water. The digest was stored in a 100 mL plastic reagent bottle for subsequent Atomic Absorption Spectrophotometric Analysis. Standard solutions of each sample of the metals were prepared according to the manufacturer's procedure.

Tissue Analysis for Hydrocarbon Accumulation

Crab sample were dissected and their muscle, digestive and ovary tissue will be removed. Tissue samples will be homogenized with Na₂SO₄ for 2 or 3 min for adequate dryness, according to the method of Abdallah (2017). The mixture was transferred to a pre-cleaned extraction thimble, and the dehydrated tissue will be extracted inside the thimble with 200 mL of n-hexane- dichloromethane in the ratio of 1:1 for 8 h in a Soxhlet apparatus, cycling 5 or 6 times/hr. Anhydrous Na₂SO₄ were extracted the same way as the sample and used as blank. The extracted solvents were concentrated down to 2 mL with a rotary evaporator at a maximum temperature of 35 °C, and then further concentrated down to 2 mL with a pure nitrogen gas stream. Cleanup and fractionation were conducted by passing the extract through a silica/alumina column. The first millilitre of the extract will pass through slurry packing of 20 mL (10 g) silica, 10 mL (10 g) of aluminium, and then 1 g of anhydrous Na₂SO₄. Elution was made with 40 mL of hexane/dichloromethane in the ratio 90:10, and then by 20 mL hexane/dichloromethane in the ratio 50:50. Eluted samples were then concentrated under a gentle stream of purified nitrogen to about 0.2 mL, before injection into a GC-FID.

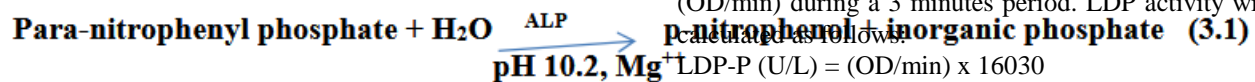


IV. ESTIMATION OF BIOMARKERS

The laboratory procedures were in keeping with the method of Ujowundu, Igwe, Alisi, Nwaogu, Ogbuagu, & Onwuliri, 2017.

1. Alkaline Phosphatase (ALP)

The principle derives from the activity of ALP in highly alkaline pH in presence of divalent Mg ions where its catalysis the hydrolysis of p-Nitrophenylphosphatase (PNPP) which results in release of p-Nitrophenol and free phosphate group. Absorbance is proportional to the serum ALP at 405 nm. The enzyme ALP hydrolyses the 4-NPP to release 4-nitrophenol, under alkaline conditions. The 4-nitrophenol formed is detected spectrophotometrically at 405 nm to give a measurement of ALP activity in the sample.



The manufacturer's kit components included the followings:

- Alkaline Phosphatase (S.L) Reagent 1 (R1), containing a mixture of 8.78 g/L HEDTA, 6 mL/L zinc sulphate, 5 g/L Magnesium acetate, and 92.6 AMP buffer;
- Alkaline Phosphatase (S.L) Reagent 2 (R2), containing 50 mmol/L of P-Nitro phenyl phosphate.

The reagent will be prepared by mixing 4 volumes of R1 with 1 volume of R2. The test parameters under Kinetic Mode and Increasing slope of reaction included wavelength of 405 nm, temperature of 37 °C, 2757 Factor, D1 Water as Blank, Linearity of 2000 U/L, Dalay time of 60 sec, sample volume of 20 µL, Reagent volume of 1000 µL, and 1 cm light path Cuvette. The agape Mult calibrator will be used for the calibration of the Auto-analyzer.

Exactly 1000 µL of the working reagent will be mixed with 20 µL of serum/plasma sample and incubated at 37 °C for 1 min. The change in absorbance will be measured per min (OD/min) during 3 min. ALP activity was calculated as follows:

$$\text{ALP (U/L)} = (\text{OD/min}) \times 2757$$

Where 2757 is a factor.

The AGAPPE qualicheck Norm and Path (11601001) will be used to verify the performance of the assay.

2. Lactate dehydrogenase (LDH)

The principle for the kinetic determination of LDH is according to the reaction:



The reagent composition included:

- LDH-P (S.L) Reagent 1 (R1), containing a mixture of 80 mmol/L Tris buffer (pH 7.4), 1.6 mmol/L pyruvate, and 200 mmol/L NaCl; and
- LDH-P (S.L) Reagent 2 (R2), containing 240 mmol/L NADH.

