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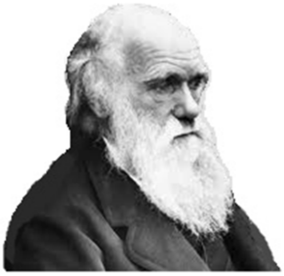

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Risk assessment of human exposure to heterocyclic aromatic compounds in sediment dwelling biota from Ibeno River Catchment, Southeast, Nigeria

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ABSTRACT

Biota and associated sediment samples were collected from the Ibeno river catchment and analyzed using the standard methods for investigation of the sources and the distribution of heterocyclic compounds in the area. The lipid content values of the biota varied from 10-29% dry weight (dw; mean = 15.8 + 7.79) in Large crab (LC) and ranged from 5-10% dw (mean = 7.6 + 1.82) in small crab (SC). Also, it ranged from 6-10% dw (mean= 7.6 + 1.52) in small shrimp (SS) and varied from 7-40% dw (mean = 23.6 + 1.47) in large shrimp (LS). High TOC (4.5%) and TIC (2.53%) were found for the associated sediment, and this was because of the silty nature of the sediment, having high adsorption capacity for organic matter. The high extractible organic matter (EOM) value recorded for the associated sediment may be linked to petroleum hydrocarbon discharge into the environment by Exxon Mobil. The biota-sediment accumulation factor (BSAF) values ranged from 0.0029-0.0059 (mean = 0.0047 + 0.00128) for LC and 0.0059-0.0085 (mean = 0.00724+0.001276) for SC. On the other hand, the values ranged from 0.0060-0.0160 (mean = 0.0037 + 0.002787) and 0.0018-0.0084 (mean = 0.0096 + 0.004079) for LS and SS respectively. This low result reviews that the contaminant uptake from the associated sediment by the biota was negligible, hence, the consumers exposed to this biota might not have experienced any serious health risk associated with the consumption of heterocyclic aromatic hydrocarbons. The aromatic fractions from column chromatography would subsequently be subjected to gas chromatography mass spectrometry analysis to identify and quantify the individual heterocyclic aromatic hydrocarbons.

***Keywords*:** Biodata, Heterocyclic aromatic compounds, human exposure, sediments

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1. INTRODUCTION

Increasing population of Nigeria is associated with increased agriculture and industrial activities have contributed a large quantity of hydrocarbons into the ecosystem with no attention on their environmental consequences. Nigeria has vast deposits of fossils in form of coal, natural gas and petroleum which derivatives are rich in aromatic hydrocarbons.

Ibeno river catchment represents one of the few environments suitable for the investigation of the diverse origins of aromatic hydrocarbons. This is because, its environmental characteristics provide a means of tracing non-natural (terrestrial and aquatic) and anthropogenic (petrogenic and pyrogenic) origins of NSO-HETs. Soil and groundwater contamination due to NSO-HETs, have been reported in Germany, Denmark and India with principal contaminants being thiophene, benzothiophene, benzofuran and carbazole (Meyer, 2011). Co-existence of NSO-HETs such as quinoline, pyridine with homocyclic PAH and phenoms of cooking plant wastewater was reported. Several hetero-PAH have been reported to exhibit toxic, mutagenic and carcinogenic activities (Lo *et al*, 2003). Several authors have reported the treatment of NSO compounds using various physiochemical techniques such as adsorption and electrochemical oxidation, although biological remediation can be a promising clean-up technique on pat with other physiochemical processes (Rasmissen, 2002).

River sediments are important compartments of aquatic ecosystems as they provide habitat and shelter do many organisms. However, sediments also act as a long-term source of contamination, as pollutants can be temporarily unavailable for uptake when bound to particles but could be gradually released to the aquatic phase. Many benthic and epibenthic organisms which represent much of the lower trophic levels may then be exposed to these substances. Adverse effects such as changes in benthic community structure or population decline have been reported.

In Nigeria, sediments are considered as part of the surface waters. According to the ordinance on water protection (Oeaux, 1998), sediments should not accumulate any persistent pollutant in order to protect aquatic life. However, contaminants analyses and studies on their toxicity potential are scare or not often undertaken. In addition, recommendation for the characterization of sediments is lacking and quality criteria missing. Thus, the present study among other topical environmental issues is the first step of a project aimed at elaborating recommendations for the assessment of sediment toxicity in Nigeria. Therefore, this study aims at providing a basis for discussion to help experts and stakeholders take decisions regarding the applicability of sediment quality assessment criteria for future management strategy in Nigeria.

