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Bioaccumulation of heavy metals and petroleum hydrocarbons in the blue crab (*Callinectes sapidus*, Rathbun 1896) from Ogu Creek in Upper Bonny Estuary, Nigeria

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Abstract

Prolonged industrial and artisanal discharge of petroleum refinery effluents into the Ogu Creek at the Upper Bonny estuary of the Niger Delta could pose a great threat to the aquatic biotopes. This research investigated bioaccumulation of selected heavy metals and petroleum hydrocarbons in the blue crab (*Callinectes sapidus*) from the creek. Crabs (N=20; average length 18±20cm; weight 150.18±0.02g) were collected with nets. Mean concentrations (mg/l) of Zn (0.044±0.01), Cd (0.002±0.00), Cr (0.001±0.00), Pb (0.001±0.00), Fe (5.82±0.29), Mn (0.001±0.00), total petroleum hydrocarbons (TPH; 40.23±2.83) and total polynuclear aromatic hydrocarbons (ΣPAHs;7.60±1.29) were lower in water than sediments (22.10±1.37, 1.98±0.04, 2.13±0.09, 4.80±0.57, 24.03±1.29, 0.04±0.01, 70.93±3.10 and 29.80±4.02mg/kg, respectively) at the impacted location. Accumulations (µg/g) in digestive tract (dtr), muscle (mus) and ovaries (ov) were as follows: Zn 10.50 (dtr), 11.00 (mus), 2.40 (ov); Cr 0.21 (dtr), 0.10 (mus), 0.04 (ov); Cd 0.01 (dtr), 0.02 (mus), 0.01 (ov); Pb 0.04 (dtr), 0.02 (mus), 0.01 (ov); Mn 0.05 (dtr), 0.03 (mus), 0.01 (ov); TPH 4.14 (dtr), 3.14 (mus), 1.20 (ov); and mononuclear aromatic hydrocarbons (MAHs=BTEX) 0.01µg/g (dtr), 0.02 µg/g (mus), 0.01 µg/g (ov). Accumulations of TPH and BTEX were significantly ($p<0.05$) higher at the impacted location ($p = 0.03$ and 0.02, respectively) than reference locations. High TQ/HQ values of 1.59, 2.82 and 1.90 were recorded for Zn, TPHs and PAHs, respectively. The results raise environmental health concerns among consumers of the aquatic food in the area.

Introduction

A creek is a shallow expanse of water with restricted circulation in a micro tidal environment (Daka *et al* 2007). Creeks are of economic importance in aquatic ecosystems, as they are used for various human activities such as fishing and domestic purposes. This aquatic resource of multiple usage could receive input of domestic and industrial wastewaters, petroleum hydrocarbons, and emissions from automobile exhausts (Orhibabor and Ogbeibu 2009), thus leading to environmental pollution problems, including bioaccumulation in aquatic biotopes.

The Ogu Creek, located in the Upper Bonny Estuary of the Niger Delta is a major habitat for aquatic organisms including fish, crabs, periwinkles, crayfish, and prawns that are consumed by inhabitants around and beyond the area. These aquatic foods are mostly consumed as local diet due to their high protein, low saturated fat and omega fatty acid contents that are known to contribute to good health (Kennedy *et al* 2009). The Port Harcourt Refining Company Ltd (PHRC), sited on the Okrika Mainland discharges petroleum effluent into the Ekerekana Creek, and the effluent spreads into the proximal Ogu and Ogan creeks. Furthermore, an increasing population of inhabitants who are attracted by the presence of this petroleum hydrocarbon industry, especially of the artisanal refiners contribute more domestic and petroleum effluents to the effluent stream discharges. Consequently, more aquatic foods are enmeshed in possible toxic pollutants, which could threaten their fecundity and survival in the ecosystem.

