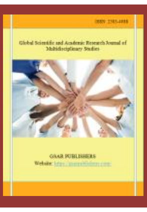

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Variation in physicochemical and hydrocarbon concentration in *callinectes sapidus* and *macrobrachium malcolmsonii* from Bolo Creek, River State

By

Orji-Kelechi, C. L.[1], Ogbuagu, D.H.[2], Nwoko, M.C.[3] and Nwachukwu, M.O.[4]

[1 2 3 4] Department Of Biology, Federal University Of Technology Owerri Imo State Nigeria

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Abstract

This study investigated the bioaccumulation of selected heavy metals and petroleum hydrocarbons in two commercially and ecologically important aquatic species, Callinectes sapidus (blue crab) and Macrobrachium malcolmsonii (prawn). Twenty blue crabs and prawns were collected with nets while, water and sediment samples were collected in 250 mL glass bottles and with 10 X 10 cm Eckman Grab from three impacted and one reference sampling locations respectively. Water quality parameters including heavy metals and hydrocarbons contents were analysed using standard methods. Data were analysed with SPSS V. 23.0 software. Mean concentrations (mg/L) of Zn (0.022±0.0039), Cd (0.002±0.0005), Cr (0.001±0.0002), Pb (0.0005±0.0002), Mn (0.0008±0.0003), total petroleum hydrocarbons (TPHs; 32.975±3.932) and total polynuclear aromatic hydrocarbons (ΣPAHs; 6.350±0.808) were lower in water than sediments (17.880±2.261, 1.488±0.259, 1.818±0.265, 3.603±0.662, 0.0275±0.006, 55.133±8.584, 22.749±3.970 mg/kg respectively) at the impacted location. Mean accumulations (µg/g) of Zn (9.00±1.80), Cr (1.14±1.01), Cd (0.13±0.05), Pb (0.03±0.01), Mn (0.33±0.14), TPH (3.62±0.57), PAH (0.11±0.03) and BTEX (0.03±0.01) were higher in combined tissues of C. sapidus at the impacted locations than at the reference locations (1.25±0.09, 0.03±0.01, 0.01±0.001, 0.002±0.001, 0.003±0.001, 0.004±0.001, 0.002±0.001 and 0.001±0.0002 µg/g respectively). Mean accumulations (µg/g) of Zn (10.02±2.97), Cd (2.16±2.02), Cr (0.21±0.052), Pb (0.43±0.01), Mn (0.64±0.05), TPHs (4.41±0.52), PAHs (0.16±0.38) and BTEX (0.05±0.12) were higher in combined tissues of M. malcolmsonii at the impacted locations than at the reference locations (1.33±1.53, 0.43±0.12, 0.01±0.002, 0.001±0.001, 0.003±0.001, 0.003±0.002, 0.001±0.001 and 0.0003±0.0003 µg/g respectively). This underscores the potential long-term health implications of consuming contaminated aquatic resources from Bolo Creek. There is an urgent need for continuous environmental monitoring and effective pollution control strategies in the area to mitigate ecological degradation and public health risks.

Keywords: *physicochemical, hydrocarbon, callinectes sapidus, macrobrachium malcolmsonii Bolo Creek, River State.*

Introduction

Aquatic ecosystems across the globe are increasingly vulnerable to contamination from anthropogenic activities, particularly those associated with industrialization, oil exploration, agricultural and urban runoff. The increasing contamination of aquatic ecosystems by these anthropogenic activities poses a significant threat to biodiversity and human health. In Nigeria, particularly the Niger Delta region, rivers and creeks like Bolo Creek in Rivers State, which lies in a zone of intense petroleum exploration and exploitation are exposed to contaminations by heavy metals and hydrocarbons which tends to persist in the environment and bioaccumulate in aquatic organisms. The creek serves as a vital resource for local communities, providing water, fish, crustaceans such as crabs and prawns, and supporting livelihoods. However, there are growing concerns over the accumulation of toxic substances such as heavy metals and petroleum hydrocarbons in aquatic organisms that form a major part of the local diet [1].

