Anthropogenic PAHs in Sediment-Dwelling Biota from Mangrove Areas of the Calabar River, SE Niger Delta, Nigeria

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) in mangrove sediment-dwelling biota (crabs and molluscs) from the Calabar River, SE Niger Delta of Nigeria were analyzed using GC-MS in order to assess the degree of contamination of the river by anthropogenic activity. The associated sediment samples (where these biota were collected) showed much higher total PAH (TPAH) concentration (16,028.3 ng/g dry weight (dw)) at the upper mangrove area (UMA) than that found toward the river mouth (MR; 1,667.5 ng/g dw). However, the mean TPAH levels were higher in molluscs (16,749.8 ng/g ww) and crabs (29,325.1 ng/g ww) at theMR, and in molluscs (28,580.8 ng/g ww) and crabs (71,782.6 ng/g ww) at the UMA than in the associated sediments, indicating occurrence of bio-accumulation/bio-concentration of PAHs in tissues of these organisms. The results revealed that molluscs are safer to consume than crabs. One way analysis of variance (ANOVA) indicated no significant relationship between lipid content or body size of organisms and contaminant load probably because of non-equilibrium situation: smaller animals accumulated more PAHs than their larger counterparts, suggesting different uptake and elimination rates for these compounds. Biota-sediment accumulation factors (BSAFs) varied among the organisms (4.84-14.98) and were generally lower for highly polluted site (UMA; fresh water area) and higher for area of low anthropogenic pressure (MR- brackish water area). Risk assessment against USEPA standard show the biota to be highly contaminated with carcinogenic PAHs and may pose life-time cancer risk, especially to residents of the riverine/coastal communities who often consume more of these organisms than those living hinterland.

Keywords: concentrations, mangrove, sources, risk assessment and biota

1. Introduction

Coastal zones are accidentally or non-accidentally exposed to pollution from industrial and petroleum related activities. On a global scale, petroleum contamination is relatively well known but on a local scale, there is lack of data for many sites, especially for tropical mangrove ecosystem (Mille, Guiliano, Asia, & Jalaluddin, 2006).

Mangroves are exposed to polycyclic aromatic hydrocarbons (PAHs) contamination from different sources including river water carrying refinery wastes, land based activity associated with urbanization, vehicle exhaust emission, ship/boat traffic, etc. Mangrove ecosystems have been shown by many researchers to represent favourable environment, where accumulation of organic matter including PAHs may occur (e.g. Ke, Yu, Wong, & Tam, 2005; Mille et al., 2006). Mangroves are also nursery and development sites for crabs, molluscs, fishes, birds, reptiles and even mammals (de Souza et al., 2008). The degree of PAH contamination were demonstrated by Ke et al. (2005) to vary significantly among mangrove sediments and even in relatively clean swamps, 'hot spot' of contamination have been identified. Furthermore, recent data concerning levels of PAHs in tropical mangrove areas are not many (Bernard, Budzinski, & Garrigue, 1995; Bernard, Pascaline, & Jeremie, 1996; Munoz et al., 1997; Orge, Porsche, Costa, Lima, & Justino, 2001; Duke & Watkinson, 2002; Zheng, Man, M. Lam, & P. Lam, 2004; ke et al., 2005; Mille et al., 2006; Ekpo, Oyo-Ita, Oros, & Simoneit, 2011).

Nigeria has the largest mangrove forest cover in Africa which ranks fourth in the world after Indonesia, Brazil and Australia. Cross River system (in which Calabar River is one of its tributaries) has about 950 km[2] mangrove

area (largest in Nigeria; Edu, Omokaro, Holzolehner, & Udensi, 2007). In order to extend the data available for tropical mangrove zones, a study of PAHs in sediment-dwelling biota from the Calabar River was undertaken.

Calabar River is recognised as one of the most productive ecosystems whose organic carbon provides energy-rich food source to organisms. In the 1970s, Calabar River was well known for its abundant and cheap aquatic animals. Recently, the relatively high cost of these edible organisms (which is almost out of reach of the poor masses) is attributed to their gradual extinction occasioned by urbanization and enhanced anthropogenic activities.