Ogbuagu *et al* (2019) observed that the long-term discharge of poorly treated petroleum refinery effluent into the point-source Ekerekana Creek, proximal to the Ogu Creek, as well as contributions from artisanal refining in the area have already created worries about safety to the health of consumers of two catfish, *Clarias gariepinus* and *Heterobranchus longifilis* from this coastal water. Their research investigated the presence and levels of some heavy metals/trace elements, petroleum hydrocarbons (TPHs,

BTEX and PAHs) and phenol in tissues of the fishes, and observed significant accumulations of Cd, Pb, Mg, Ca, K, Na, Fe and Mn in *C. gariepinus* and Zn, Cd, Pb, Mg, Ca, K, Na, Fe and Mn in *H. longifilis*. High toxicity/hazard quotients that exceeded regulatory limits of the World Health Organization (WHO), United Nations Environmental Programme (UNEP), and the Food and Agricultural Organization (FAO) were recorded for Cd in both fish species and Zn in *H. longifilis*.

However, whether these trace elements and hydrocarbons are also bioavailable in tissues and organs of the commonly consumed crab delicacy or not is largely unconfirmed, as there is paucity of research and data on their bioaccumulation status in the creeks. This work therefore attempted to close the gap in knowledge, by investigating the presence of some toxic and persistent pollutants in the tissues of *Callinectes sapidus* sourced from the Ogu creek.

Materials and methods

Study area

The Ogu Bolo Local Government Area (LGA) is a predominantly low-lying pluvial location in the eastern flank of the Niger Delta, on the ocean-ward extension of the Benue Trough. The typical Niger Delta environment features many mangrove swamps and rainfall is generally seasonal, variable, heavy, and occurs between March and November; with a short dry season covering the rest of the year (Osuji and Opiah 2007). Rainfall amounts of up to 2400-2600mm are common, average temperatures are typically between 25 and 28°C, and relative humidity rarely dips below 80% and fluctuates between 90% and 100% for most of the year. Oil exploration and production operations have been ongoing for over 30 years in the area, and inhabitants are well known for fishing, farming, petty trading, artisanal labour, and in few cases civil service.

The Ogu creek in Ogu Bolo LGA; one of the creeks close to the refinery effluent-laden Ekerekana creek in Okrika Mainland is also a popular location with enormous abundance of marine organisms for economic fishing activities. It is also a source of transportation, tourism, and other industrial activities to neighbouring communities. The creek is impacted by petroleum hydrocarbon pollution spread mainly from the nearby Ekerekana Creek, which receives direct effluent discharges from the Port Harcourt Refining Company (PHRC), and from many artisanal refineries by local operators.

Three locations designated as IMP1, IMP2 and IMP3 nearest to the mouth of the creek were within the PHRC effluent-contaminated/impacted vicinity. A reference point (sited about 1km away from the mouth of the creek) designated Ref. was also studied for comparison. Table 1 shows the Geographical Positioning Coordinates (GPS) of the sampling points in the creek while Figures 1 and 2 show map of the sampled impacted and reference sites.

Table 1. GPS Coordinates of the sampling locations in Ogu Creek

Sampling points	Coordinates	Description
IMP 1	07°07'27.336''E; 04°40'45.401''N	Near point-source of pollution
IMP 2	07°06'55.822''E; 04°40'43.456''N	Near point-source of pollution
IMP 3	07°06'6.161''E; 04°40'48.282''N	Near point-source of pollution
Ref.	07°08'20.505''E; 04°39'50.582''N	1km away from IMP 1

In situ determination of 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 metre at each sampling location.

Water samples collected at 5cm depth in 250mL glass bottles at each location for the determination of hydrocarbon contents were fixed with concentrated H_2SO_4, while those for the determination of heavy metals were fixed with concentrated HNO_3, 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.

Twenty adult female blue crabs- *Callinectes sapidus* (average length 18±20cm, average weight 150.18±0.02g) were collected with nets from the locations around the creek. Samples of approximately uniform sizes were collected in order to minimize possible error due to size differences. Samples were labelled, wrapped with aluminium foil and transported to the laboratory on the same day for confirmatory identification in the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Owerri, Nigeria.

