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Petroleum Hydrocarbons Accumulation Potential of Shellfishes from Littoral Waters of the Bight of Bonny, Niger Delta, Nigeria

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Abstract: Total hydrocarbons concentration in four commercially available shellfishes (*Ocypoda africanus*, *Macura reptantia*, *Procambarus clarkii* and *Penaeus notialis*) from coastal waters of the Niger Delta region of Nigeria were investigated between June 2003 and February 2004 using standard methods. Levels of total hydrocarbons varied significantly depending on biota species, feeding habit and season. The seasonal mean concentrations reported were: 3.98±0.69, 7.95±1.18, 2.24±0.39 and 5.85±0.57 $\mu g\ g^{-1}$ dry weight for *M. reptantia*, *O. africanus*, *P. clarkii* and *P. notialis* respectively during the wet season. Enhanced concentrations recorded during the dry season were 7.81±2.32, 11.59±2.63, 6.16±2.12 and 9.69±1.90 $\mu g\ g^{-1}$ dry weight for *M. reptantia*, *O. africanus*, *P. clarkii* and *P. notialis*, respectively. The results obtained indicated seasonal variations which might have been associated with an incidental crude oil spill recorded during the investigation. In general, *O. africanus* showed higher levels of total hydrocarbons than other species investigated. However, biota species demonstrated hydrocarbon bioaccumulation potential. Regression between the monthly concentrations of total hydrocarbons in each of the biospecimens with the levels in the surface water and sediments indicated statistically significant ($p<0.05$) relationships. Moreover, comparison of the seasonal mean concentrations using paired sample t-test at 95% confidence level indicated that the concentrations between the dry and wet seasons were statistically significant.

Key words: Total hydrocarbons, oil spill, marine pollution, Niger Delta, Nigeria

Introduction

In aquatic environments, studies on hydrocarbons contamination have been based on the analyses of organisms, pelagic column and sediments (Kayal and Connell, 1995; Topping *et al*., 1997; Zheng and Richardson, 1994; Muniz *et al*., 2004; Chindah *et al*., 2004). Moreover, sedimentary hydrocarbon studies have received much attention due to enhanced bioaccumulative potential of hydrocarbon components onto colloidal surface of sediments which allows for spatial and temporal distribution of hydrocarbons to be determined relative to their sources (Volkman *et al*., 1992; Law and Biscaya, 1994; Medeiros *et al*., 2005). Although epipellic and benthic sediments act as principal sinks for hydrocarbons in aquatic environments (Bouloubassi *et al*., 2001), it is likely that a route of exposure for sediment dwelling organisms is through ingestion from interstitial water (Roesijadi *et al*., 1978; Law and Klungsøyr, 2000) and pelagic dwellers such as fishes also appear to absorb most hydrocarbons direct from the water phase (NRCC, 1983; Meador *et al*., 1995).

Contamination of aquatic ecosystems by natural and anthropogenic hydrocarbon inputs is attributable to industrial and maritime activities. Coastal ecosystems are episodically exposed to this challenge owing to their proximity to the sources of pollution. Organic chemicals such as hydrocarbons

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are major constituents of petroleum (Tissot and Welte, 1978; Philp, 1985) and can enter marine environments through oil spills, riverine discharges, off-shore oil production and transportation, land-derived organic matter, sewage disposal as well as biomass burning (Wakeman, 1996; Azevedo *et al*., 2002; Medeiros *et al*., 2005). In general, hydrocarbons are commonly found in the environment as complex mixtures derived from multiple sources (Medeiros *et al*., 2005).

Although certain hydrocarbons could be produced biogenically from bacteria and chemical degradation of naturally occurring lipids, petrogenic sources constitute the major hydrocarbon inputs to littoral water pollution cases (Law and Klungsøyr, 2000; Medeiros *et al*., 2005). Hydrocarbons are biopersistent, bioaccumulative and can cause deleterious effects to aquatic fauna and flora as well as to humans (Jones *et al*., 1998; Klerks *et al*., 2004; Chindah *et al*., 2004).