Crabs and prawns are benthic organisms, meaning they live and feed at the bottom of water bodies. This ecological behaviour makes them particularly susceptible to bioaccumulation of pollutants present in sediments and water.

*Corresponding Author: **Orji-Kelechi, C. L**

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Heavy metals such as lead, cadmium, mercury, arsenic and many more are non-biodegradable and can persist in the aquatic environment for long periods. These metals accumulate in the tissues of aquatic organisms, and over time, their concentrations may reach toxic levels that pose health risks to both the organisms and the humans who consume them (Ray & Vashishth, 2024). Similarly, hydrocarbons derived from petroleum especially polycyclic aromatic hydrocarbons (PAHs) can accumulate in aquatic fauna and cause mutagenic and carcinogenic effects [2].

Bolo Creek is located in the heart of the Niger Delta, where frequent oil spills, gas flaring, and industrial discharges are common. The region has been the focus of numerous environmental impact assessments, but there remains a significant knowledge gap in localized studies specifically investigating the levels and health implications of contaminant accumulation in edible crustaceans such as *Callinectes sapidus* (blue crab) and *Macrobrachium malcolmsonii* (prawns). While some studies have assessed the physico-chemical characteristics of the creek or evaluated contamination in fish species, comprehensive studies on bioaccumulation in crabs and prawns remain limited [3]. These species not only contribute to the biodiversity of the creek but also constitute a major protein source for the surrounding communities, making their contamination a potential public health crisis.

Moreover, chronic exposure to even low levels of heavy metals and hydrocarbons through dietary intake may lead to adverse health effects such as kidney damage, neurological disorders, immune suppression, and increased cancer risk [4]. Local consumers, often unaware of the long-term health risks, may regularly ingest contaminated seafood without any regulatory monitoring or intervention. Therefore, assessing the extent of heavy metal and hydrocarbon bioaccumulation in crabs and prawns from Bolo Creek is critical for environmental risk assessment, food safety, and policy-making.

Given this context, the present study aims to evaluate the concentration of selected heavy metals and hydrocarbons in crabs and prawns from Bolo Creek. The findings will contribute to understanding the ecological and health risks associated with contaminant bioaccumulation in the area and will serve as a foundation for public health advisories and environmental remediation strategies. This research is both timely and necessary, especially as the demand for seafood continues to rise amidst growing environmental degradation in oil-producing regions.

Materials and Methods

Study area

The study area is Bolo Creek in the southern part of Ogu-Bolo Local Government Area, sharing boundaries with Tai, Gokana and Bonny Local Government area of Rivers State, in the Niger Delta of Nigeria. Bolo is an island settlement with several archipelago type of villages and fishing ports straddled along its numerous and dense network of creeks. Bolo is the host to two oilfields: Bodo west and parts of Bomu II oilfields with associated product pipelines including the trans Niger pipeline. These pipelines are being tapped illegally and, in some cases, leading to spills and fires.

The study area is confined within the humid-hot equatorial climate, and lies between latitudes $4°34^{l}$ S and $4°45^{l}$ N and longitudes $7°10^{l}$ E and $7°15^{l}$ E (Fig. 3.1). It occupies an area of 905.2 sq.km with a population of over 150,000 (NPC, 2006). Weather conditions over the area are governed by the moist tropical maritime currents from the Atlantic Ocean wave fronts and dry wind and dust-laden tropical continental air mass from the northern part of Nigeria (Edokpa, 2018). Prevalent wind direction in the area is south westerly, with speed ranging from 0.3 to 4.5 m/s and north-easterly, with speed between 0.3 and 1.5 m/s.

The area has two main seasons, the dry season and the wet season. The dry season begins from November and ends in March, while the wet season stretches from mid-March to October. Fresh water is generally supplied by heavy precipitation resulting in mean annual rainfall of above 2600 mm. However, mean annual rainfalls is variable, with maximum amounts estimated to be 4455 mm. The peaks of the rains occur in the months of June, July, September and October, and most significant factor that influences it within the area is the tropical maritime air mass moisture.