As time increases, the deposition of contaminants led to the differences of NSO-HETs at different depth intervals, (i.e., the distributions a reason diverse in different years). Previous studies indicated that the contaminated characteristics of NSO-HETs in sediments were clearly diverse in different years due to the differences in energy structure and economic development (Guo, 2010). Therefore, it is very important to explore the historical trends of NSO-HETs in sediment cores. So far, the temporal historical trends of NSO-HETs in sediment cores have been studied extensively around the world (Guo, 2010), and many of them show that the temporal trends corresponded to the vertical distributions in sediment cores. However, the knowledge of historical trends of NSO-HETs sources in developing countries is relatively limited. Specifically, previous studies on the estuary focused mainly on fisheries (Moses, 1989), ecology (Enyenihi, (1987), water quality (Allan, 1993). Organic geochemical characterization of lipids in surface sediments (Oyo-ita, 2006) distribution of persistent organic pollutants in sediment from calabar river, southeastern Nigeria (Adie, 2010), inorganic geochemistry of water, suspended particulate matter and sediments from Southeastern Niger Delta estuaries (Helen, 2015).

Organic matter in sediment refers to the detritus (organic debris from decomposing plants and animals) which were in existence millions of years ago. Although the organic aggregate found in sediments is probably in part land delivered, the ocean waters appear to be by far the most important source. Phytoplankton and bacteria can be said to be the main producer of organic matter and thus account for most of the organic matter found in recent and ancient sediments. The organic matter may be minor or major constituent of carbohydrate, proteins, liquid, and pigments. They type of organics matter is dependent on depositional environment since different environments are colonized by different flora and fauna (Williams and Winters, 1969). It can be inferred that the origin of organic matter is merely determined from both natural and anthropogenic sources which are either aquatic or terrestrial. They are finally stored in sediments (Gagosian and Lee, 1981).

The preservation of organic matter is exclusively restricted to aquatic sediments. Depending on the environment and process of formation, these sediments may contain varying proportions of materials from pre-exiting rocks. The same principle applies to organic material sediments, that may contain allocthonous organic matter (that was transported to the site of disposition from elsewhere) or may contain autochthonous organic matter that originated at the site of deposition. Whereas the autochthonous organic material resembles more closely the primary biological products, the allochthonous fraction is mainly composed of diagenetically formed secondary reaction products, such as buried acids and kerogen (Didyk, 1978).

The materials represent the more stable residual organic matter that theft has already experienced at least part of the sedimentary cycle. Heterocyclic aromatic compounds are widely distributed pollutants in soil, air, sediments, surface water and groundwater, as well as in animal and plant tissues (Brack, 2003). They may be of natural origin (e.g., alkaloids), but high environmental concentrations mainly result from human activities. Industrialized areas, such as creosote contaminated sites, represent important sources of tar oil pollutants (Webwer, 2008). Creosote represents a complex mixture of over 10,000 single organic substances which are formed by thermal processes related to coal and fossil fuels (Blotevogel, 2008). Besides technical and chemical processes that involve tar oil, heterocyclic compounds are also present in dyestuff (Cripps, 1990), pesticides and pharmaceuticals (Fernandez, 2002). While creosote contains only 5–13% heterocyclic compounds (Dyreborg, 1997), up to 40% of their water-soluble fraction consists of these heterocyclic compounds (Licht, 1997). The higher polarity and water solubility of the heterocyclic substances is based on the substitution of one carbon atom by nitrogen, sulfur or oxygen (NSO-HET) (Meyer, 2000). These chemical properties lead to increased bioavailability and mobility as compared to the homologous polycyclic aromatic hydrocarbons (PAH).

Ibeno Local Government Area was created out of the defunct Uquo-Ibeno Local Government Area on December 4, 1996 by the Federal government instrument. Before the creation of Uquo-Ibeno Local Government Area, the people of Ibeno were in Eket Local Government Area – Ibeno Edoh, Uquo Ibeno respectively. Ibeno Local Government Area has thus graduated from being part of Eket local government area to part of Ibeno-Edor with headquarters at Inua-Eyet Ikot Ibeno (now defunct) to being part of Uquo Ibeno with headquarters at Uquo (now headquarters of Esit Eket Local Government Area) to a distinct and separate local government area, Ibeno Local Government Area with headquarters at Upenekang. It is one of the 31 local government areas in Akwa Ibom State.