Pollution of the littoral waters of the Niger Delta region of Nigeria has in recent times received much attention because of the high degree of environmental degradation and aquatic perturbations posed by petroleum exploration activities in the oil bearing states. The incidence of accidental oil spillage has been very rampant especially in the oil producing communities in Akwa Ibom, Rivers and Delta States, all located within the Niger Delta region. However, the potential environmental impacts due to the contamination of aquatic ecosystems as well as the possible adverse effects on fishes, mussels, crabs and wild life have received less study. Petroleum hydrocarbons from oil spills and human mediated activities are usually incorporated into sediments where they can persist for years (O' Clair *et al*., 1996; Moles and Norcoss, 1998).

The effects of these hydrocarbon-laden sediments on aquatic organisms of the coastal waters of Niger Delta have received less attention and literature on the levels of hydrocarbons in biota from these water bodies is scanty. The majority of studies to date in the coastal waters of Akwa Ibom State in the Niger Delta region of Nigeria are focused on water and sediment quality of the rivers, estuaries and creeks, viz-a-viz their heavy metal load (Ntekim *et al*., 1992; Udosen, 2001; Udosen and Benson, 2006). Essien and Antai (2005) have reported on the deleterious effects of crude oil spill on the beach micro algae of the Qua Iboe Estuarine ecosystem.

This study therefore focused on elucidating the present statuses of total hydrocarbons in some economically and commercially available organisms from the littoral waters of Akwa Ibom State. This is the first time the total hydrocarbon data have been collected, quantified and evaluated in seafood from the coastal waters of the region. The principal aim is to provide information on seafood that readily bioaccumulate hydrocarbons in the crude oil impacted Niger Delta ecosystem.

Materials and Methods

Location of Study Area

The Bight of Bonny is situated on the Southern Atlantic coast of Nigeria (4°15′- 4° 35′ N; 7°30′- 8°25′ E) (Fig. 1). This coastal aquatic system is about 100 km long and more than 30 km wide and constitutes the eastern flank of the Niger Delta (Ntekim *et al*., 1992), spanning the shorelines of Akwa Ibom, Cross River and Rivers States of Nigeria. The entire ecosystem is made up of extensive network of rivers, estuaries, creeks and tributaries which together form a significant commercial hydrographic feature within the region. The estuaries with the major creeks constitute a rich assemblage of fluvial ecohydrological biotopes, dominated predominantly by intertidal mangrove and nipa forested wetlands. The study area is exposed to significant anthropogenic inputs such as oil spills, untreated sewage and industrial waste waters. Several other smaller fluvial and domestic effluents are also disposed in the shallow marginal estuarine reaches and creeks.

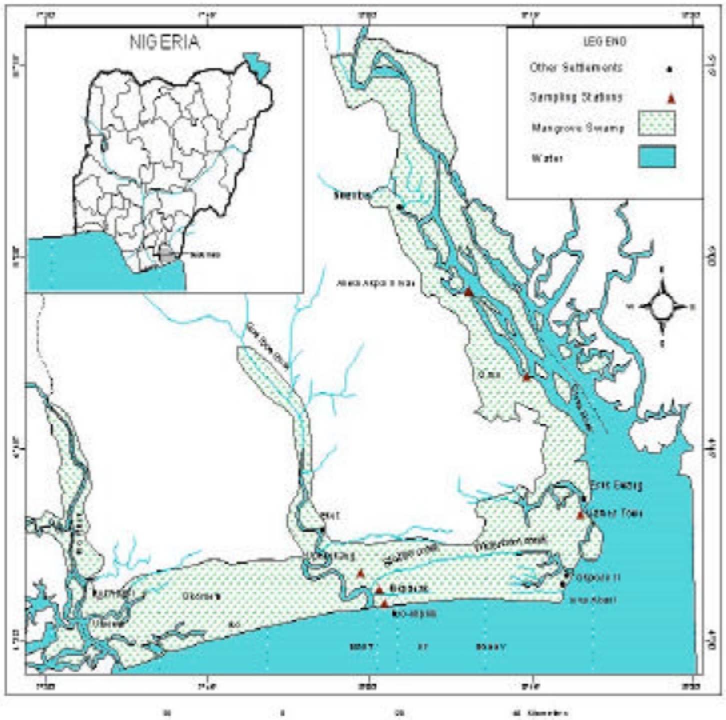

Fig. 1: Littoral aquatic ecosystems of the southeastern coast of Nigeria showing the sampling locations. Insert: The map of Nigeria showing the study area