Physiological factors such as age, sex, body size, lipid content and health status of organisms have been shown to regulate the accumulation of organic pollutants (e.g. Yang et al., 2006; de souza et al., 2008). Bioavailability and nature of chemical contaminants also play significant role in the bioaccumulation of these compounds in biota (Muncaster, Hebert, & Lazar, 1990). In order to establish a relationship between bioaccumulation and observed biological abnormalities in aquatic biota, it is essential to investigate the bioavailability of organic pollutants in the river. As an important local sink for organic contaminants, sediments increase the contaminant exposure for bottom-dwelling biota that indiscriminately ingests sediment while foraging, which may be transferred to higher trophic levels through food chain.

The use of molluscs (clam) and crabs in the present study was due to the fact that these biota provide reliable information about the extent of contamination of the study area, and also because they are the most abundant group of sediment-dwelling macro-fauna in the study area in terms of their numbers and biomass. They are also mostly consumed in the region. Etim and Akpan (1991) demonstrated the ability of edible clam, *Egeria radiata* to accumulate metals in this same river system. Thus, accumulation of PAHs in this organism has never been reported and is here reported for the first time for the river.

Consumption of food represents an important pathway for exposure to contaminants from a variety of sources. Recent studies have shown that exposures to contaminants may pose a public health risk. For example, Maclntosh, Spengler, Ozkaynak, Tsai and Ryan, (1996) have demonstrated that some adult population may be exposed to various contaminants in food at concentrations above those measured by the Council of the National Academy of Sciences (NAS). Environmental Working Groups have also found that children exposure to contaminants could be high enough to cause immediate adverse health effects (Wiles, Davies, & Campbell, 1998). Human health risk assessments have been undertaken worldwide to examine the potential health risk due to exposure to toxic contaminants in different environmental compartments and foodstuff (de Souza et al., 2008; Liu, Zhang, Tao, & He, 2010).

The recent quest by the Nigerian government in order to extensively embark on commercial fishery for domestic consumption and export to reduce over-dependent on imported seafood products as well as the importance of these biota to human health globally, it has become imperative that information concerning the state of PAHs contamination in sediment-dwelling animals be urgently provided. This will give an insight into the impact of PAHs on consumers, particularly the inhabitants of the riverine/coastal communities who are known to consume a lot of the biota. Deposition of PAHs to water bodies and their health implications on humans who consume aquatic organisms have spurred many researchers worldwide to investigate quality assessment of these biota, hence; this present study was undertaken for the first time on crabs and molluscs in the Calabar River.

The study focuses on : (i) determination of the extent of anthropogenic PAH contamination in the study area, (ii) probing the factors that influence the accumulation of these compounds in sediment-dwelling animals, (iii) determining biota-sediment accumulation factors (BSAFs) in order to assess the bio-availabilities of these compounds in the river and (iv) carrying out risk assessment related to the consumption of the biota in order to identify potential health risk.

2. Materials and Methods

2.1 Study Area and Samples

Detail of the study area is as described by Asuquo, (1989). Briefly, as one of the tributaries of Cross River System, Calabar River, which discharges its content into the Atlantic Ocean through the Gulf of Guinea (Figure 1), is located between latitudes $04^0 54'$ and $05^0 50'$ N and longitudes $08^0 00'$ and $08^0 20'$E. It originates in the hilly regions of northern Cross River State of Nigeria and drains through heavily forested landscapes with a catchment area of approximately 1,662 km^2 (CRBDA, 1982) and a discharge of between 879 and 2533m^3/sec (Lowenberg & Kunzel, 1992). The lower reaches of the river are influenced by semi-diurnal ocean tides with a tidal range of 3.0 m with a shallow depth ranging from 1 m to 10 m at flood tide and about 8 m maximum at ebb tide (Asuquo, 1989; Ekpo & Ibok, 1998).

Industrial and agricultural activities along the riverbank contribute high input of trace metals (Ntekim, Ekwere, & Ukpong, 1993; Ekpo & Ibok, 1998) and organic pollutants (Ekpo, Oyo-Ita, & Wehner, 2005; Ekpo & Wehner, 2010) into the river sediments. Calabar has witnessed a surge in economic and industrial activities in the last decade. Striding the river bank are the Free Trade Zone (FTZ), Nigerian Port Authority (NPA) that houses many industrial operations, and the famous Tinapa (a hub of business and commerce). Jetties for shipment of refined petroleum products also dot the riverbank.