Four volumes of R1 were mixed with 1 unit of R2. The decreasing slope of kinetic reaction parameters used will wavelength 340 nm, temperature 37 °C, 16030 Factor, D1 Water Blank, Linearity 2400 U/L, 60 seconds Delay time, 3 number of readings, 60 seconds interval, sample volume 10 µL, Reagent volume 1000 µL, and 1 cm light path Cuvette.

Exactly 1000 µL of the working reagent will be mixed with 10 µL of serum sample and incubated at 37 °C for 1 min. The change in absorbance was measured per min (OD/min) during a 3 minutes period. LDP activity will be calculated as follows:

$$\text{LDP-P (U/L)} = (\text{OD/min}) \times 16030$$

3. Alanine Aminotransferase (ALT)

The principle behind the kinetic determination of ALT is according to the reaction:



The composition of the reagent is:

- SGPT (S.L) Reagent 1 (R1), containing a mixture of 110 mmol/L Tris buffer (pH 7.5), 600 mmol/L L-Alanine, and >1500 U/L Lactate dehydrogenase (LDH); and
- SGPT (S.L) Reagent 2 (R2), containing 16 mmol/L alpha-ketoglutarate and 0.24 mmol/L NADH.

Four volumes of R1 were mixed with 1 unit of R2. The kinetic reaction parameters were determined using wavelength of 340 nm, temperature 37 °C, 1745 Factor, D1 Water Blank, 350 U/L Linearity, 60 sec Delay time, 3 number of readings, 60 sec interval, 100 µL sample volume, 1000 µL Reagent volume, and 1 cm light path Cuvette. One thousand µL of the working reagent will be mixed with 100 µL of serum sample and incubated at 37 °C for 1 min. The change in absorbance will be measured per minute (OD/min) during a 3 min period. SGPT (ALT) activity will be calculated as follows: SGPT (U/L) = (OD/min) x 1745

4. Aspartate Aminotransaminase (AST)

The kinetic determination of AST is based on the following reaction:





The reagent is composed of:

- SGPT (S.L) Reagent 1 (R1), containing a mixture of 88 mmol/L Tris buffer (pH 7.8), 260 mmol/L L-Aspartate, >1500 U/L Lactate dehydrogenase (LDH); and >900 U/L Malate dehydrogenase (MDH); and
- SGPT (S.L) Reagent 2 (R2), containing 12 mmol/L alpha-ketoglutarate and 0.24 mmol/L NADH.

Four volumes of R1 was mixed with 1 volume of R2. The decreasing slope kinetic reaction parameters used will be 340 nm wavelength, 37 °C temperature, 1745 Factor, D1 Water Blank, 350 U/L Linearity, 60 sec Delay time, 3 number of readings, 60 sec interval, 100 µL sample volume, 1000 µL Reagent volume, and 1 cm light path Cuvette.

Exactly 1000 µL of the working reagent was mixed with 100 µL of serum/plasma sample and incubated at 37 °C for 1 min. The change in absorbance will be measured per min (OD/min) during a 3 min period. SGOT (AST) activity was calculated as follows:

$$\text{AST (U/L)} = (\text{OD/min}) \times 1745 \quad (3.6)$$

5. Malondialdehyde (MDA)

The principle behind the estimation of serum lipid peroxidation product Malondialdehyde involves the reaction of Thiobarbituric acid (TBA) with MDA and end product lipid peroxidation, under slight acidic condition to produce a red trimetrone complex which absorbs maximally at 532 nm wavelength with a spectrophotometer. The equation for the reaction is as follows:

$$\text{AST (U/L)} = (\text{OD/min}) \times 1745 \quad (3.6)$$

Exactly 0.25 mL of serum and 1.25 mL of 10% trichloroacetic acid will be added to a clean centrifuge tube and allowed for 10 min after this. Exactly 1.25 mL of 0.05M H₂SO₄ and 1.5 mL of 0.67 TBA will be added to the tube and mixed properly. The tubes were placed in a boiling water bath for 1 hour. They were then cooled under a running water tap and 2 mL of butan-1-ol was added. TBA reactive material will be extracted and absorbance read at 532 nm wavelength. Values of the TBA-reactive material will be extrapolated from the standard curve.