Choice of Biospecimens and Sampling Strategy

In order to determine the total hydrocarbon levels in commercially available seafood harvested from the area, 72 of freshly harvested biospecimen samples of *Ocypoda africanus, Macura reptantia, Procambarus clarkii* and *Penaeus notialis* were collected monthly from local fishermen between June and September 2003 (wet season), and between November 2003 and February 2004 (dry season). The harvested samples were wrapped in aluminum foil, labeled and stored in ice-packed coolers and transferred immediately to the laboratory for pretreatment and analyses. These biological samples are important commercially available seafood and are commonly consumed within the Niger Delta region and beyond.

Sample Extraction and Analyses

The analytical procedure outlined below is based on FEPA's Method (FEPA, 2001). In the laboratory, about 10 g of each dried and ground biospecimens spiked with squalene and C32-alkane were serially extracted with methyl isobutyl ketone (Analar grade). The solvent was allowed to settle and later centrifuged and decanted. The extracts were concentrated on a rotary evaporator maintained at 20°C to a volume of about 5 mL. A sample volume of 1 μL of each extract was subjected to a GC-MS analysis using Hewlett Packard Model 5890 GC coupled with a DB-5 high resolution capillary column (25 m×0.25 mm, 0.25 μm film thickness) directly interfaced to a quadruple MS Hewlett Packard model 5970, with nitrogen as carrier gas. The GC conditions were: Injection port temperature 250°C; carrier gas flow rate 30 mL min^{-1}. The oven temperature was programmed from an initial temperature of 60°C (2 min hold) to 300°C and isothermal at 300°C for 15 min. Detector port temperature was 320°C. Concentrations of total hydrocarbons were quantified relative to the total peaks as these were converted to weights using hydrocarbon standard calibration. Blank analyses were carried out and all values have been corrected for these concentrations.

Statistical Analyses

Comparative and continuous summary descriptives of data were performed using Analyze-It +1.73© version, with level of significance maintained at 95% for each test.

Results

Table 1 reports the summary descriptives of total hydrocarbon levels found in shellfishes obtained during the wet and dry seasons. The data in Table 2 show the 95% confidence level paired samples t-test for each biological specimen. Results of TPH concentration in the shellfishes during the wet and dry seasons are presented in Fig. 2 and 3. The results revealed that in crab-*Ocypoda africanus*

Table 1: Continuous summary descriptives of total hydrocarbon levels in seafood. Concentrations in $\mu g\ g^{-1}$ dry weight

	Wet season				Dry season			
Parameter	*M. reptantia*	*O. africanus*	*P. clarkii*	*P. notialis*	*M. reptantia*	*O. africanus*	*P. clarkii*	*P. notialis*
Mean	3.98	7.95	2.24	5.85	7.81	11.59	6.16	9.69
95% CI of Mean	3.54-4.41	7.20-8.69	1.99-2.49	5.49-6.21	6.34-9.28	9.92-13.26	4.81-7.51	8.48-10.90
Variance	0.47	1.39	0.16	0.32	5.36	6.93	4.51	3.60
SD	0.69	1.18	0.39	0.57	2.32	2.63	2.12	1.90
SE	0.19	0.34	0.11	0.16	0.67	0.76	0.61	0.55
CV (%)	17	15	18	10	30	23	34	20
Range	2.10	4.02	1.11	1.97	7.87	7.06	6.00	5.60
IQR	0.95	1.23	0.65	0.75	3.38	3.18	3.15	1.92
25th percentile	3.46	7.18	1.95	5.49	6.64	10.64	4.76	9.18
50th percentile	3.86	7.83	2.32	5.91	7.65	12.21	6.99	10.21
75th percentile	4.41	8.41	2.60	6.24	10.02	13.82	7.91	11.09
Shapiro Wilk coeff.	0.944	0.934	0.917	0.965	0.975	0.818	0.878	0.854
Shapiro Wilk prob.	0.551	0.421	0.262	0.854	0.956	0.015	0.082	0.042
Skewness	0.425	0.832	-0.345	0.089	-0.317	-1.016	-0.565	-0.963
Kurtosis	-0.876	0.593	-1.403	0.121	-0.403	-0.412	-1.122	-0.381