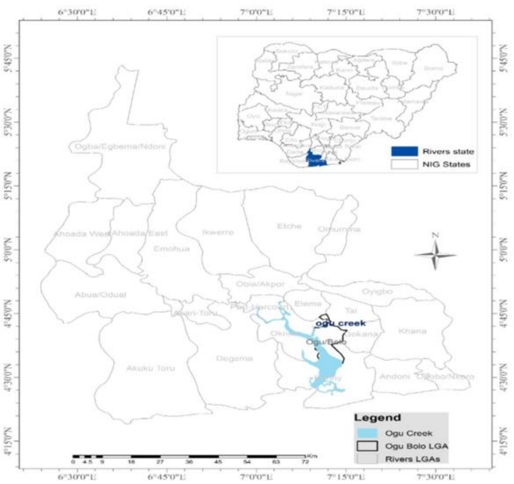

Figure 1: Aerial map showing the Ogu creek in the Upper Bonny Estuary of the Niger Delta

Relative humidity is usually above 85 percent in the rainy season, but decreases to 45percent in the dry season. Ambient air temperature ranges from 24.5 to 32^{o}C in the rainy season and from 25 to 36^{o}C in the dry season. Bonny River and its tributaries and creeks are accountable for the drainage around the deltaic plain belt area. The dockyard creek in the south, Amadi and Okpoka Creeks in the east, and the Channel of Bonny River (Port Harcourt Harbour) by the west drain the area. These creeks flow in the N-S direction into the Bonny River, which eventually flow to the west. Each of the creeks that border Port Harcourt area can be subdivided into three sections, the head water (usually fresh water streams), the down streams (saline) and the brackish water (in between them [5].

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Sampling locations

Three locations designated as IMP1, IMP2 and IMP3 nearest to the mouth of the creek were sited within crude oil effluent-contaminated/impacted vicinity. Another location, sited about 1 km away from the mouth of the creek and designated as Reference location was also studied for comparison (Fig. 2).

Ethical Consideration and Permission

The study proposal was approved by the Ethical Clearance Committee of the Department of Biology, Federal University of Technology, Owerri. Blue crab (*Callinectes sapidus*) and prawn (*Macrobrachium malcolmsonii*) were obtained from the fishermen from the study area.

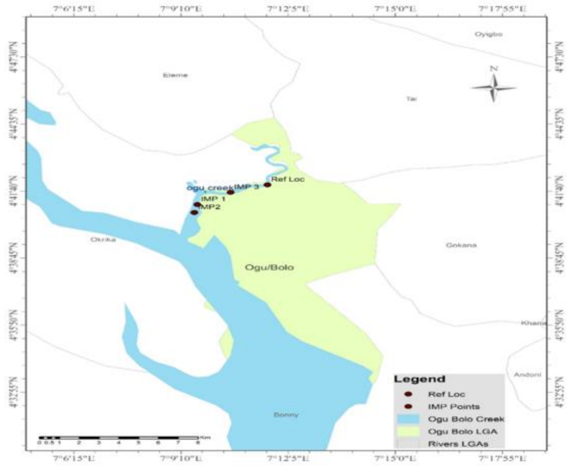

Figure 2: The sampling points designated as IMP1 – IMP3 (Impacted) and REF (reference/control) in Ogu Bolo Creek, River State.

Permission from local district fisheries and officers were obtained before sampling in their areas.

Field Sample Collection

***In Situ* Measurements**

In situ determination of surface water temperature, pH, electrical conductivity (EC), salinity, total dissolved solids (TDS), and dissolved oxygen (DO) were made with a pre-calibrated HANNA HI9828 pH/ORP/EC/DO meter at each sampling location.

Water Sample Collection

Water samples for the determination of heavy metals were collected at 5 cm depth in 250 mL glass containers against the flow of current and fixed with concentrated Nitric acid $(HNO)_3$. Those for the determination of hydrocarbon concentrations (TPHs, PAHs, MAHs) were fixed with concentrated Sulfuric acid (H_2SO_4) each in the ratio of 2:500. Samples were replicated, labelled and taken to the laboratory for analyses [6]

Sediment Sample Collection

Sediment samples collected with 10 x 12 cm Eckman Grab at each of the sampling locations samples were replicated and transferred to the laboratory in labelled polythene bags (Ogbuagu et al. 2023).