The area under investigation is a section of the Ibeno Beach, which is a part of the coastal portion of the Atlantic Ocean exposed in Ibeno Local Government Area of Akwa Ibom State and lies entirely on the coastal plain of Southeastern Nigeria. Ibeno Beach is one of the beaches on the Atlantic Ocean along the Ibeno shoreline. It is a low gradient (<5°), mainly featureless and longest sandy beach type in West Africa; it spans up to 45km in length and 103m in width during low water tide. The study area is characterized by semi-diurnal tide with a meso-tidal range of 2.4m. Ibeno beach, which stretches 45 km from Ibeno to James Town, Akwa Ibom, is the longest beach in Africa. It is the most popular beach in Akwa Ibom State, attracting a lot of tourist and also houses Exxon Mobile operational Headquarter, which is a multi-international oil company in West Africa and other service companies influence activities both upstream and downstream. The prime occupation of the people is fishing. However, farming, and petty trading enjoy appreciative notice. Ibeno beach is highly rated because of its peaceful and safe surroundings. Ibeno was the landing site for one of the most successful Christian missions in Nigeria - the Qua Iboe Christian mission from Scotland which arrived in the early 20th century. Relics of that missionary expedition also attract tourists to the area. In 2013, the federal government constructed the Ibeno Bridge to attract tourists and improve other economic activities in the area.

This study is designed for the determination of bulk parameters such as total organic carbon (TOC), total inorganic carbon (TIC), extractible organic matter (EOM)/ lipid content and grain size distribution in sediments at Ibeno River Catchment, Southeast, Nigeria. It is expected to evaluate the relationship among these variables and determine the biota-sediment accumulation factor (BSAF). Ultimately, the risk impact of human exposure to the heterocyclic aromatic compounds in sediment dwelling biota are assessed.

2. MATERIALS AND METHODS

Geology of the study area

The geology of the area shows that it is part of the Coastal Plain sediment in Nigeria belonging to the Tertiary Benin Formation, that spans along coastal sedimentary basins in Nigeria. Ibeno occupies the largest Atlantic coastline of more than 129 km. in Akwa Ibom State. Located in the mangrove swamp forest (Figure 1).

Climate and vegetation of the study area

Ibeno river catchment, which is located in a mangrove swamp forest, has rain throughout the year with the peak between May and September. The climatic condition in Ibeno is favorable all year round for fishing and farming.

Hydrogeology of the study area

The whole of Akwa lbom State is underlain by sedimentary formations of Late Tertiary and Holocene ages (Edet, 1993; Esu et al, 1999). Deposits of recent alluvium and beach ridge sands occur along the coast and the estuaries of Qua lboe River, and also along the flood-plains of creeks. Upwards, the geologic succession passes imperceptibly into thick sequences of clays, sands and gravel (Udom, 2002).