Laboratory analysis

Analysis of petroleum hydrocarbons in sediment and water samples were analysed using standard methods of APHA (2000) and Anaero-Nweke *et al* (2018). In sediments, sample extraction procedure involved weighing out 5g 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, 50ml of the sample was measured into 1l separating funnel, a drop of concentrated H_2SO_4 was added to the sample in the separating funnel to release the hydrocarbon components and exactly 5ml of analytical grade N-hexane (as solvent) was subsequently added. Samples were vigorously shaken for 5 minutes and allowed to stand for another 20 minutes. 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

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

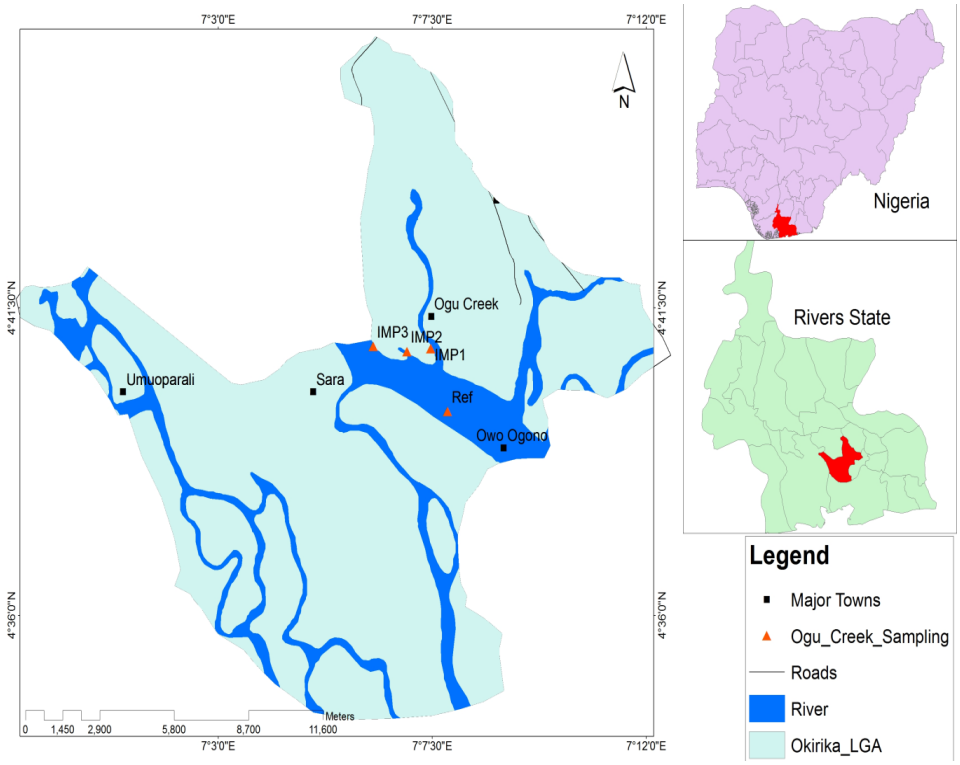

Figure 2. The sampling points [designated as IMP1-IMP3 (Impacted) and Reference (Reference/Control)] in Ogu Creek, Ogu-Bolo Local Government Area of Rivers State, Nigeria

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 GC analysis. The GC was calibrated using commercially prepared external standards having 16 components of PAHs with concentration of 1000ppm per component. The GC parameters used included helium as carrier gas, air and hydrogen as fuel gases, nitrogen as back-up gas, detector temperature of 35ºC, in-let temperature of 25ºC, initial oven temperature of 5ºC, final oven temperature of 300ºC, hydrogen flow rate of 30ml/mins., air flow rate of 300ml/mins., nitrogen flow rate of 30ml/mins., and helium flow rate of 30ml/mins. The GC parameters were set and a PAH extract loaded using a micro-syringe to prompt the GC interphased with Flame Ionization Detector (GC-FID) to run for about 41 minutes.