Crab and Prawn Sample Collection

Twenty adult female blue crabs and twenty adult prawns were collected with drag nets from the locations around the creek. Samples of approximately uniform sizes were collected in order to minimize possible error due to size differences. Each prawn and crab were properly cleaned by rinsing with water to remove debris, plankton and other external adherents. They were immediately placed in a plastic container with their habitat water and transported alive to the laboratory for further analyses.

Samples were identified by Dr. Ojo Akinrotimi of the Nigerian Institute for Oceanography and Marine Research (NIOMR), Onne, Eleme, Rivers State.

Laboratory Procedures

Determination of Heavy Metal contents in Water and Sediments

This was carried out according to the method of [7]. To determine the heavy metal concentration, 250 mL of water sample was filtered and digested with 10 mL concentrated analytical grade HNO_3. 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 HNO_3 in the ratio/proportion of 2 g of sediment sample to 5 mL of acid. The mixture was gently heated in a water bath at a temperature of 150 ^{0}C until the sediment became bleached. The mixture was diluted to 20 mL with distilled water, decanted and filtered for analysis. The extracts from water and sediment samples were analysed for heavy metals with the Perkin Elmer (Analyst 2000 Version 6.0) Atomic Absorption Spectrophotometer (AAS).

Determination of Hydrocarbons in Water and Sediments

The analytical procedure was in keeping with standard methods of APHA (2000) and Anaero-[8] In sediments, sample extraction procedure involved weighing out 5 g each of sediment sample into a beaker and adding 10ml of analytical grade hexane to the samples. The mixture was shaken for 5 minutes, filtered, and filtrates used for Gas Chromatography (GC) analysis.

For water samples, 50 mL of a sample was measured into 1 L separating funnel, a drop of concentrated H_2SO_4 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 biogenics, and then collected for Gas Chromatography (GC) analysis.

GC was calibrated using commercially prepared external standards having 16 components of PAHs with concentration of 1000 ppm per component. The GC parameters used

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included helium as carrier gas, air and hydrogen as fuel gases, nitrogen as back-up gas, detector temperature of 35^{o}C, in-let temperature of 25^{o}C, initial oven temperature of 5^{o}C, final oven temperature of 300^{o}C, hydrogen flow rate of 30 mL/mins., air flow rate of 300 mL/mins., nitrogen flow rate of 30 mL/mins., and helium flow rate of 30 mL/mins. The GC parameters were set and a PAH extract loaded using a micro-syringe to prompt the GC interphase with Flame Ionization Detector (GC-FID) to run for about 41 minutes.

Statistical Analysis

SPSS V.23.0 (DBI, 2023) and MS Excel 2017 tools were used to analyse the data collected from the study. The differences in heavy metals and hydrocarbon concentration between the mainstream (impacted) and the upstream(reference) locations was assessed using the One-way Analysis of Variance (ANOVA) at $p<0.05$. Student's t-test was used to analyse variations in the different organisms with T-value of t=0.05. In all cases, treatments were considered significantly different at $p<0.05$.

Results and Discussion

Results

Spatial Variations in Physicochemical Parameters of Water and Sediments

The physicochemical properties of water and sediments of the Bolo creek are presented in Table 4.1. In water column, mean temperature, pH and Electrical Conductivity (EC) varied from 30.33 ± 0.47^{0}C at the Reference location (REF) to 31.42±0.41^{0}C at the Impacted (IMP 3) location, 6.00±0.48 at IMP 2 to 6.68±0.08 at REF and 2608±4.29 µS/cm at REF to 4912±4.07 µS/cm at IMP 1 respectively. Salinity varied from 434.67±3.21 at REF to 818.67±5.18 ($^{0}/_{00}$) at IMP 1, Total Dissolved Solid (TDS) varies from 1304±2.50 mg/L at REF to 2456±4.71 mg/L at IMP 1 while Dissolved Oxygen (DO) varied from 4.26±0.21 at IMP 1 mg/L to 4.72±0.10 mg/L at REF. However, in sediments pH varied from 6.60±0.03 at IMP 1 to 6.70±0.06 at REF. The mean levels of EC, Salinity and TDS were above the World Health Organization (WHO) permissible limits of 100 (µS/cm), 150 ($^{0}/_{00}$), and 250 (mg/L).