The riverine/coastal communities of the area engage in extensive fishing activities using engine boats, and a good number of them cultivate vegetables, pineapple, banana and plantain. As a result, runoff of agricultural waste could significantly contribute to the pollutants load of the river. However, following the recent restriction by the Cross River State government on bush burning and tree logging (being hitherto the common practice by the inhabitants of the hinterland for agriculture and domestic cooking purposes), aimed at conservation of forest/wildlife and promotion of ecotourism, the extent of contamination by biomass burning is expected to be reduced.

Sediment-dwelling samples (crabs and molluscs-clam) were caught from the mangrove area towards the river mouth (MR site; brackish water) and from the upper mangrove area (UMA; fresh water) using woody pike and by hands. The associated surface sediments were simultaneously obtained using stainless steel trowel. These two sites were chosen on the basis of presumed differential in the degree of hydrocarbons contamination. Each biota sample contained at least 20 individuals of different sizes. Two sizes (small- < 2 cm and large - > 2 cm; determined by measuring the weight and length of the animals) were chosen. At UMA site (fresh water), the crab and mollusc species collected for analysis were *Micropipus depurator and Musculin secures*, respectively, whereas the species *Egeria radiata* (Mollusc-clam*)* and *Collinectes amicola* (crab*)* were collected at MR site (brackish water). The biota samples were separated according to their type and left overnight in filtered seawater. This allowed them to empty their guts without losing hydrophobic contaminants. The following morning, the animals were removed and stored at -20°C before further processing.

2.2 Experimental

2.2.1 Materials and Quality Assurance

The analytical grade solvents/chemicals used for this study including dichloromethane (DCM), methanol (MeOH), hexane, acetone and toluene as well as molecular sieves and potassium hydroxide (KOH) were purchased from Merck (Darmstad, Germany). Per-deuterated polycyclic aromatic hydrocarbons used as internal standards and surrogates: [d_2]-benzo(a)anthracene, [d_{10}]-fluoranthene, [d_{12}]-benzo(ghi)perylene, [d_{10}]-pyrene and [d_{12}]-perylene were purchased from Cambridge isotope laboratories (Andoyer, MA, USA) and [d_{10}]-anthracene from Dr. Ehrenstofer GmbH(Augsburg, Germany).

The vessels used for analysis were pre-cleaned with water and detergent, rinsed with ethyl acetate, heated at 300^{0}C overnight and then rinsed again with acetone. One analytical blank was run for every batch of 15-20 samples to check background contamination during the extraction and purification steps. To remove background contamination, PAH concentrations were corrected by subtracting the mean of the analytical blanks.
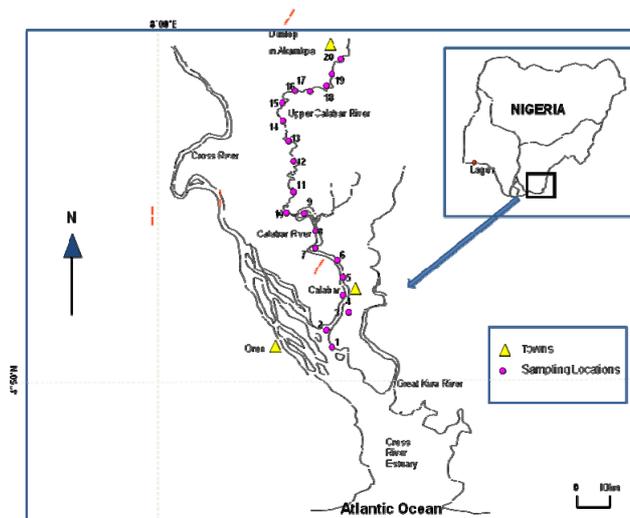

Figure 1. Map of the study area showing sample locations

2.2.2 TOC Determination, Extraction and PAHs Analysis

Prior to extraction protocol, freeze-dried associated sediment samples were decarbonated in 37% hydrochloric acid repeatedly until bubbling stopped and rinsed in deionised water until neutral pH. Flash combustion at 1024°C, followed by thermal conductivity detection in triplicate in a CHNS Elemental Analyser, Carlo Erbar 1108 was used for TOC determination.