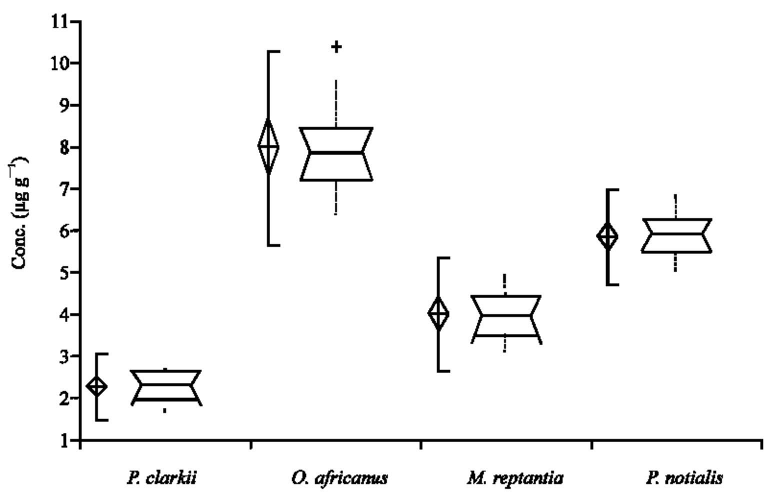

Fig. 2: Total hydrocarbon levels in biospecimen samples from littoral ecosystems of Niger Delta during the wet season

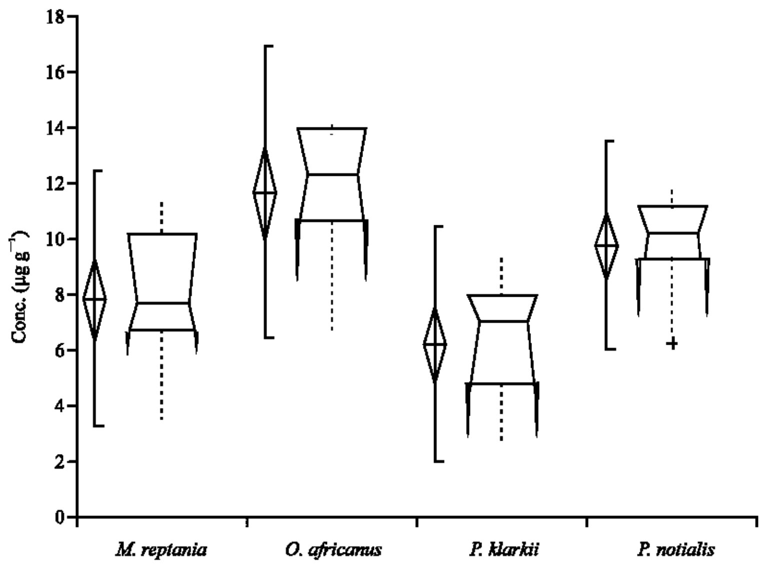

Fig. 3: Total hydrocarbon levels in biospecimen samples from littoral ecosystems of Niger Delta during the dry season

the levels during the wet season months ranged between 6.40 and 10.42 µg g^{-1} dry weight, while a range of 6.94-14.00 µg g^{-1} dry weight was recorded during the dry season. The mean seasonal concentration of total hydrocarbons in *O. africanus* was relatively higher during the dry season with 11.59 µg g^{-1} dry weight (95% confidence limit of 9.92-13.26), while 7.95 µg g^{-1} dry weight (95% CI = 7.20-8.69) was recorded for the wet season (Table 1).