Variations in Heavy Metals and Hydrocarbons concentrations in Water and Sediments

The variations in heavy metals and hydrocarbons in water and sediments of the creek is represented in Table 4.2. In water of the Bolo Creek, Zn, Cd, Cr, Pb and Mn varied from 0.002-0.0038 (0.022±0.0039), 0.000-0.004 (0.002±0.0005), 0.000-0.002(0.001±0.0002), 0.000-0.001 (0.0005±0.0002) and 0.000-0.002 (0.0008±0.0003) mg/L respectively (Table 4.2). TPH varied from 11.190-45.210 (32.975±3.932) and PAHs varied from 2.590-9.430 (6.350±0.808) mg/L.

In sediments, Zn, Cd, Cr, Pb and Mn varied from 5.18-24.80 (17.880±2.261), 0.000-2.070 (1.488±0.259), 0.000-2.30 (1.818±0.265), 0.000-5.930 (3.603±0.662) and 0.000-0.051(0.0008±0.0003) mg/kg respectively. TPH varied from 6.40–76.00 (55.133±8.584)

Table 1: Physicochemical properties of Water and Sediments of the Bolo Creek in the Niger Delta

Parameters (units)	Sampling Locations				WHO (2016)
	IMP 1	IMP 2	IMP 3	REF.	
Temperature (^{0}C)	31.20±1.38	31.33±1.02	31.42±0.41	30.33±0.47	30.00 -32.00
pH	6.29±0.91	6.00±0.48	6.20±0.41	6.68±0.08	7.00 - 8.50
EC (µS/cm)	4912.00±4.07	3783.90±5.02	2988.55±4.62	2608.00±4.29	100.00
Salinity ($^{0}/_{00}$)	818.67±5.18	630.65±5.71	498.09±3.71	434.67±3.21	150.00
TDS (mg/L)	2456.00±4.71	1891.95±5.77	1494.27±4.81	1304.00±2.5	250.00
DO (mg/L)	4.26±0.21	4.56±0.61	4.68±0.04	4.72±0.10	> 5.00
Sediment					
pH	6.60±0.03	6.65±0.41	6.65±0.51	6.70±0.06	7.00 - 8.50

EC=Electrical conductivity, TDS=Total Dissolved Solids, DO=Dissolved Oxygen

Table 2: Descriptive Statistics of Heavy Metals and Hydrocarbons in Water (mg/L) and Sediments (mg/Kg) of the Bolo Creek

Heavy metals & Hydrocarbons	Concentrations			
	Minimum	Maximum	Mean	S.E
Water				
Zn	0.002	0.038	0.022	0.0039
Cd	0.000	0.004	0.002	0.0005
Cr	0.000	0.002	0.001	0.0002

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Pb	0.000	0.001	0.0005	0.0002
Mn	0.000	0.002	0.0008	0.0003
TPHs	11.19	45.210	32.975	3.932
ΣPAHs	2.590	9.430	6.350	0.808
Sediments				
Zn	5.180	24.810	17.880	2.261
Cd	0.000	2.070	1.488	0.259
Cr	0.000	2.310	1.818	0.265
Pb	0.000	5.930	3.603	0.662
Mn	0.000	0.051	0.0275	0.006
TPHs	6.370	79.950	55.133	8.584
ΣPAHs	1.590	37.830	22.749	3.970

TPH=total petroleum hydrocarbons, PAHs=total polynuclear aromatic hydrocarbons, SE=standard error

mg/kg, PAHs varied from 1.59-37.83 (22.749±3.970) mg/kg, and BTEX (Benzene, toluene, ethylbenzene, and xylene) were not detected at all the sampling locations.

Accumulations of Heavy Metals and Hydrocarbons in Tissues of Organisms

Accumulations of the selected heavy metals and hydrocarbons in the digestive, muscle and Ovary tissue of *C. sapidus* as well as those in the head, internal organ and muscle of *M. Malcolmsonii* varied in the tissues sampled.