Extraction of the biota samples was according to Yan et al. (2006). Each biota sample was defrosted and homogenized wet in a blender. The small crabs were homogenized with their whole bodies, while large crabs and molluscs were homogenized using the inner tissues. 5g of the homogenized wet sample was saponified with 10 ml of 2M KOH overnight and was extracted with acetone: *n*-hexane (1:1, v/v), (3 x 30 ml) for 20 minutes in an ultrasonic bath. The extracts were combined and evaporated *in vacuo* to 5 ml at 40^{0}C. This was further fractionated on a multi-layer silica gel column prepared following the order: anhydrous sodium sulphate (bottom layer, 1 cm), 8 g of silica gel (activated at 450^{0}C for 4 hours, allowed to cool in a desicator) and anhydrous sodium sulphate (top layer, 2 cm) after the column was pre-washed with 15 ml of n-hexane. The extract was added and slowly eluted with 50 ml n-hexane. After concentration *in vacuo*, the fractions were further purified by treatment with concentrated sulphuric acid after which the acid was removed using separating funnel. The acid treated fraction was again purified on a multi-layer silica gel column with n-hexane and finally reduced to about 2 ml by exposing to the laboratory atmosphere. The extraction of the associated sediments was carried out according to Albaiges et al. (2006).

2.2.3 Instrumental Analysis

GC-MS (Trace THERMO instruments, Manchester, UK) with a 60 m capillary column coated with 5% phenyl-95% methyl-polysiloxane (0.25 mm id and 0.25 μm film thickness; HP-5, Hewlett-Packard, CA, USA) was used for identification and quantification of the individual PAH compounds. The carrier gas was Helium at a flow of 2.1 ml/min. Injection port and transfer line temperatures were 300°C. Fractions in toluene were injected in splitless mode. The following oven temperature program was used: the program started at 90°C and held for 1 min., raised to 150°C and held for another 1 min., then at 4°C held for 1 min. to 320°C and a final holding time of 30 min. The quadrupole mass spectrometer was operated in EI mode (70eV), with an ion-source temperature of 200°C. Selected ion monitoring (SIM) of the PAHs was carried out using retention time windows.

A calibration curve (detector response versus amounts injected) was performed for each compound to be quantified. The linear range of the detector was estimated from the curve generated by plotting detector signal versus amount injected. All measurements were performed in the linear ranges for each target compound. In few cases, the fractions were re-diluted and re-injected for fitting within the linear range of the instrument. The quantitative data were corrected for surrogate recovery; recoveries were in the range 70%-110 % (average 85%). Procedural blanks were lower than 5 ng/g, the limit of detection (LOD) in the SIM mode ranged from 0.04 to 0.13 ng/g dw. The lower limit of determination of PAHs was estimated from the smallest peak (signal-to-noise ratio > 3:1 in the chromatograms) that could be integrated. Accuracy of PAHs quantification was validated by analysing with the certified reference materials "CRM-104" (Resource Technology Corporation, USA), obtaining accuracy from 75% to 107% compared with the certified value (Grimalt et al., 2004).

3. Results and Discussion

3.1 PAH Concentrations in Associated Mangrove Sediments

Characteristic features of the environment and analytical data for the biota samples including lipid content (LC) and size of biota are presented in Table 1. TOC contents of sediments were 2.10% at MR and 5.21% at UMA. The mean LC for the molluscs and crabs were in the range 1.98±0.16 - 2.32±0.24% and 1.87±0.17 - 2.02± 0.41%, respectively at MR site whereas at UMA site 2.58±0.21 - 3.14±0.32% and 3.23±0.67 - 4.34±0.42% were found for molluscs and crabs, respectively. The following parent PAH compounds were identified and quantified in sediment and biota samples from the river: naphthalene (naph), acenaphtylene (Acy), acenaphthene (Ace), flourene (F), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), Pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chrys), benzo(b)fluoranthen (BbFla), benzo(k)fluoranthene (BkFla), benzo(a)pyrene (BaP), benzo(e)pyrene (BeP), indeno(1, 2, 3-cd)pyrene (InP), benzo(ghi)perylene (BghiP), perylene (Pery) and biphenyl (BPh). Others were the alkylated homologues including 1-methylnaphthalene (1-MNaph), 2-methylnaphthalene (2-MNaph), 2,6-dimethylnaphthalene (2,6-DMNaph), 2,3,5-trimethylnaphthalene (2,3,5-TMNaoh), 1-methylphenanthrene(1-MPhe), 2-methylphenanthrene(2-MPhe), 3-methylphenanthrene (3-MPhe), 2,6-dimethylphenanthrene (2,6- DMPhe) and 9/4-methylphenanthrene (9/4-MPhe).