Analysis of hydrocarbons in organism

The method of Abdallah (2017) was employed for the determination of hydrocarbons in animal tissues. The tissue samples were homogenized with $NaSO_4$ for 2-3 minutes for adequate dryness. The mixture was transferred to a pre-cleaned extraction thimble inside, which the dehydrated tissue was extracted with 200ml of *n*-hexane-dichloromethane in the ratio of 1:1 for 8 hours in a Soxhlet apparatus cycling 5-6 times/hr. Anhydrous $NaSO_4$ was also extracted the same way as the sample and used as blank. The extracted solvents were concentrated with a rotary evaporator down to 2ml at a maximum temperature of 35ºC, and then further concentrated with a pure nitrogen gas stream down to 2ml. Clean-up and fractionation were conducted by passing the extract through a silica/alumina column. The first millilitre volume of the extract was passed through slurry packing of 20ml (10g) silica, 10ml (10g) of aluminium, and then 1g of anhydrous $NaSO_4$. Elution was made with 40ml of hexane (for the aliphatic fractions, F1), followed by 40ml hexane/dichloromethane in the ratio 90:10, and then by 20ml hexane/dichloromethane in the ratio 50:50 (for unsaturated and aromatic hydrocarbon fractions, F2). Eluted samples were then concentrated under a gentle stream of purified nitrogen to about 0.2mL, before injection into a Gas Chromatograph interfaced with Flame Ionization Detector (GC-FID).

Analysis of Heavy metal contents in Water and Sediments

Exactly 250ml of water sample was filtered and digested with 10ml 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 2g of sediment sample to 5ml of acid. The mixture was gently heated in a water bath at a temperature of 150ºC 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 analysed for heavy metals (Zn, Cd, Cr, Pb and Fe) with the Perkin Elmer Atomic Absorption Spectrophotometer (AAS) (Analyst 2000 Version 6.0).

Analysis of heavy metals in organism

The method of Wangboje and Ikhuabe (2015) was employed for the determination of heavy metals in animal tissues. The crab samples were dissected and their muscle, digestive and ovary tissues harvested. Tissues were oven-dried at 70ºC for 48hrs, milled separately with a porcelain mortar and pestle, and kept in foils. Two grams, each of the tissue samples was weighed into 250ml conical flask, into which 5ml of $HClO_4$ and 15ml HNO_3 were added. The mixture was heated until a clear solution was formed and 5ml of 20% HCl added. The mixture was filtered into a 100ml 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 100ml plastic reagent bottle for subsequent AAS analysis. Standard solutions of each sample of the metals were prepared according to the manufacturer's procedure.

Statistical analysis

Data were analysed with the SPSS© V.22.0 software. Descriptive statistics was used to compute means and standard errors of the data set. The test of homogeneity in mean variance of accumulations in tissues was conducted with the One-Way Analysis of Variance (ANOVA) at the 95% confidence interval. Variation plots were used to represent accumulation of the persistent pollutants in the tissues. Toxicity/Hazard Quotient (TQ/HQ) was computed as follows:

$$\text{TQ/HQ} = \frac{\text{Concentration of pollutant}}{\text{Health based criteria}}$$ (Newstead *et al* 2002)

Results

Physicochemical parameters in water and sediments

The results of physicochemical parameters were surface water temperature (31.20-31.42°C), pH (6.00-6.29), electrical conductivity (2988.55-4912.00µS/cm), salinity (498.09-818.67‰), TDS (1494.27-2456.00mg/l) and DO (4.26-4.68mg/l) at the impacted location (Table 2). In sediments, pH ranged from 6.50 to 6.60 at the impacted locations, and was 6.70 at the reference location. In water column, mean concentrations of Zn, Cd, Cr, Pb, Fe and Mn were 0.03±0.01, 0.002±0.00, 0.001±0.00, 0.001±0.00, 5.82±0.29 and 0.001±0.00mg/l, respectively while those for TPH and ΣPAHs were 40.23±2.83 and 7.60±1.29mg/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±0.01, 70.93±3.10 and 29.80±4.02 mg/kg, respectively.