6. Total Proteins

The colorimetric determination of total protein will be based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue cooler is proportional to the protein concentration. The 2x50 mL Total Protein Reagent (TPR) is composed of 6 mmol/L potassium iodide, 21 mmol/L potassium sodium tartarated, 6 mmol/L copper sulphate, and 58 mmol/L NaOH. The 1x3 mL Total Protein Standard (TPS) is composed of 6 g/dL TPS concentration. The autoanalyzer systems

parameters include End point mode of reaction, Increasing slope of reaction, 546 nm wavelength, 37°C temperature, 6 g/dL standard concentration, 15 g/dL linearity, Reagent blank, 10 min incubation time, 20 µL sample volume, 1000 µL reagent volume, and 1 cm light path Cuvette.

The reagent blank will be mixed with 20 µL of serum sample and 1000 µL reagent sample, and incubated at 37 °C. The absorbance of standard and sample will be measured against reagent blank. The total protein was calculated as follows:

$$\text{Total protein concentration (g/dL)} = \frac{\text{Absorbance of sample} \times 6}{\text{Absorbance of standard}} \quad (3.8)$$

Statistical Analysis

All data collected were analyzed using SPSS v.23.0 and MS Excel 2009 software, possible homogeneity in the mean-variance of the biomarker levels, heavy metals and hydrocarbons in the tissues of organism were explored with the One-way ANOVA and their separation was achieved with a post hoc Duncan's Multiple Range Test at $p < 0.05$. Pearson's correlation coefficient (r) was used to explore the possible association of the inductor physicochemical attributes of the creeks with biomarker levels. Student t-test was used for comparison between wet and dry seasons. Pollution index was explored to compare pollutants levels across sampling locations while Toxicity quotient was used to compares the concentration of the pollution to a reference value such as regulatory standard.

V. RESULT

Water temperatures were between 30.33 at the ISD and 31.42 at OSD C, pH was between 6.00 at OSD B and 6.68 at ISD, while Electrical conductivity (EC) was between 2608.00 µS/cm at ISD and 4912.00 µS/cm at OSD C locations (Table 1). Salinity, total dissolved solids (TDS) and dissolved oxygen (DO) varied from 434.67 0/00 at ISD to 818.67 0/00 at OSD A, 1304.00 mg L⁻¹ at ISD to 2456.00 mg L⁻¹ at OSD A and 4.26 mg L⁻¹ at OSD A to 4.72 mg L⁻¹ at ISD locations respectively. Total polynuclear aromatic (=polyaromatic) hydrocarbons (PAHs) varied from 0.07 at ISD to 10.23 mg L⁻¹ at OSD A. However, in sediments, the hydrocarbons (PAHs and MAHs) varied from 6.20 and negligible mg/kg at ISD to 52.70 and 0.25 mg kg⁻¹ at the OSD C locations. pH in sediments varied from 6.50 at OSD B and OSD C to 6.70 at the ISD locations

In water column, mean concentrations of Zn, Cd, Cr, Pb, Fe and Mn were 0.03±0.01, 0.001±0.00, 0.001±0.00, 0.001±0.00, 5.82±0.29 and 0.001±0.00mg/l, respectively at the impacted locations (Table 3). However, in sediments, they were 22.10±1.37, 1.98±0.04, 2.13±0.09, 4.80±0.57, 24.03±1.29, 0.04±0.01mg/l, respectively.



Accumulation of Pollutants in Tissues

At the impacted location, the accumulation of Zn was 11.00µg/g in the digestive tract, 10.50µg/g in the muscle and 2.40µg/g in the ovaries, and at the reference location they were 1.31, 1.21 and 1.00µg/g, respectively (Table 4). Accumulation of Cr was 0.21µg/g in the digestive tract, 0.10µg/g in the muscle and 0.04µg/g in the ovaries at the impacted location, and 0.02, 0.01, 0.03µg/g, respectively at the Reference location.

At the Impacted location, the accumulation of Cd was 0.01µg/g in the digestive tract, 0.02µg/g in the muscle and 0.01µg/g in the ovaries, whereas at the Reference location they were 0.005, 0.004, 0.006µg/g, respectively. That for Pb was 0.04µg/g in the digestive tract, 0.02µg/g in the muscle and 0.01µg/g in the ovaries at the impacted location, and 0.003, 0.002 and 0.01µg/g respectively at the Reference location. At the impacted location, the accumulation of Mn was 0.05 µg/g in the digestive tract, 0.03µg/g in the muscle and 0.01µg/g in the ovaries, and at the Reference locations, they were 0.005, 0.003, and 0.001µg/g respectively.