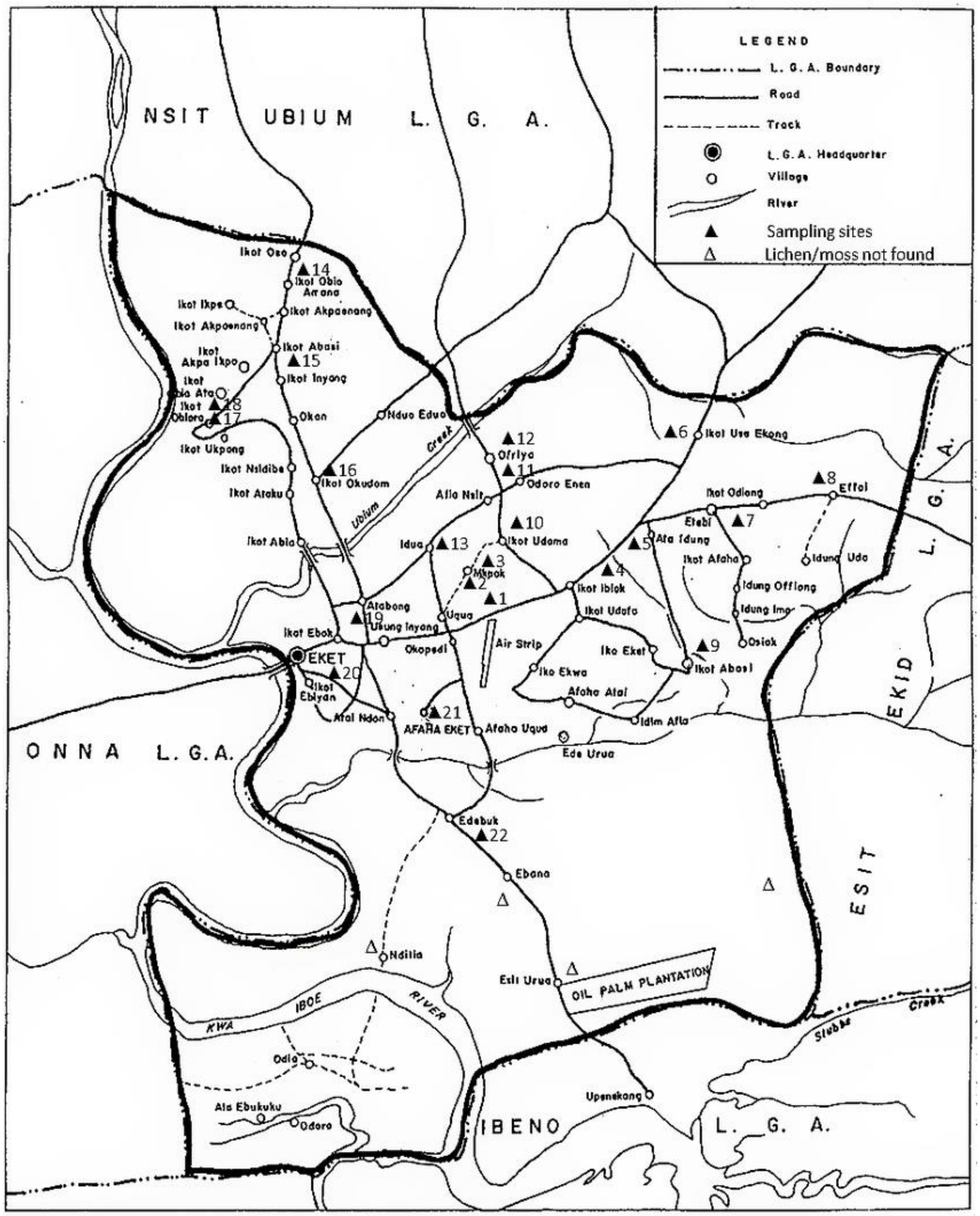

Figure 1. Map of the study area

Materials and reagents

The following analytical grade solvents/materials: methanol, n-hexane, dichloromethane and sulphuric acid of BDH laboratory supplies, England, were purchased from a chemical vendor in Aba. Others include phosphosric acid, potassium dichromate, calcium chloride and sodium fluoride. Perdauterated PAHs such as [d_8]-napthalene, [d_{12}]-benzo(a)anthracene, [d_{10}]-fluoranthene, [d_{12}]-benzo(ghi)perylene, [d_{10}]-pyrene and [d_{12}]-perylene used as standards were of Cambridge isotope laboratories (Andoyer, MA, USA) and [d_{10}]-anthracene was of Dr. ehrenstofer GmbH laboratory (Augsburg, Germany), all purchased by the Institute of Environmental Assessment and Water Research, Spanish Council of Scientific Research (CSIC) Barcelona, Spain. The internal standard used was triphenylamine purchased from Merck, Hohenbrunn, Germany. Silica gel 40 (0.063-0.200 mm), Anhydrous sodium sulphate and neutral alumina 90 active (0.063-0.200 mm) were the column chromatography adsorbent used were also purchased from Merck.

Sample preparation

In the laboratory, samples from the wrapped aluminium foil were placed on a lean aluminium foil and labeled for easy identification. The sediment sample was air-dried for seven days, sieves of about 20g of the sediment were mechanically crushed and wrapped with foil for sediment grain size analysis.

Preparation of 85% orthophosphuric acid

A 1000 cm^3 of orthophosphuric acid was dissolved in 15 ml of distilled water and the resulting solution was added with more distilled water to 180 ml mark.