Accumulation of pollutants in tissues

At the impacted location, the accumulation of Zn was 10.50µg/g in the digestive tract, 11.00µg/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 some physicochemical properties of water and sediments of the Ogu Creek in the Niger Delta

	Sampling locations			
	IMP 1	IMP 2	IMP 3	Ref
			Water	
Temperature (ºC)	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 ($^0/_{00}$)	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
			Sediment	
pH	6.60	6.50	6.50	6.70

Table 3. Concentrations of Heavy metals and Hydrocarbons in Water (mg/L) and sediments (mg/kg) of the Ogu Creek in the Niger Delta

Locations	Zn	Cd	Cr	Pb	Fe	Mn	TPH	∑PAHs
				Water				
IMP 1	0.037	0.004	0.002	0.001	5.25	0.001	45.20	8.30
IMP 2	0.020	0.002	0.001	ND	6.00	ND	40.10	5.10
IMP 3	0.030	0.001	0.001	0.001	6.20	0.002	35.40	9.40
Mean	0.029	0.002	0.001	0.001	5.817	0.001	40.23	7.60
Ref	0.002	ND	ND	ND	2.20	ND	11.20	2.60
				Sediment				
IMP 1	24.80	2.05	2.30	5.90	21.60	0.05	76.00	37.80
IMP 2	21.10	2.00	2.10	4.50	26.00	0.02	71.50	26.50
IMP 3	20.40	1.90	2.00	4.00	24.50	0.04	65.30	25.10
Mean	22.10	1.98	2.13	4.80	24.03	0.04	70.93	29.80
Ref	5.22	ND	ND	ND	5.80	ND	6.40	1.60

TPH=total petroleum hydrocarbons, ∑PAHs=total polynuclear aromatic hydrocarbons, ND=Not detectable

Table 4. Concentrations of heavy metals and hydrocarbons in tissues of the blue crab (*Callinectes sapidus*) from the Ogu Creek, Rivers State

	Impacted locations			Reference location		
Conc.(µg/g)	Digestive	Muscle	Ovary	Digestive	Muscle	Ovary
Zn	10.50	11.00	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	0.001
ΣPAHs	0.09	0.06	0.02	0.004	0.001	0.001
BTEX	0.01	0.02	0.01	0.001	ND	0.001

TPH=Total Petroleum Hydrocarbons, PAH=Polycyclic Aromatic Hydrocarbons, BTEX=Benzene, ethylbenzene, toluene and xylene, Poll=Pollutant, ND=Not detectable

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. Polynuclear 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).

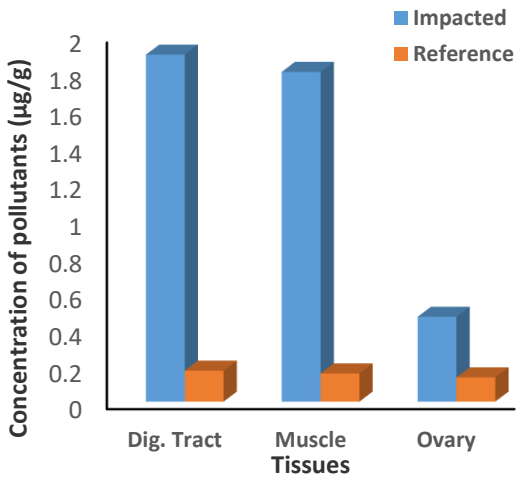

Figure 3. Mean accumulation of the combined pollutants in tissues of the blue crab, *Calinectes sapidus* from the Ogu Creek, Rivers State

The ANOVA test revealed that the accumulations of TPH and BTEX differed significantly in tissues of crab between the Impacted and Reference locations (p =0.03 and 0.02, respectively).

Toxicity and hazard quotients (TQ and HQ)

At the Impacted location, combined mean accumulations of Zn, Cr, Cd, Pb, & Mn in all the tissues were 7.97±2.79, 0.12±0.05, 0.05±0.03, 0.02±0.01, and 0.03±0.01µg/g respectively, and those of TPHs, PAHs, and BTEX were 2.82±0.86, 0.06±0.02, and 0.01±0.00µg/g, respectively (Table 5). Consequently, the mean accumulations of Zn, TPH and PAHs exceeded the Maximum Permissible Limits of 5.0, 0.001 and 0.03µg/g, respectively by various regulatory agencies. The highest TQ/HQ value of 2.82 was recorded for TPH, while the least value of 0.12 was recorded for Pb and Cr.