Table 2: Concentrations of hydrocarbons and some physicochemical attributes in water and sediments of the Ekerekana and Iwofe creeks in the Niger Delta.

	Sampling locations			
	OSD A	OSD B	OSD C	ISD
	Water			
Temperature (°C)	31.20	31.33	31.42	30.33
pH	6.29	6.00	6.20	6.68
EC (µS/cm)	4912.00	3783.90	2988.55	2608.00
Salinity (‰)	818.67	630.65	498.09	434.67
TDS (mg/L)	2456.00	1891.95	1494.27	1304.00
DO (mg/L)	4.26	4.56	4.68	4.72
TPH	45.20	40.10	35.40	11.20
∑PAHs (mg/L)	10.23	9.55	10.10	0.07
∑MAHs (mg/L)	0.00	0.00	0.00	0.00
	Sediment			
pH	6.60	6.50	6.50	6.70
TPH	76.00	81.50	75.30	6.40
∑PAHs (mg/kg)	37.85	51.67	52.70	6.20
∑MAHs (mg/kg)	0.18	0.22	0.25	0.00

Table 3: Concentrations of Heavy metals in Water (mg/L) and sediments (mg/kg) of the Ekerekana and Iwofe creeks in the Niger Delta

Locations	Zn	Cd	Cr	Pb	Fe	Mn
	Water					
OSD A	0.037	0.004	0.002	0.001	5.25	0.001
OSD B	0.020	0.002	0.001	ND	6.00	ND
OSD C	0.030	0.00	0.001	0.001	6.20	0.002
Mean	0.029	0.00	0.001	0.001	5.817	0.001
ISD	0.002	ND	ND	ND	2.20	ND
	Sediment					
OSD A	24.80	2.05	2.30	5.90	21.60	0.05
OSD B	21.10	2.00	2.10	4.50	26.	0.02
OSD C	20.40	1.90	2.00	4.00	24.5	0.04
Mean	22.10	1.98	2.13	4.80	24.3	0.04
ISD	5.22	ND	ND	ND	5.80	ND

Table 4: Concentrations of heavy metals and hydrocarbons in tissues of the blue crab (Callinectes sapidus) from the Ekerekana and Iwofe creek in the Niger Delta

Poll. (µg/g)	Tissues					
	Ekerekana (Impacted locations)			Iwofe (Reference location)		
	Digestive	Muscle	Ovary	Digestive	Muscle	Ovary
Zn	11.00	10.50	2.40	1.31	1.21	1.00
Cr	0.21	0.10	0.04	0.02	0.01	0.03
Cd	0.10	0.02	0.01	0.005	0.004	0.006
Pb	0.04	0.02	0.01	0.003	0.002	0.001
Mn	0.05	0.03	0.01	0.005	0.003	0.001
TPH	4.14	3.14	1.20	0.006	0.002	0.004
∑PAHs	0.09	0.06	0.02	0.004	0.001	0.001
∑MAHs	0.01	0.02	0.01	0.001	ND	0.001

Total Petroleum Hydrocarbons was 4.14µg/g in the digestive tract, 3.14µg/g in the muscle and 1.20µg/g in the ovaries at the Impacted location. Polycyclic Aromatic Hydrocarbons was 0.09µg/g in the digestive tract, 0.06µg/g in the muscle and 0.02µg/g in the ovaries at the Impacted location. The combination of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) was 0.01µg/g in the digestive tract, 0.02µg/g in the muscle and 0.01µg/g in the ovaries at the Impacted location, and 0.001, 0.000 and 0.001µg/g, respectively at the Reference location.

However, mean accumulations of combined pollutants (heavy metals & hydrocarbons) in the digestive tract at the Impacted locations was 1.89±1.33µg/g, and 0.17±0.16µg/g at the Reference location; in the muscle was 1.80±1.37µg/g at the Impacted location and 0.15±0.15µg/g at the reference location; and in the ovary was 0.46±0.31µg/g at the impacted location and 0.131±0.12µg/g at the reference location (Figure 3)

However, At the impacted location, cumulative mean accumulations of Zn, Cr, Cd, Pb and Mn in tissues of C. sapidus were 7.60±1.60, 0.13± 0.28, 0.50±0.18, 0.22± 0.06, and 0.277±0.00694 µg/g respectively (Fig. 3). At the Reference location, the cumulative mean accumulations of the respective heavy metals were 1.023±0.134, 0.183±0.000, 0.004±0.001, 0.001± 0.0003 and 0.003±0.008 µg/g respectively.