Associated sediment sample from UMA site showed much higher total PAH (TPAH; sum of parent and alkyl

PAHs), ΣPar (sum of parent PAHs), ΣAlkyl (sum of alkyl PAHs), ΣPAH$_{carc}$ (sum of 8 carcinogenic PAHs) and ΣPAH$_{EPA}$ (sum of 16 USEPA priority listed PAHs) levels (16,028.8, 1,922.2, 6,646.4, 2,972.5 and 394.5 ng/g dw, respectively) than that for MR site (2,441.8, 224.5, 459.5, 90.3 and 567.5 ng/g dw respectively).

Table 1. Characteristic features of the environment, size and lipid contents of biota

Site feature	Species	Sample code	Weight (g)	LC (%)	Length (cm)	EOM (mg/kg)
Mouth of river (MR); oil leakage,agricultural waste, garbage, vehicle emission	*Egeria radiata* (molluscs-clam)	SMK	3.2± 0.2	1.78± 0.16	1.65± 0.2	800
		LMK	4.4±0.21	1.72± 0.24	2.17±0.25	
	Callinectes amicola (crab)	SCRB	3.1± 0.42	1.87± 0.17	1.80±0.31	
		LCRB	12.5± 0.5	1.62± 0.41	3.91±0.39	
Upper mangrove area (UMA): offshore oil spill influx.	*Musculin securis* (molluscs-clam)	SMM	3.9±0.25	1.58± 0.21	1.85±0.41	2350
		LMM	5.2± 0.22	1.44± 0.32	1.97±0.35	
	*Micropipus depurator(*Crab*)*	SCRM	3.1± 0.44	2.02±0.67	2.0± 0.68	
		LCRM	14.4±0.41	1.95± 0.42	5.77±0.52	

N/B: SMK= small molluscs from MR site, LMK= large molluscs fron MR site, SCRB= small crab from MR site, LCRB= large crab from MR site, SMM= small molluscs from UMA site, LMM= large mollusks from UMA site, SCRM= small crabs from UMA site, LCRM= large crab from UMA site.

The degree of PAH contamination in the upper mangrove swamp area (UMA) was about 8 order of magnitude higher than those found in mangrove swamp area in Hong Kong (Ke et al., 2005). Field observation revealed that UMA site is exposed to various contaminations including runoff from agricultural wastes, oil leakages and vehicle exhaust emission from commercial fishery as well as garbage dumps. Our data reported elsewhere (unpublished work) revealed high levels of organochlorine compounds such as HCHs (eg. lindane), and DDTs used as herbicides/pesticides for agricultural purposes and malaria vector control at this site.

It appears that PAHs contamination load was localized and confined to geographical locations and contaminants reduce significantly once away from the sources. In other words, PAH contamination load was not dependent on regional influx or atmospheric deposition from industrial emissions and/or biomass burning. This assertion is evident in the observed high percentage composition (63%) of 2-ring PAH species at UMA whereas at MR, 3-ring counterparts predominated (60%) the PAHs profile (Table 2), suggesting different petroleum related activity (source) at these two sites. In addition, the concentrations of PAHs according to ring size also varied considerably between the two sites. Generally, the low molecular weight PAHs (2-3 rings) were the dominant species. The high molecular weight (HMW) PAHs exhibited low total grouping concentrations. For instance, 6-ring compounds showed values that were 5.2 ng/g dw at MR and 30.5 ng/g dw at UMA, representing a group percentage of about 1% at each site (Table 2). The dominance of 2-3 ring compounds and their alkyl homologues in sediments suggests that petroleum input may be the main source of PAH contamination in these two sites.

3.2 PAH Concentrations in biota and BSAFs

The mean TPAH concentrations measured in the biota are presented in Table 3 and varied from a minimum of 16,382.8 ng/g wet weight (ww) in large size molluscs (LMK) at MR to a maximum of 86,331.3 ng/g ww in small crabs (SCRM) at UMA. Similar trends were observed for other related PAH parameters such as mean ΣPAH$_{EPA}$, ΣPAH$_{carc}$, ΣPar and ΣAlkyl (Table 3). Comparatively, the average level of ΣPAH$_{carc}$ was the least among related PAH parameters, with LMK showing least accumulation and the highest by SCRM. Generally, alkyl PAHs were more dominant in the organisms than their parent counterparts, showing greater accumulation of petroleum derived PAHs than pyrolytic types, reflecting predominance contamination of the study area by petroleum derived PAHs. This idea is supported by the detection of high levels of 2- and 3-ring PAHs in the biota (Figure 2), usually observed to be present in higher proportion in petroleum than in pyrolytic products.