Table 5. Mean accumulation, maximum permissible limits and toxicity/hazard quotients of some persistent pollutants in *Callinectes sapidus*

Pollutant	Pb	Cd	Zn	Cr	TPH	PAHs
Conc. (µg/g)	0.02	0.05	7.97	0.12	2.82	0.06
MPL (µg/g)	0.20[a]	0.05[a]	5.00[a]	1.00[a]	0.001[b]	0.03[c]
TQ/HQ	0.12	0.98	1.59	0.12	2.82	1.90

[a]=WHO/UNEP/FAO (UNEP 1986); [b]=WHO (Clinton *et al* 2009); [c]=EU (2014), MPL=Maximum Permissible Limit

Discussion

Results from this work which investigated bioaccumulation of selected heavy metals and petroleum hydrocarbons in the blue crab (*C. sapidus*) from the Ogu Creek reflects the fact that water bodies are the ultimate repository of wastes generated by industrial and artisanal activities. Allocthonous inputs of the pollutants from these sources proximal to the creek obviously accounts for their elevated levels in the tissues of the detritus organism. Research findings from several authors also implicated similar industrial, domestic, and municipal sources of pollutants in water bodies (Ikem *et al* 2003; Olowu *et al* 2010; Alam *et al* 2012; Oladele and Jenyo-Oni 2015).

The observed significantly higher pollutants concentrations recorded in tissues of crab from the Impacted than Reference locations clearly indicate that the effluents from the nearby refinery which are discharged directly into the surrounding creeks, and those from artisanal refining activities along the coastlines obviously contained a variety of pollutants, including hydrocarbons and heavy metals. This observation was also made by Wangboje and Ikhuabe (2015). Although many of these metals have been reported to be essential for the growth of organisms at trace levels, they become essentially toxic when their concentrations exceed critical levels. Elevated pollutant concentrations are maintained in both water column and sediments of the creek. The presence and levels of the pollutants in crab tissues sampled from the Reference locations, farther away from the point of discharge of industrial effluents reflects transport by tides, causing mixture of the pollutants further upstream, and from the surrounding lands. Additionally, during rainfalls, runoffs which are usually rich in certain trace metals and other pollutants contributed to pollutant loading in the coastal water.

Zinc and Total Petroleum Hydrocarbons showed the greatest tendency to bioaccumulate in the crab tissues from the creek. Wangboje and Ikhuabe (2015) 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 organism.

The accumulations and toxicity/hazard quotients of Zn and the petroleum hydrocarbons (TPH and PAHs), which are greater than unity are of great health concern, especially to man as a tertiary consumer of the aquatic food. Incidents of endocrine disruption and carcinogenicity from heavy metals and hydrocarbon pollutants have severally been indicated (Brooks *et al* 2004; Giri and Singh, 2014; Anaero-Nweke *et al* 2018). Wangboje and Ikhuabe (2015) had also observed that Zn has a great tendency to bioaccumulate in tissues of fish sampled from the River Niger, while Giri and Singh (2014) had also recorded HQ values above unity for Cr in shrimps from the Subarmarekha River in India. Heavy metals, including Cd, Cu, Fe and Mn had revealed less than unity HQ values in the work of Bandowe *et al* (2014) in fish species from Ghana.

Conclusion

The current study indicated that bioaccumulation of some trace/heavy elements and hydrocarbons occurred in the blue crab-*Callinectes sapidus*. The pattern of accumulation was proximally and tissue-related; with more accumulations occurring closest to the point source of pollution and in the digestive tissues. Petroleum hydrocarbon effluents from the nearby Port-Harcourt Refinery Company (PHRC), as well as those from artisanal refining activities should be properly treated before discharging into the Ekerekana and other nearby creeks in the area.

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