Preparation of 017m. Potassium heptaoxodichromate (vi) solutions

49.98g of potassium heptaoxodichromate (vi) was weighed out into 1000 cm^3 capacity volumetric flask and dissolved with sufficient distilled water. The resulting solution was shaken vigorously and more distill water was added to the calibration mark.

Preparation of 0.25m ferrous ammonium tetraoxosulphate (vi) solutions

71g of ferrous ammonium sulphate was placed in a 1000 cm^3 capacity volumetric flask, followed by addition of sufficient amount of distilled water, shaken vigorously and then more distilled water was added to the calibration mark of the flask.

Preparation of diphenylamine indicator

1g of diphenylamine was dissolved in 100 cm^3 concentrated tetraoxosulphate (iv) acid and shaken vigorously to effect solubilization.

Determination of sediment particle size

The sediments were air-dried for about a week and the grain size analysis was done with the aid of a sieve shaker machine. The sieves were of the range 2 mm, 1 mm, 0.50 mm, 0.10 mm, 0.05 mm pan. Each sample weighed 50g was placed on the arranged laboratory test sieves, inserted into the sieve shaker machine, and shaken for 10 min. After this duration, the particles that passed through and/or retained on the standard set of sieves of various sizes were measured.

The procedure was repeated for each sample and the grain size percentage was calculated using the formula.

Grain size percentage = sieve weight/total weight × 100 …................................. (1)

Total inorganic carbon (tic) determination

The total inorganic carbon was determined experimentally by Schumacher methods (Schumacher, 2002). The weight of the empty test tube was measured and recorded. Sediment sample of 2.0g, sieved, grounded and air-dried was placed in the pre-weighed test tube and 98% H_2SO_4 (dully prepared by diluting 55 cm^3 of concentration H_2SO_4 with 500 cm^3 of distilled water) was added to the content of the test tube in drips and the in excess until effervescence stopped. The treated sample was then washed thoroughly with distilled water until a neutral pH was attained. It was then transferred to crucible and allowed to dry in a dedicatory and reweighed. The procedure was repeated for all the others. The percentage of total inorganic carbon was calculated from the following relation:

$$\% \text{ TIC} = \{(B-A)/B\} \times 100$$

% TIC = percentage of total inorganic carbon

A = Weight of sample after treatment

B = Weight of sample before treatment

Total organic carbon (toc) determination

The total organic carbon (TOC) can be measured by either Walkley Black titration method or by high temperature combustion of pulverized rock followed by removal of carbonate minerals by acid leaching (Scheele, 1980). In this study, the TOC was evaluated by a modified Walkley Black Titration method (Schumacher, 2002). A sieved, grounded, and air-dried sample weighing 1.0g was placed in a 250 cm^3 conical flask and 5 cm^3 of 0.17M solution of $K_2Cr_2O_7$, followed by the addition of the 10 cm^3 concentrated H_2SO_4 to oxidize the organic matter. An electrothermal magnetic stirrer at a temperature of 100^oC was employed to improve the digestion process (Schumacher, 2002). The resulting solution was then allowed to stand for about 30mins and then diluted with 100 cm^3 of distilled water. About 9.5 cm^3 of 85% syrupy orthophosphoric acid was added to the diluted solution, followed by the addition of 0.2g sodium fluoride and two drops of diphenylamine indicator. The resultant solution was then treated with 0.25M iron (II) ammonium sulphate hexahydrate $(Fe(NH_4)_2(SO_4)_2.6H_2O$ solution with the aid of an electro-thermal magnetic stirrer. A bright green colour was observed at the end point. The procedure was repeated, and the average tire value was calculated. The entire process was then repeated, and the average was then repeated for all the remaining samples.

Percentage total organic carbon in each sample was calculated using the equation:

$$\text{TOC } (\%) = 10(1-G/H)$$

where G = Sample tire value
H = standard or blank tire value

Extraction by sonification of associated sediment

Prior to the extraction, all solvents were distilled to remove any form of organic contamination. (Ekpo, 2010) all glass wares were thoroughly washed with water and detergent and rinsed with acetone. Samples weighed 2.0g each, were placed in a test tube and about 4 cm^3 of distilled dichloromethane and 2 cm^3 of distilled methanol added to each of the weighed samples. The test tubes were put in a beaker and placed in an ultrasonic bath to agitate for 30 minutes. The test tubes and their contents were then inserted into a centrifuge machine set at 2000 revolution per minute for 15 min to allow settling of suspended particles. The extracts from each test tube were decanted into another test tube. The process was repeated three times for effective extraction from the sample. The decanted extract was vacuum-evaporated and weighed.