The ranges of total petroleum hydrocarbons in shrimp-*Penaeus notialis* obtained from the littoral waters were 4.98-6.95 µg g^{-1} dry weight and 6.28-11.88 µg g^{-1} dry weight during the wet and dry seasons respectively. The mean seasonal levels in *P. notialis* were 5.85 µg g^{-1} (95% CI of mean = 5.49-6.21) and 9.69 µg g^{-1} (95% CI of mean = 8.48-10.90). Moreover, the concentrations of total hydrocarbons in *P. clarkii* were remarkably low, although relatively higher concentrations were recorded during the dry season. The ranges of concentrations found were 1.62-2.73 µg g^{-1} dry weight and 3.00-9.00 µg g^{-1} dry weight, during the wet and dry seasons, respectively. Seasonal mean concentrations in *P. clarkii* were computed as: 2.24 µg g^{-1} (95% CI = 1.99-2.49) for wet season and 6.16 µg g^{-1} dry weight (95% CI = 4.81-7.51) for the dry season.

The ranges of TPH found in *M. reptantia* were 3.05-5.15 µg g^{-1} dry weight and 3.43-11.30 µg g^{-1} dry weight during the wet and dry seasons respectively. The lowest levels of hydrocarbons in the tissues of *M. reptantia* were obtained during the wet season (June 2003-September 2003), while relatively high values were obtained 30 days after the November 22, 2003 crude oil spill. The mean total hydrocarbon concentrations were: 3.98 µg g^{-1} dry weight (95% CI of mean = 3.54-4.41) and 7.81 µg g^{-1} dry weight (95% CI of mean = 6.34-9.28) for the wet and dry seasons, respectively.

However, a comparison of the seasonal mean concentrations using paired sample t-test at 95% confidence level indicated that variation in concentrations between the dry and wet seasons were statistically significant ($p<0.05$) (Table 2). Regression between the monthly concentrations of total hydrocarbons in each of the biospecimens with the levels in the surface

Table 2: Paired sample t-Test between TPH levels in both wet and dry seasons

	M. reptantia	*O. africanus*	*P. clarkii*	*P. notialis*
Mean wet season ($\mu g\ g^{-1}$)	3.976	7.949	2.242	5.854
Mean dry season ($\mu g\ g^{-1}$)	7.810	11.591	6.161	9.690
Difference ($\mu g\ g^{-1}$)	3.834	3.642	3.919	3.836
95% Confidence interval	2.307-5.362	1.949-5.334	2.714-5.124	2.623-5.049
t-Statistics	5.52	4.74	7.16	6.96
2 tailed p-level	0.0002	0.0006	<0.0001	<0.0001

Table 3: Relationship of total hydrocarbons in biota, surface water and sediment samples from the littoral waters of Niger Delta

	Linear relation	r	R^2 (%)
REP(y) vs WAT(x)	y = 0.93x + 3.57	0.72	51.22
REP(y) vs SED(x)	y = 0.02x + 3.81	0.88	76.85
OAF(y) vs WAT(x)	y = 0.82x + 7.72	0.59	35.74
OAF(y) vs SED(x)	y = 0.03x + 7.76	0.80	63.28
PCL(y) vs WAT(x)	y = 0.63x + 2.68	0.49	24.17
PCL(y) vs SED(x)	y = 0.03x + 2.08	0.92	85.29
PEN(y) vs WAT(x)	y = 0.62x + 6.23	0.51	26.20
PEN(y) vs SED(x)	y = 0.03x + 5.76	0.91	82.62

WAT- surface water; SED- sediment; REP- *M. reptantia*; OAF- *O. africanus,* PCL- *P. clarkii*; PEN- *P. notialis*; r-correlation coefficient; R^2- coefficient of determination

water and sediments indicated statistically significant ($p<0.05$) relationships. The correlations between TPH concentrations in surface water, sediments and the biological specimens investigated are given in Table 3.