At the impacted location, cumulative mean accumulations of TPH, ∑PAHs and ∑MAHs in tissues of the organism were 2.823±0.427, 0.583± 0.128, and 0.010±0.026µg/g respectively (Fig 4.). However, at the reference locations, the cumulative mean accumulations of the respective hydrocarbons were 0.003±0.007, 0.002± 0.001, and 0.0005±0.00022µg/g.

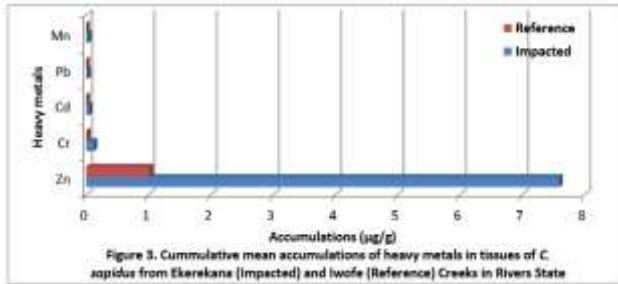


Figure 3. Cumulative mean accumulations of heavy metals in tissues of *C. sapidus* from Ekerekana (Impacted) and Iwofe (Reference) Creeks in Rivers State

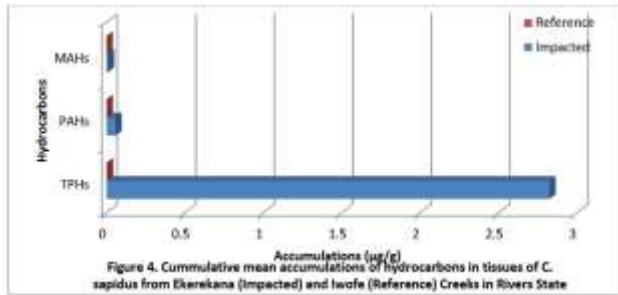


Figure 4. Cumulative mean accumulations of hydrocarbons in tissues of *C. sapidus* from Ekerekana (Impacted) and Iwofe (Reference) Creeks in Rivers State

The ANOVA test revealed that the accumulations of the heavy metals and hydrocarbons in the various tissues of *C. sapidus* differed markedly between the seasons at $p < 0.05$. Their significant values were 0.002 (Zn), 0.004 (Cr), 0.027 (Cd), 0.008 (Pb), 0.005 (Mn), 0.000 (TPHs), 0.001 (PAHs) and 0.04 (MAHs).

Concentrations of biomarkers in tissues: Table 5, shows the concentrations of the biomarkers measured in the blue crab across the sampling locations.

At OSD A, the mean levels of LDH, ALT, AST, ALP, Total protein and MDA were 48.500 ± 4.048 U/L, 42.133 ± 3.653 U/L, 60.383 ± 0.662 U/L, 20.367 ± 0.592 U/L, 5.550 ± 0.2432 g/dL and 29.333 ± 0.333 mmol/L respectively (Fig 5). At OSD B, the mean levels of the respective biomarkers were 40.833 ± 1.6210 U/L, 31.933 ± 0.2996 U/L, 73.250 ± 3.199 U/L, 18.183 ± 0.252 U/L, 5.400 ± 0.257 g/dL and 22.000 ± 1.461 mmol/L. However, at OSD C, the mean levels of the respective biomarkers were 41.333 ± 1.542 U/L, 29.983 ± 0.32395 U/L, 60.000 ± 1.9905 U/L, 24.583 ± 0.9779 U/L, 5.2000 ± 0.1238 g/dL and 25.833 ± 1.682 mmol/L

At ISD, the mean levels of the respective biomarkers were 29.833 ± 1.078 U/L, 22.833 ± 0.691 U/L, 43.500 ± 1.158 U/L, 11.917 ± 0.315 U/L, 5.017 ± 0.133 g/dL and 11.167 ± 0.401 mmol/L respectively.

The One-Way ANOVA test revealed that the mean levels of LDH, ALT, AST, ALP and MDA (Sig. values=0.000each) differed markedly in *C. sapidus* across the sampling of the creeks studied.