Callinectes sapidus

In *C. Sapidus*, mean accumulations of Zn, Cr, Cd, Pd and Mn in the digestive tissue were 5.91±4.60, 0.12±0.09, 0.05±0.004, 0.02±0.02 and 0.03±0.02 µg/g respectively (Fig. 4.1). In the muscles, mean accumulations of the respective heavy metal were 6.10±4.90, 0.06±0.05, 0.01±0.01, 0.01±0.01 and 0.02±0.01 µg/g. However, in the ovary tissues, the mean accumulations were as follows: Zn 1.70±0.70, Cr 0.04± 0.01, Cd 0.01±0.002, Pb 0.01±0.01 and Mn 0.01±0.004 µg/g.

Of the hydrocarbons, mean accumulations of TPHs were 2.07±2.06 µg/g, in the digestive tissues, 1.57±1.56 µg/g in the muscle tissue and 0.60±0.59 µg/g in the ovary tissue of the organism (Fig. 4.2). Accumulations of the PAHs were 0.05±0.04 µg/g in in the digestive tissues, 0.03±0.02 µg/g in the muscle tissue and 0.01 ± 0.01 µg/g in the ovary tissue of the organism. However, accumulations of BTEX were 0.01±0.01 µg/g in the digestive tissues, 0.01±0.01 µg/g in the muscle tissue and 0.01±0.004 µg/g in the ovary tissue of *C. Sapidus.*

The ANOVA test revealed that mean accumulations of the heavy metals and hydrocarbons did not differ significantly in the tissues of *C. Sapidus* sampled at $p < 0.05$.

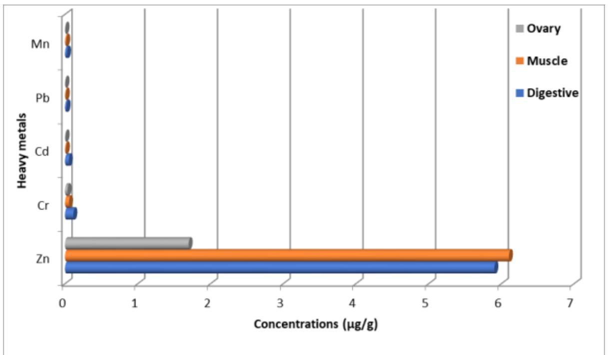

Fig. 1. Mean accumulations of heavy metals in tissues of *C. callinectes* from the Bolo Creek, Rivers State

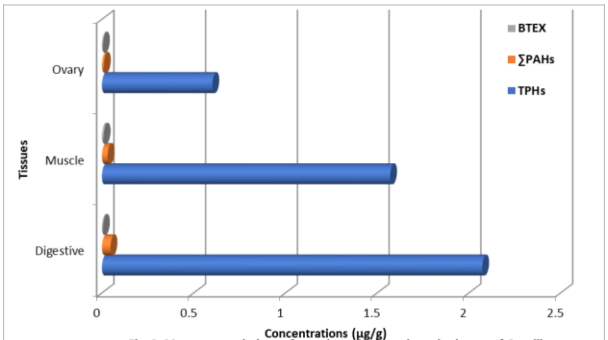

Fig. 2. Mean accumulations of petroleum hydrocarbons in tissues of *C. callinectes* from the Bolo Creek, Rivers State

Discussion

In this study, physicochemical properties of the water samples revealed variations in parameters assayed. The mean temperature of water samples in the current study fell within the World Health Organization (WHO, 2016) acceptable range for aquatic life and household activities, including drinking purposes. [9] reported that the temperature of the Turag River ranged from 30.23°C to 31.52°C from January to March, which agrees with the findings of this study. Temperature variation in the Turag River may not act as a

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limiting factor for the survival of aquatic populations and the biotic community [10].

pH indicates the intensity of the acidic or basic character of a solution and is controlled by the dissolved chemical compounds and biochemical processes in the solution. It is an important indicator of water quality and pollution level in the aquatic environment. In the present study, the pH values across the sampling stations were slightly acidic. According to [11] pond water released into the environment at this pH is not likely to pose any harm to the environment. These values are therefore within the permissible limit of 6.5 – 8.5 by WHO for drinking and irrigation purposes, respectively. Similar observation had been reported by [12]