Discussion

Hydrocarbons are ubiquitous organic toxicants (Raoux *et al*., 1999; Kucuksezgin *et al*., 2006). They show marked resistance to biodegradation and possess distinct hydrophobic properties (Soclo *et al*., 2000; Jack *et al*., 2005). These characteristics make possible the bio-transfer of hydrocarbons in pelagic column into the tissues of aquatic organisms through direct ingestion of hydrocarbon-laden water. Many aquatic indigenous organisms such as shell- and fin-fishes, mussels and oysters which reside in nearshore sediments and some benthic feeders are particularly vulnerable to synergistic and antagonistic effects from hydrocarbons impacted media because of direct contact with the pollutants (Moles and Norcoss, 1998). Therefore, biomonitoring of total hydrocarbons using biota, sediments and surface waters could prove to be a reliable and efficient way of ascertaining the integrity of aquatic ecosystems viz-a-viz hydrocarbon pollution (Chindah *et al*., 2004; Kucuksezgin *et al*., 2006; Ponce-Velez and Botello, 2006).

The concentrations of hydrocarbons in the shellfishes were considerably high and may have wide environmental implications bothering on bioconcentration in their tissues. The relatively higher concentrations of total hydrocarbons in tissues of the biota than in the pelagic column is suggestive of the bioaccumulative potency of hydrocarbons by the investigated biological organisms, which could have been acquired through direct ingestion and feeding (Micheel and Zengel, 1998). Several aquatic organisms such as shellfishes, spine fauna and finfishes have been recognized as excellent bioaccumulators of organic and inorganic pollutants (King and Jonathan, 2003). Biota including bivalves, crabs and shrimps have been identified as standard bioindicators of aquatic pollution owing to their capability to bioaccumulate and bioconcentrate organic pollutants in addition to heavy metals in their target organs at levels higher than background concentrations (Osibanjo *et al*., 1994; Etuk *et al*., 2000).

As observed in this study, *O. africanus* (crab) which feed on contaminant laden sediments accumulated the highest levels of total hydrocarbons in its tissues during both dry and wet seasons. Other organisms also demonstrated excellent hydrocarbon bioaccumulation potential. But in all the cases, the amounts of TPH accumulated during the dry season were comparatively higher than wet season. These observed differences in concentration following seasonal gradient might have been associated with factors such as episodic crude oil spill, feeding, tidal effects and nature of sediments scavenged (Law and Klungsøyr, 2000, Chindah *et al.*, 2004). Moreover, the seasonal concentrations in total hydrocarbons were higher in the dry than wet season. This observation was however, contrary to earlier report by Chindah *et al.* (2004) and might have been influenced by crude oil spillage which was reported in the ecosystem under study on November 22, 2003. Thus, the observed anomalous seasonal total hydrocarbons concentration in the littoral aquatic organisms and the entire ecosystem is a strong evidence of human-mediated sources. However, these organisms (*O. africanus*, *M. reptantia*, *P. clarkii* and *P. notialis*) have demonstrated greater propensity to bioaccumulate hydrocarbons from their environment and could therefore be used as possible bioindicators of hydrocarbon pollution for monitoring crude oil spills which is very rampant in the Niger Delta coastal waters. In addition, the concentration of total hydrocarbons recorded in this study indicated significant levels which when compared with WHO recommended concentration of 0.01 mg g^{-1} (Jack *et al.*, 2005), reveals that the entire littoral water bodies have been polluted by toxic organic compounds. The levels of total hydrocarbons in biota, surface water and sediment in this study indicate that the activities of adjacent developments (oil exploitation, transportation and storage) have considerably altered their quality. Therefore, the monitoring of the ecosystems becomes an issue of great concern in order to acertain the environmental integrity and health of the ecosystems.

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