It appears that the levels of PAHs contamination in the animals are dependent on geographical location and sources. Our data show higher levels in biota samples from fresh water area (UMA) and lower in brackish water area (MR; Table 2). It was also generally observed that smaller individual organisms accumulated more PAJs

than their larger counterparts, showing that levels of PAHs in biota are affected by the body size. This observation may be a result of the hypothesis that smaller individuals have faster rates of hydrophobic contaminants uptake and probably slower rate of elimination than their larger counterparts (Yang et al., 2006). Besides the effect of body size on PAHs accumulation in sediment-dwelling organisms, other factor such as lipid content (LC) was also examined. LC has been shown to be an important factor that determines hydrophobic compounds accumulation as they are a primary site for these contaminants storage (Muncaster et al., 1990). The relative size of lipid pool has been shown to affects movement of hydrophobic compounds into biota tissues, implying that higher LC causes faster uptake and slower elimination of these contaminants. This gives rise to greater accumulation of contaminants in high LC tissues than those tissues with low LCs (Hanson et al., 1978). However, this fact was not observed in our case study.

One way analysis of variance (ANOVA) of PAH concentrations in biota at a confidence level of 95% was performed in order to test the significance of the relationship between LCs or body sizes of organisms and the contaminant body burden. The calculated F values were 1.53 for the body size (df=7; p=0.05) and 1.65 for the LC (df=7; p=0.05). These values are less than the critical F value of 2.01, implying that there were no significant relationships between these two paired variables. These results may be a reflection of the non-equilibrium situation in which LCs in the biota have not changed proportionally with contaminant load (Kelly & Campbell, 1994; Yang et al., 2006).

Biota sediment accumulation factor (BSAF) was taken in this study as a measure of bio-availabilities of PAHs in the river and were estimated according to Wang et al. (2003). The calculated BSAF values were in the range 4.84-14.98, with the highest value found in large size crabs (LCRB) at MR site, even though the associated sediment exhibited lower TPAH concentration and TOC content. On the other hand, the lowest BSAF value was observed in small molluscs (SMM) at UMA site, with higher associated sedimentary TPAH concentration and TOC content (Table 4). This scenario is consistent with reports by Ferguson and Chandler (1990); Lake et al. (1990); Yang et al. (2006) who found lowest BSAF values in highly polluted, high TOC sediments and highest values in sediments with low contaminant levels and low TOC contents. This phenomenon most likely is a reflection of strong affinity of PAHs toward sedimentary organic carbon. Hence, despite their high loads in sediments at UMA, PAHs in this area would therefore be less bio-available for the sediment-dwelling organisms.

Table 2. PAH concentrations in associated sediments by number of rings and related PAH parameters

Site	TPAH	ΣPAH_{EPA}	ΣPAH_{carcc}	ΣPAR	$\Sigma Alkyl$	2-ring	3-ring	4-ring	5-ring	6-ring
MR	2,441.4	567.5	90.3Σ	224.5	459.5	301.5 (18%)	989.5 (60%)	314.4 (19%)	32.5 (2%)	17.1 (1%)
UMA	16,028.5	2,972.5	394.5	1,922.2	6646.4	10,256.6 (63%)	4,703.2 (29%)	1,135.4 (7%)	114 (!%)	14.3 (<1%)

N/B: values in parenthesis represent percentage composition by number of rings.

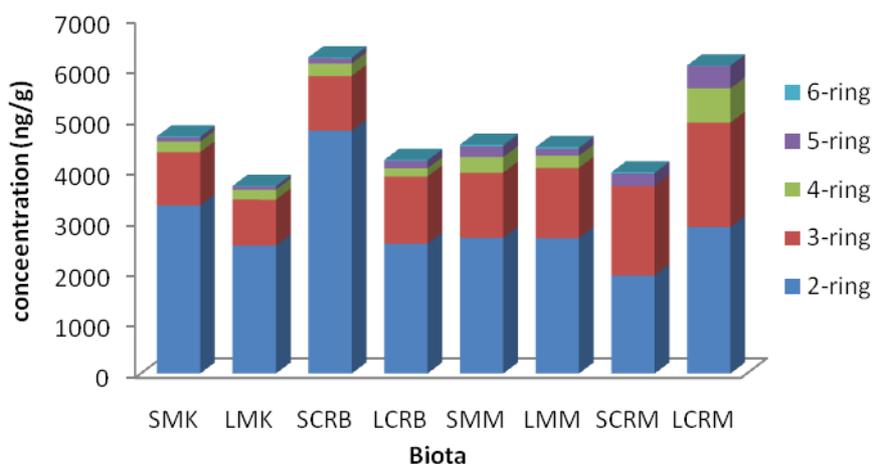Figure 2. Distribution of PAHs in biota by number of rings

