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POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) PROFILE IN THE TISSUES OF PERIWINKLE (Tympanotonus fuscatus) IN IDEMA CREEK BASIN, BAYELSA STATE NIGERIA.

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ABSTRACT

The study of Polycyclic aromatic hydrocarbons (PAHS) Profile in the tissues of Periwinkle (Tympanotonusfuscatus) of Idema Creek Basin was investigated from April, 2017 to March, 2018. PAHS concentration were measured by gas chromatography with flame ionization detector (GC/FID) in the above named Macrofauna. The ∑PAHs concentrations recorded in T. fuscatus ranged from 0.10431/.µg/g d. wt in station (1) for the month of February, 2018 to 1.95775 µg/g d. wt for April, 2017 in station (1). Benzo (a) Pyrene recorded the highest mean value of 0.034 ± 0.02 µg/g d. wt and Indeno (1,2,3-cd) pyrene recorded the lowest mean value of 0.00038 ± 0.00µg/g .d. wti. The result from PAHs analysis showed some level of bioaccumulation of 15 PAHs from below detection to appreciable limits in the tissues of the studied macrofauna. Seven low molecular weight PAHs and eight high molecular weight PAHs were identified in the tissues of T. fuscatus, indicating pyrogenic and petrogenic origin or source as a result of activities of crude oil bunkering and local refining in the study area. Fifteen of the identified PAHS are among the 16 listed PAHs by USEPA as priority pollutants in aquatic and terrestrial ecosystems and seven of those PAHs may cause cancer in humans. Although the PAHs level analysed in the macrofauna in Idema Creek Basin are below or within the limit permitted or allowed in sea food by WHO,EU/USEPA, but if the activities of illegal bunkering and local refining are allowed to continue unabated for years, it will pose a serious health risk to the inhabitants of Idema creek basin.

KEYWORDS: Polycyclic aromatic hydrocarbons, profile, bioaccumulation, Periwinkle, Idema, crude oil, kpo -fire, pollution

INTRODUCTION

Polycyclic Aromatic Hydrocarbon (PAH) is a large class of persistent organic pollutants that is made up of two or more fused benzene rings. They are known to be ubiquitous in both marine and terrestrial environments (Bouloubassi et al., 2009), and are included in the EU and USEPA priority pollutant lists due to their mutagenic and carcinogenic properties (Nwaichi and Onyeike (2010). PAHs are chemically stable and lipophilic compounds (Bouloubassi et al., 2009), they can easily cross lipid membrane and have the potential to bioaccumulate in aquatic organisms. PAHS are classified into High Molecular Weight (HMW) and Low molecular weight (LMW) types based on their physical and biological properties. Those consisting of 4-6 aromatic rings are termed HMW and have been shown to be less readily bio-degraded by native micro-organisms and can bioaccumulate in the aquatic organisms like fish and mussels. While the LMW PAHs consists of 2-3 aromatic rings and less carcinogenic than HMW type (Brown and Peak, 2006). Wilson et al., 1992 reported the possibility of using periwinkles and oysters as pollution bio monitors because they are sedentary or bottom feeders and are good accumulators of heavy substances.

European Union established a maximum level of 1µgg-1 wet weight for benzo (a) pyrene in foodstuff and is used for carcinogenic risk of PAHs in fish (EFSA, 2006) but recently, it was attributed to dibenz (a,1) Pyrene, a carcinogenic potency that is about 100 times that of benz (a) pyrene (Okona-Mensah et al.,2005)

PAHS are derived from petroleum sources, combustion products, and natural synthesis by organisms (Laflamme and Hites 1978). They accumulate in sediments and biota that are unable to efficiently eliminate them.