Extraction by sonification of biota

A recently developed procedure based on ethylacetate-aqueous sample suspension partition step (kamilia et al. 2011) was used for the extraction of the biota samples for heterocyclic analysis. Briefly 1ml of distilled water and 2 ml of Ethyl acetate were added to 2g of homogenized biota samples and shaken vigorously for 1 min. Then 0.8g of magnesium sulphate and 0.4g of sodium chloride were added to the extract. The tube containing the sample mixture was shaken for another 1min and centrifuged (10 min at 3000 rpm) and an aliquot was removed from the upper organic phase. The aliquot evaporated to the last drop under room temperature.

Clean up by column chromatography of associated sediment

Column chromatography is essentially liquid chromatography in which the stationary phase is a surface active solid, or a liquid immobilized on a solid support held in a column. The solutes are absorbed differently on the surface of active adsorbent or partitioned between the movie liquid phase and the stationary liquid content immobilized on the solid support (Etuk, 1987). The glass column was first washed with water and detergent and rinsed with acetone to minimize contamination. Glass wool was extracted with n-hexane using ultrasonic bath (to also reduce contamination) and pushed down the glass column using a glass rod. Thereafter, the column was filled with 45 cm^3 of. N-hexane and packed with alumina activated at 300 °C (bottom). The n-hexane was allowed to run out through the packed column. The column was then loaded with the extract and about 40 cm^3 dichloromethane (DCM) was then used as eluent to elute the aromatic fraction. The fraction gotten was allowed to evaporate in vacuum, weighed for injection into GC/MS.

Clean up by column chromatography of biota

The extraction process was followed by mini-column silica cleanup as optimized in Kamila et al. (2011) was used for the purification of biota samples for heterocyclic analysis. A handmade mini-column cleanup was performed using a Pasteur pipette filled with glass wool. 1g of alumina and a thin layer of sodium sulphate based on the lipid content of the analyzed biota (biota with lipid content up to 2% (<0.8g). Then the extract was applied onto the mini-column, and the analytes were eluted with 8 ml of the mixtures of n-hexane:dichloromethane (3:1, v/v). The collected eluent was carefully evaporated under the gentle stream of nitrogen to dryness.

3. RESULTS AND DISCUSSIONS

Grain size distribution

Grain size distribution is a list of values or a mathematical function that defines the relative amount, typically by mass, of particles present according to size. The associated sediment sample from the location was 100% silt fraction with a value of 50.00 (Table 1).

Table 1. Grain size distribution

SAMPLE ID	SILT (%)	SAND (%)	CLAY (%)
BS	50.00	0.00	0.00

Total organic carbon

Total organic carbon (TOC) refers to the amount of carbon found in an organic compound. Total organic carbon value is 4.5% for the associated sediment sample collected from Ibeno river catchment. Comparing the TOC value with other water systems, the total organic carbon was observed to be very high, and this is because of the fact that the sediment in the study is silty and thus exhibited high adsorption capacity for organic matter. And due to the presence of Exxon Mobil anthropogenic activity in the area.

Total inorganic carbon

Total inorganic Carbon (TIC) is the sum of inorganic carbon species in a solution. Total inorganic carbon values obtained is 2.53% for the associated sediment sample (BS) collected from Ibeno river catchment. Comparing the total inorganic carbon value with other water systems, the total inorganic carbon was observed to be very high, and this is because the sediment in the study is silty and thus exhibited high adsorption capacity for organic matter. And due to the presence of Exxon Mobil anthropogenic activity in the area.

Lipid content

Lipid content are those components that are soluble in organic solvents. The lipid content values of the biota varied from 10-29% dry weight (dw; mean = 15.8 + 7.79) in large crab (LC) and ranged from 5-10% dw (mean = 7.6 + 1.82) in small crab (SC) (Figure 1).