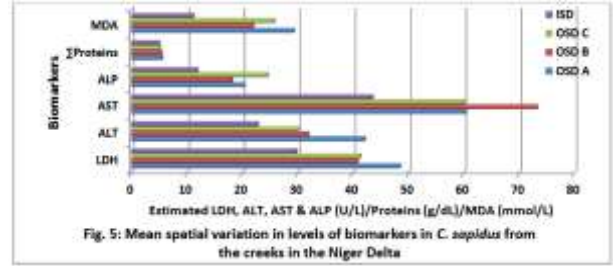


Fig. 5: Mean spatial variation in levels of biomarkers in *C. sapidus* from the creeks in the Niger Delta

Table 6: Biomarker levels in tissues of the blue crab *Callinectes sapidus* from the Ekerekana and Iwofe creeks of the Niger Delta (Rainy Season)

Replicates	Sampling locations											
	OSD A			OSD B			OSD C			ISD		
Biomarkers	1	2	3	1	2	3	1	2	3	1	2	3
LDH (U/L)	60	40	40	35	45	40	38	40	47	32	30	25
ALT (U/L)	47.2	39.2	48.5	31.0	32.0	31.2	28.5	39.8	30.0	20.8	25.9	22.5
AST (U/L)	60.2	58.1	62.8	79.8	77.2	60.6	52.5	62.8	63.3	45.5	42.2	42.1
ALP (U/L)	20.9	21.0	17.8	17.0	18.6	18.5	22.2	23.2	27.2	11.8	12.8	12.2
Protein (g/dL)	5.2	6.4	6.1	5.1	6.6	5.0	5.5	5.1	5.6	5.5	5.1	4.9
MDA (mmol/L)	29	28	30	28	18	20	21	29	30	13	11	11

LDH: Lactate dehydrogenase, **ALT:** Alanine aminotransferase, **AST:** Aspartate aminotransferase, **ALP:** Alkaline phosphatase and **MDA:** Malondialdehyde. Of the biomarkers, the activities of ALT (sig. = 0.032), AST (sig. = 0.045) and ALP (sig. = 0.007) and the concentration of total proteins (sig. = 0.036) and MDA (sig. = 0.005) all differed markedly across the location at the 95% confidence limit. A post-hoc Duncan Multiple Range test revealed that the observed differences in LDH and ALT, were between OSD A, ISD and all the other impacted locations (OSD B and OSD C) (Table 4.5). In AST, the differences were between OSD A=OSD C and the other locations (OSD B and ISD) while in ALP and MDA, the difference was between all the sampling locations.

Relationships between physicochemical attributes of the Creeks and biomarkers in organism

The Pearson's correlation (r) between the physicochemical attribute, heavy metals and Hydrocarbon contents of the Creeks and the biomarker levels in *C. sapidus* are shown in Table 7. At $p < 0.05$, MAHs in water correlated negatively with ALT ($r = -0.584$), AST ($r = -0.615$) and ALP ($r = -0.509$), pH in water, correlated negatively with AST ($r = -0.519$), DO in water, correlated positively with AST ($r = 0.552$). Cr in water, correlated negatively with AST ($r = -0.513$). while pH in water correlated negatively with Total proteins ($r = -0.553$). At $p < 0.01$, the MAHs in water correlated negatively with MDA ($r = -0.634$).

Table 7: Correlation (r) matrix between the physicochemical attributes of heavy metal and hydrocarbons contents of the creeks and biomarkers levels in *C. sapidus*



Temp_s pH_w ECW Salw TDSW DO_w TPH_w PAH_w MAH_w pH_{sed} TPH_{sed}
 PAH_{sed} MAH_{sed} Zaw Cdw Crw

LDH	0.144	-0.173	0.059	-0.045	0.081	0.104	0.104	0.075	-0.466	-0.043	0.037
0.048	0.035	0.107	-0.157	0.0767							
ALT	0.165	-0.286	-0.131	-0.218	-0.170	0.261	-0.064	-0.131	-0.584*	-0.023	-0.100
0.065	-0.113	0.206	-0.379	-0.208							
AST	0.265	-0.519*	-0.417	0.353	-0.348	0.553*	-0.204	-0.212	-0.615*	-0.170	-0.191
0.109	-0.204	-0.399	-0.513*	-0.421	ALP	0.008	-0.073	-0.195	-0.174	-0.093	0.293
0.255	-0.280	-0.509*	0.197	-0.311	-0.300	-0.344	-0.239	-0.247	-0.095	Protein	
0.445	-0.553*	0.133	0.202	0.158	0.291	0.242	0.229	-0.110	-0.373	0.218	0.194
0.191	0.129	0.015	0.306								
MDA	0.101	-0.320	-0.102	-0.138	-0.053	0.319	-0.134	-0.217	-0.634**	-0.025	-0.204
0.208	-0.281	-0.0286	-0.340	-0.191							