Table 3. Mean concentrations (ng/g w/w) of related PAHs in biota from the Calabar River

Parameter	SMK	LMK	SCRB	LCRB	SMM	LMM	LCRM	SCRM
TPAH	17,116.8	16,382.8	30411.8	28238.3	23546.7	33614.8	57234.5	86331.3
ΣPAH_{EPA}	4,586.2	3702.3	6214.4	4182.8	4482.3	4407.3	4154.5	5784.4
$\Sigma PAH_{(carc)}$	155.7	134.6	212.4	162.7	372.6	250.4	380.4	887.4
ΣPar	2,060.7	1,420.6	2051.8	1888.5	1934.3	1988.5	2358.1	3062.9
$\Sigma Alkyl$	11,799.2	11,855.7	22893.6	22765.1	17935.2	27716.2	51250.7	78126.9

Despite this relatively low bioavailability of these compounds at UMA site, occurrence of bioaccumulation was evident, reflected in higher PAH loads in organisms in relation to the associated sediments. The observed higher levels of PAHs in the organisms may be due to a long resident time of the animals in the sediments, a consequence of low mobility of the biota.

Results of epidemiological survey have established that exposure to PAHs can be linked to a wide range of adverse effects on human health (WHO, 2006). In the present work, ΣPAH_{carc} and bemzo(a)pyrene were used in the assessment of risk of long-term adverse health effect. The choice of benzo(a)pyrene was on the recognition that this compound was considered as a marker for the occurrence and effect of carcinogenic PAHs in aquatic food products while ΣPAH_{carc} have been reported to be one of the most reliable indicators of carcinogenic potency of PAHs in foods (IPCS, 2006). PAHs have not only been detected in all biota samples but also their concentrations were excessively high. All samples had mean residual levels of benzo(a)pyrene that exceeded the maximum permissible value of 10.0 ng/g ww for molluscs and 5.0 ng/g ww for crabs established by the European Union (e.g. Bineli & Provini, 2003; Table 5). Thus, due to the widespread distribution of benzo(a)pyrene, further monitoring efforts are needed, particularly in other Sub-Saharan African regions where high levels of benzo(a)pyrene may be found, to ensure the long-term safety of consumers of sediment-dwelling animals.

If the aquatic food product consumption data provided by Food and Agriculture Organisation fact sheet (http /faostat.fao.org/site/346/default.aspx) are taken into consideration and the estimated daily intake (EDI; which is usually used to assess human exposure to organic contaminants) derived from the mean levels from the study area are used, then the EDIs of benzo(a)pyrene and ΣPAH_{carc} were calculated according to the equation:

$$EDI = \frac{EDC \times CC}{BW} \text{ (Liu et al., 2010)} \tag{1}$$

where EDC is food daily consumption, CC is mean contaminant concentration and BW is body weight for which 60 kg is typical (IPCS, 2006). To estimate the extent of crab and mollusc consumption in the region, 250 questionnaires were prepared and sent to families living in Calabar city and its environs including inhabitants of the riverine/coastal communities. Information on the consumption frequencies, quantities of crabs and molluscs consumed and number of people (including children) were included in the questionnaires. The overall consumption information for the region was obtained after statistical analysis of generated data and is presented in Table 5. The amounts ranged between 15.5 and 29.6 g/day, with a mean of 17.7 g/day for crabs, and between 10.5 and 22.4 g/day, with a mean of 14.5 g/day for molluscs. Lower comparison values were foind in our study when compared with the average consumption value of fishery food from the China Statistical Yearbook [38.90 g/day (NBSC, 2008)]. From these data, average EDIs for benzo (a) pyrene and ΣPAH_{carc} via crabs and molluscs consumption were calculated; the selected PAH concentrations used were the overall mean values in both crab and mollusc samples (Table 5). Potential public health risks as a result of exposure to PAHs via crab and mollusc consumption were assessed using the US Environmental Protection Agency (USEPA) cancer benchmark concentrations (CBCs) that represent exposure concentrations at which lifetime cancer risk is 1 in 1 million (USEPA, 2006). A benchmark concentration indicates the daily concentration below which the probability of developing adverse health effect is low (Lui et al., 2010).