Also, it ranged from 6-10% dw (mean= 7.6 + 1.52) in small shrimp (SS) and varied from 7-40% dw (mean= 23.6+ 1.47) in large shrimp (LS). The result obtained from the biota shows that LC and LS are high, thus, the quality and quantity of organic matter is high. Whereas those for SS and SC are Low (Figure 2).

Biota-sediment accumulation factor

Biota-sediment accumulation factor (BSAF) was taken in this study as the measure of bioavailabilities or biotic fate of heterocyclic aromatic compounds in the river. BSAF was calculated according to Wang et al. (2004) using the expression below.

$$\text{BSAF} = \frac{C_{b/F_l}}{C_{s/F_{oc}}}$$

where C_b is the aromatic fraction, F_l is the fraction obtained from the biota, C_s is the column fraction obtained from EOM and F_{oc} is the organic carbon fraction of the sediment.

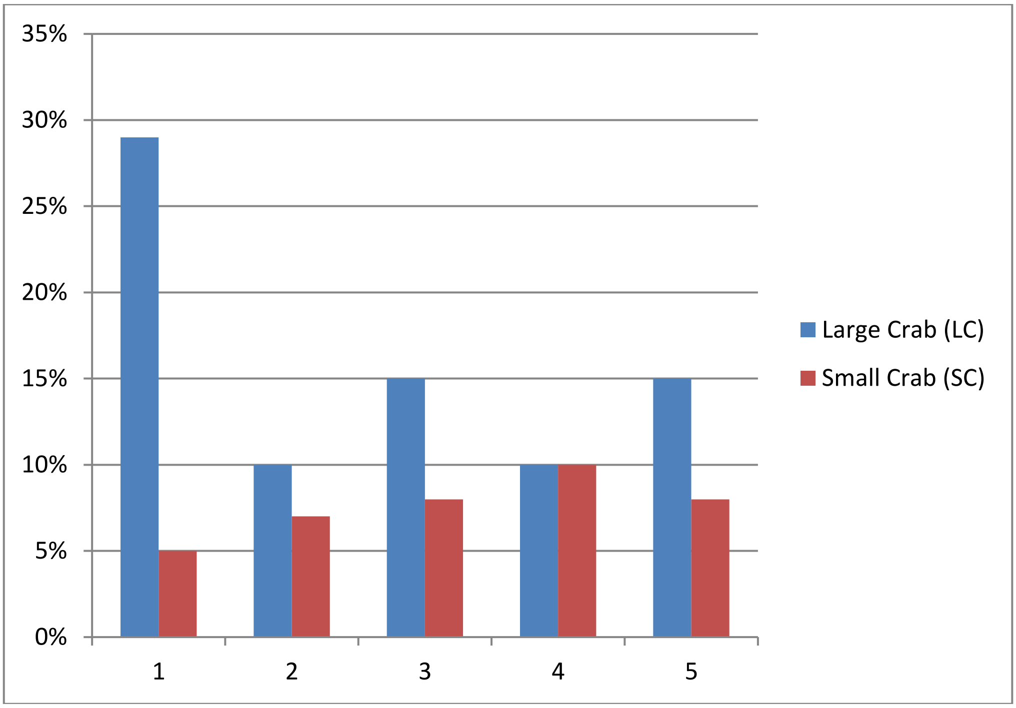

Figure 1. Distribution of Lipid concentration of large crab (LC) and small crab (SC)