M _{sed}	Pb _w	Fe _w	Mn _w	Zn _w	Cd _w	Cr _w	Pb _{sed}	Fe _{sed}
LDH	0.259	-0.179	0.378	0.041	-0.050	-0.032	0.084	0.005
0.148								
ALT	-0.194	-0.266	-0.094	-0.209	-0.230	-0.228	-0.119	-0.0015
0.249								
AST	-0.206	-0.423	-0.163	-0.362	-0.295	-0.00	-0.248	-0.286
0.249								
ALP	0.029	-0.472	0.161	-0.266	-0.334	-0.314	-0.236	-0.334
0.004								
∑Protein	0.062	-0.137	0.080	0.115	0.198	0.178	0.272	0.044
MDA	0.196	-0.455	-0.243	-0.263	-0.295	-0.303	-0.167	-0.262
-0.141								

*=Significant at p<0.05, **= significant at p<0.01, EC=electrical conductivity, TDS=total dissolved solids, DO=dissolved oxygen, TPH=total petroleum hydrocarbons, ∑PAH=total polycyclic aromatic hydrocarbons, ∑MAH=total mononuclear aromatic hydrocarbons (n-benzene, toluene, ethylbenzene, xylene) LDH=lactate dehydrogenase, ALT=alanine aminotransferase, AST=aspartate aminotransferase, ALP=alkaline phosphatase, MDA=malondialdehyde.

VI. DISCUSSION

The results of the present study corroborate existing knowledge that water bodies act as the ultimate sink for pollutants generated by industrial and artisanal activities (Ogbuagu et al., 2019). The accumulation of hydrocarbons in the tissues of the organisms sampled can be attributed to allochthonous input from industrial and artisanal pollution sources near the creek. Both local (Olowu et al., 2010; Oladele & Jenyo-Oni, 2015) and international research (Ikem et al., 2003; Alam et al., 2012) support the idea that contaminants enter aquatic ecosystems through various pathways, including industrial, domestic, and municipal run-offs and leachates. Alam et al. (2012) and Akan et al. (2012) further reported that untreated effluents from industrial processes discharged into nearby water bodies can severely affect water quality and cause both acute and chronic effects on resident organisms.

For example, the findings of this study, particularly on a spatial basis, demonstrate that these anthropogenic discharges have significantly impacted the water's electrical conductivity, salinity, total dissolved solids, total petroleum hydrocarbons (TPHs), and polynuclear aromatic hydrocarbons (PAHs) content. These impacts were also observed in the sediments, where elevated levels of TPHs, PAHs, and mononuclear aromatic hydrocarbons (MAHs) were found. On a seasonal scale, there was a marked increase in surface water temperature, dissolved oxygen, pH, and Fe ions, reflecting the uneven spatio-temporal distribution of pollutants in the impacted and reference creeks. Effluents from industrial processes are often hot and contain dissolved inorganic ions, with additional contributions from artisanal and domestic sources exacerbating the pollution. These elevated levels of pollutants were linked to significant bioaccumulation of hydrocarbons in the tissues of the test organism. Bioaccumulation and bioconcentration of stable and

persistent pollutants occur when ambient levels in the surrounding media exceed those within the organism.

The notably higher concentrations of pollutants in the tissues of crabs from the impacted creeks compared to those from the reference creek further indicate that the nearby refinery discharges effluents directly into the creeks, alongside pollutants from artisanal refining activities along the coastline. This finding aligns with the work of Wangboje and Ikhuabe (2015). While some metals are essential for organism growth at trace levels, they become toxic when concentrations exceed certain thresholds. The higher concentrations of pollutants in the water and sediments of the impacted creek were reflected in the tissues of the organisms. The lower pollutant levels found in the tissues from the reference Iwofe Creek, located further from the impacted Ekerekana Creek, suggest that tides transport pollutants upstream, mixing them with the surrounding lands, and that runoff during rainfall, which often carries trace metals and other pollutants, contributes to this loading in coastal waters.

Hydrocarbons are known to cause adverse health effects such as cancer, respiratory diseases, fatigue, headaches, and other conditions when they enter the food chain and are consumed by higher organisms (Copat et al., 2012; Ujowundu et al., 2014a). The significantly higher hydrocarbon concentrations in crabs from the impacted creeks point to the presence of petroleum pollutants in effluents discharged by the nearby PHRC refinery and from artisanal refining activities along the coastline.