Electrical conductivity reflects the ability of water to conduct electricity. It is due to substances dissolved in the water which breaks down into positively and negatively charged ions and is influenced by the presence of dissolved salts, minerals and contaminates. The EC values at the sampling locations were significantly higher at the impacted locations than the reference location. Similar trend had been observed by [13] In related to this study, [14] observed high and low values of conductivity in impacted locations than reference location.

Total Dissolved Solids of water is the amount of dissolved inorganic salts and organic matter present in water. Water containing more than 500 mg/L of TDS is not considered as a potential source of pollution. Water with high TDS is undesirable or harmful for human and aquatic life. It may taste bitter, salty, or metallic and may have unpleasant odours. High TDS water is also less thirst-quenching and interferes with the taste of [14]. The present study showed that mean TDS values varied in the three sampling sites with impacted environment having slightly higher values than the reference site. These values were within the permissible limits of WHO. This corroborates the finding of [15] in their study on bioaccumulation of heavy metals and petroleum hydrocarbons in the blue crab. The elevated levels of TDS recorded in the water might be due to agricultural runoff, discharge of wastes from the town, and other human activities like washing of different vehicle at and around the study areas.

Dissolved oxygen plays a role of regulator of metabolic activities of organisms and thus governs metabolism of the biological community as a whole and used as an indicator of tropic status of the water. Thus, in the present study, there was no significant difference in the DO from the three study locations. DO recorded in this study was within WHO permissible limits of >5 mg/L. These results are similar to the reports of [15] in their study on a comparative study of gut helminths of *Tilapia Zilli* and Clarias gariepinus from river Uke, Plateau State, Nigeria.

Higher salinities were recorded in impacted locations than the reference location. However, the mean values for salinity were higher than the WHO benchmark of 150.00. Salinities observed in this study fell within the range of values reported by [16].

The study also established the accumulations of heavy metals and hydrocarbon accumulation in the tissues of crab and prawn samples from Bolo Creek. Traces of heavy metals were observed in tissue of both crab and prawn. Crabs are often found at the riverbanks, and sometimes, burrow sediments close to the river and may probably feed on the contaminated sediments. The contamination could also be attributed to the anthropogenic activities of the local fishermen, such as oil spillage, gas flares, industrial discharges, agricultural runoff and home effluents.

Zn and Total Petroleum Hydrocarbons showed the greatest tendency to bioaccumulate in the crab and prawn tissues sampled. [2] had also observed that Zn showed great bioaccumulation tendencies in crab tissues sampled from River Niger at Agenebode, Delta State, Nigeria. The observed significant difference in tissue accumulations of most of the pollutants between the Impacted and Reference locations further reflects elevated levels of pollutants in water and sediments enmeshing the organisms. This result is similar to findings by [3] on the determination of physico-chemical parameters and heavy metals in water samples from Ita Oghobohi Area of Ondo State, Nigeria. High concentration of Zn is needed for optimum functioning of certain metabolic activities in the body. Hence, the deficiency its deficiency could lead to retarded growth, loss of appetite and skin changes, amongst others [5]. However, large dosage of Zn could affect the nervous system causing headache, irritability, epigastria pain, diarrhoea, nausea and vomiting [2]. Prawn tissues exhibited a higher mean accumulation potential than crab tissues. This suggests that prawns may be more susceptible to bioaccumulation or may inhabit areas with higher pollutant exposure [9]. Such differences could be attributed to species-specific feeding habits, habitat preferences, and metabolic rates.

The following recommendations are made:

1. Government should enforce the laws that ensure standard waste treatment by industrial plants before they are discharged into water bodies.
2. There should be a constant monitoring of the physicochemical parameters in future because of the increase in anthropogenic activities around the study area.
3. More studies on heavy metal metabolism rates in organs of crabs and prawn should be conducted.

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*Corresponding Author: **Orji-Kelechi, C. L**
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