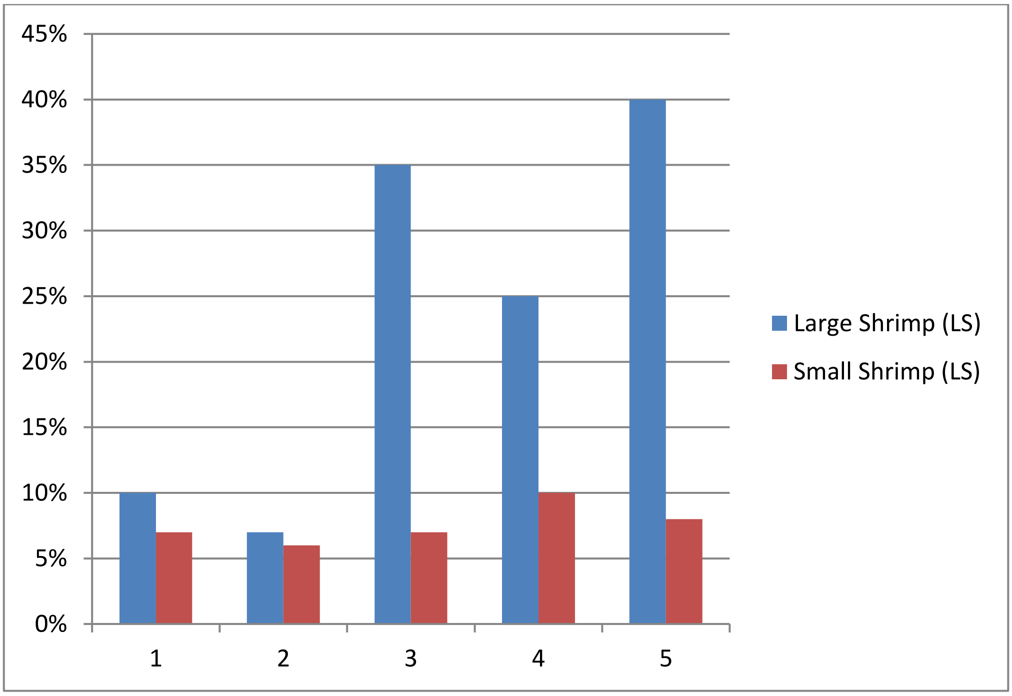

Figure 2. Distribution of Lipid concentration of large shrimp (LS) and small shrimp (SS)

The calculated BSAF values ranged from 0.0029-0.0059 (mean = 0.0047 + 0.00128) for LC and 0.0059-0.0085 (mean = 0.00724 + 0.001276) for SC. On the other hand, the values ranged from 0.0060-0.0160 (mean = 0.0037 + 0.002787) and 0.0018 - 0.0084 (mean = 0.0096 + 0.004079) for LS and SS respectively. This low result reviews that the contaminant uptake from the associated sediment by the biota was negligible, hence, the consumers exposed to this biota might not have experienced any serious health risk associated with the consumption of heterocyclic aromatic hydrocarbons.

Extractable organic matter

It was shown that the EOM content of the sediment is 1589 as presented in Table 2. It was observed to be very high, and this is because the sediment in the study is silty and thus exhibited high adsorption capacity for organic matter. The site is very active with fishing; hence there is frequent movement of boats passing through the site and maybe linked to petroleum hydrocarbon discharge into the environment by Exxon Mobil.

Table 2. Characteristics feature and bulk geochemical properties, and biota-sediment accumulation factor

SAMPLE ID.	BSAF	AROMATIC FRACTION (mg/kg)	TOC (%)	TIC (%)	EOM (mg/kg)	EOM/ TOC	COORDINATE	CHARACTERISTICS
BS		5	4.5	2.53	1589	353.11	N04º33m11.8s. E007º57m27.2s	Incomplete combustion, Gas flaring, Tidal movement, Fishing, Burning and Exxon mobile
LC1	0.0029	30						
LC2	0.0059	21						
LC3	0.0047	25						
LC4	0.0057	20						
LC5	0.0043	23						
SC1	0.0085	15						
SC2	0.0073	18						
SC3	0.0085	24						
SC4	0.0059	21						
SC5	0.0060	17						
LS1	0.0061	30						
LS2	0.0102	25						
LS3	0.0097	22						

LS4	0.0160	20						
LS5	0.0060	27						
SS1	0.0084	15						
SS2	0.0041	18						
SS3	0.0018	24						
SS4	0.0023	21						
SS5	0.0019	17						

4. CONCLUSION

Biota and associated Sediments samples obtained from Ibeno Beach which is a part of the coastal portion of the Atlantic Ocean in Ibeno Local Government Area of Akwa Ibom State, Southeastern Nigeria were investigated to interpret their bulk properties such as Total Organic Carbon, Total Inorganic Carbon, Extractible Organic Matter, lipid content and grain size as well as determine the biota-sediment accumulation factor (BSAF).

This low result reviews that the contaminant uptake from the associated sediment by the biota was negligible, hence, the consumers exposed to this biota might not have experienced any serious health risk associated with the consumption of heterocyclic aromatic hydrocarbons.

The aromatic fractions from column chromatography would subsequently be subjected to gas chromatography mass spectrometry analysis to identify and quantify the individual heterocyclic aromatic hydrocarbons.

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