Among the pollutants measured, Zn and TPHs exhibited the highest tendency for bioaccumulation in the tissues of crabs from the impacted creeks. Wangboje and Ikhuabe (2015) similarly observed significant bioaccumulation of Zn in crab tissues from the River Niger in Agenebode, Delta State, Nigeria. The pronounced differences in pollutant concentrations between the impacted and reference creeks underscore the elevated pollutant levels in both water and sediments surrounding the organisms studied.

The increased pollutant loads in the water and sediments of the impacted creek appear to have disrupted the biochemical and physiological processes of the test organisms, as indicated by elevated levels of biomarkers in the crabs from the Ekerekana Creek. A significant increase in total protein levels was also observed during the rainy season. These spatio-temporal variations in pollutant inputs reflect greater contamination in the Ekerekana Creek compared to the reference Iwofe Creek. Elevated levels of physicochemical variables, including heavy metals and hydrocarbons, were correlated with changes in biomarker levels in the organisms. For instance, slight acidification of the water was linked to decreases in AST (aspartate aminotransferase) and total protein levels, while higher dissolved oxygen levels appeared to affect AST activity. Elevated Cr ion levels were associated with



decreased AST synthesis and activity, and increased MAHs seemed to reduce the synthesis of ALT (alanine aminotransferase), AST, ALP (alkaline phosphatase), and MDA (malondialdehyde). Such biochemical changes could indicate disruptions in protein synthesis, a process critical for cellular function and energy production.

Increases in AST activity may indicate the effects of oxygen stress, as AST is a glycolytic enzyme responsive to oxygen levels (Mishra & Shukla, 2003). ALP, a nonspecific phosphatase involved in various physiological processes including carbohydrate metabolism, growth, and molting in crustaceans (Vijayavel & Balasubramanian, 2006), was also affected by hydrocarbon pollutants in this study.

During protein synthesis, enzymes like ALT and AST facilitate the interconversion of amino acids and α -keto acids, essential for maintaining the amino acid pool in organisms. Variations in the activity of these enzymes, as seen in this study, reflect cellular damage caused by pollutants. These changes may also be due to metabolic disruptions induced by petroleum pollutants, an observation also made in similar studies of naphthalene exposure in estuarine crabs (Vijayavel & Balasubramanian, 2006).

Polycyclic aromatic hydrocarbons (PAHs) and other pollutants undergo biotransformation in organisms, converting them into less harmful substances. However, during this process, more toxic intermediates may be generated, which can bind to cellular macromolecules like DNA, RNA, and proteins, causing toxic reactions.

The high pollutant concentrations found in water, sediments, and crab tissues in this study, along with the alarming pollution indices (PI) and hazard quotients (HQ) values, confirm that the Niger Delta aquatic ecosystems are heavily contaminated with petroleum hydrocarbons and heavy metals, consistent with findings by Ogbuagu et al. (2019) in nearby Ogu Creek. The elevated PI and HQ values for Zn, TPHs, and PAHs, which exceed unity, represent significant health risks, especially to humans who are tertiary consumers in the food chain. Such high pollution indices are linked to risks of endocrine disruption, carcinogenicity, and other toxic effects associated with heavy metals and hydrocarbons (ATSDR, 1994; EPA, 1998; Brooks et al., 2004; Giri & Singh, 2014; Ujowundu et al., 2014b). Wangboje and Ikhuae (2015) also found high bioaccumulation of Zn in fish tissues from the Niger River, and Giri and Singh (2014) reported HQ values above unity for Cr in shrimps from the Subarnarekha River in India. Other metals such as Cd, Cu, Fe, and Mn showed lower HQ values, consistent with findings by Bandowe et al. (2014) in fish from Ghana.

VI. CONCLUSION

The results of this study confirm that the estuarine environments of Rivers State are severely impacted by pollution from both industrial and artisanal sources. The bioaccumulation of pollutants in *Callinectes sapidus* serves as a useful indicator of environmental pollution, providing a means for assessing the ecological health of the region. The high pollutant levels found in both the aquatic environment and the organisms sampled highlight the need for urgent intervention, including improved waste management practices, stricter environmental regulations, and remediation efforts to mitigate the ongoing pollution in the Niger Delta region. The health risks posed by these pollutants to aquatic organisms and human populations underscore the importance of ongoing monitoring and assessment to safeguard the integrity of these vital ecosystems.

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