The EDIs for benzo (a) pyrene and ΣPAH_{carc} were higher than the USEPA CBC values, suggesting a perceptible health risk associated with the consumption of these biota in the region. This implies that life-time cancer risk is possible for inhabitants of coastal/riverine zones who are likely to consume more sediment-dwelling animals than those living hinterland. In addition, much of the EDI of each of these marker contaminants comes from crabs (Table 5). For example, 69.7% of the benzo (a) pyrene is associated with crabs. This suggests that molluscs

are safer than crabs to consume based on their contributions to the EDI of organic contaminants.

With little amounts of data available on PAHs in molluscs and crabs from coastal waters of the African countries, the use of consumption data from FAO fact sheet would allow a comparison of EDIs among the population in African region especially with the high quantity of molluscs and crabs consumed and the high PAH concentrations in these biota. The EDIs of PAHs via aquatic food consumption in the SE Niger Delta, Nigeria is higher than those for some countries in the Europe and Asian pacific region. Since molluscs do not entirely represent bivalves, this comparison may not be representative and may lead to bias results. Nevertheless, the results could provide preliminary baseline for the assessment of the status of human exposure to PAHs via consumption of sediment-dwelling biota among different countries in African region. In addition, our health risk assessment was quite encompassing as it did consider children exposure which may contribute significantly to the possibility of developing cancer among the inhabitants of the catchments.

Table 4. Sedimentary PAH concentrations, total organic carbon (TOC) contents and BSAFs

Site	Species	Sample code	BSAF	TPAH (ng/g)	TOC (%)
Mouth of the river(MR)	*Egeria radiate* (molluscs-clam)	SMK	8.27	2441.5	2.10
		LMK	8.19		
	Callinectes amicola (crab)	SCRB	13.99		
		LCRB	14.98		
Upper mangrove area (UMA)	*Musculin securis* (molluscs-clam)	SMM	4.84	16,028.5	5.21
		LMM	7.59		
	Micropipus depurator (Crab)	SCRM	9.51		
		LCRM	13.39		

Table 5. Comparison of the estimated daily intake from the present work with cancer benchmark concentration (CBC) developed by the US Environmental Protection Agency

Contaminant	Species	Consumption (g/day)	Average EDI (ng/kg.day)	CBC (ng/kg.day)
Benzo(a)pyrene	Molluscs	14.5	9.7	0.00012
	Crabs	17.7	18.6	--
$\sum PAH_{carc}$	Molluscs	14.5	55.2	0.00077
	Crabs	17.7	121.2	--

4. Conclusions

The present study represents the first investigation on the extent of PAHs contamination and accumulation in sediment-dwelling animals of the Calabar River. PAH concentrations found in associated sediment from the upper mangrove swamp area (UMA; fresh water) were much higher than those found towards river mouth (brackish water). The dominant PAHs source appears to be derived from petroleum related activity through localized run-off, oil spill and leakages arising from boating activity for commercial fishery rather than regional influx or atmospheric deposition from industrial emissions/biomass burning.

There was no significant relationship between lipid content or body size and PAH concentrations in these organisms: smaller individuals accumulated more PAHs than their larger counterparts. BSAFs varied amongst biota and were generally lower in highly polluted site (UMA) and higher in low impacted area. Hence, despite their high loads in sediments at UMA, PAHs would therefore be less bio-available for the sediment-dwelling organisms in this site. However, occurrence of bioaccumulation was also evident, reflected in higher PAH loads in organisms in relation to the associated sediments.

EDI results for benzo(a)pyrene and $\sum PAH_{carc}$ revealed life-time cancer risk, especially to inhabitants of the

riverine/coastal areas who often consume more sediment-dwelling biota than those living hinterland. Therefore, for purposes of protecting public health, it is absolutely necessary to continually monitor the occurrence and levels of PAHs in this environment. Although the present investigation was not focused on the relationship between various species of these biota in fresh or brackish water in the region and contaminant body burden, further monitoring efforts should be aimed at identifying certain species of crabs and molluscs with possibility of possessing higher accumulation potential.

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