The term bioaccumulation is used to describe build-up of pollutant in the body of an aquatic organism by uptake in food and directly from the surrounding water. Through food chain, chemicals like polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT), dioxins, and mercury build up in the bodies of the fish (Chase et al., 2013). During oil spill, hydrocarbons called polycyclic hydrocarbons (PAHs) can accumulate in sea animals. Bioaccumulation or bio-concentration is known to occur in fishes especially in the immature ones than the adults. Perhaps because the polycyclic aromatic hydrocarbons (PAHs) break down system were not fully developed and at times, because of a high percentage of lipid tissues. Fish also have inherent enzyme system for clearing hydrocarbons. This though slow in cold water fish, there is a chance of accumulating hydrocarbons (Irwin et al., 1997).

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There is ample evidence that fish exposed to petroleum sediments, water or through their diet accumulate hydrocarbon in tissues and body fluids. Some of the aromatic hydrocarbon are converted metabolically (metabolites) and remain in liver tissues for prolonged periods. Their concentration in the organ or tissues depends on the concentration of the water soluble components of hydrocarbons that get into their tissues (Jeerderma, 2005). These aromatic hydrocarbons are water soluble and very toxic resulting in cardiac dysfunction, in fold defects, oedema, anaemia, and infracranal haemorrhage (Ramachandian et al., 2005). Hydrocarbons can persist in near shore sediments for decades or longer and imposing long term effects on aquatic ecosystem (Neff, 1985).

PAHs can be linked to cancer in humans that eat fish and shellfish and adversely affect survival, growth and the ability to fight disease in the organism (WHO, 2004).

Knowledge of the processes of bioaccumulation is important for the following reasons : bioaccumulation in organisms may enhance the persistence of industrial chemical in the ecosystem because they can be fixed in the tissues of organisms, stored chemicals are not exposed to direct physical, chemical or biochemical degradation, stored chemicals can directly affect an individual's health and lastly, predators of those organisms that have bioaccumulated harmful substances may be endangered by food chain effect.

The Idema creek basin has been subjected to oil spills for the past two years, resulting from the activities of oil and gas related operations (illegal bunkering and petroleum refining) that has polluted the area and adversely affected the flora, fauna and the inhabitants of the ecosystem which depend on the fisheries resources for their livelihood.

Rather than produce good quality protein and mineral salts, fin and shell fishes from most contaminated Nigerian waters, are transferring through the food chain, bioaccumulated, biomagnified, heavy metals and hydrocarbon at toxic levels that are mutagenic, tetratogenic and carcinogenic to humans. Polycyclic Aromatic hydrocarbons are often linked to oil spills and include carcinogen and chemicals that pose various health risks to human life.

The Idema River Basin is the largest brackish water fisheries in Ogbia Local Government Area, occupied by the Abureni people of the Niger Delta, spanning through Rivers and Bayelsa states of Nigeria. The fisheries provide aquatic resources; fishes, wood, income, employment, domestic water, means of transport and several economic activities for the people of Idema, Obeduma, Eboh, Okoroba, Agrisaba, Nembe and Emago-kugbo. Hence, the need to protect the ecosystem and its inhabitants that depend on these resources for their protein needs.

The aim of this research was to determine the level of bioaccumulation of PAHs in the tissues of *Tympanotonus fuscatus of the Idema creek basin in Ogbia Local Government Area of Bayelsa state and the health implication of the inhabitants that depends on this important biota for their proteins needs.*

There is no specific report on the bioaccumulation of PAHs in the above named macrofauna in the studied ecosystem, thus this study will provide useful information and background in future studies on the bioaccumulation of PAHs in other macro-invertebrates in the contaminated Idema creek and other Rivers of the Niger Delta.

MATERIALS AND METHODS

Description of Study Area

The Idema Creek Basin is located in Ogbia Local Government Area of Bayelsa State in the Niger Delta Area of Nigeria. The river is located within latitude E6o 27’ 37” and longitude N4o 37’ 48” in the Niger Delta of Nigeria. The basin is interconnected with several creeks (Idema, Okoroba, Agrisaba, Nembe and Kugbo creeks), which in turn link into San Barbara and San Bartholomew River through which it empties into the Bonny estuary. It comprises of many networks of creeks and outlets and the largest estuary in Ogbia Local Government Area.

The vegetation of Idema Basin is dominated by red mangrove (*Rhizophora racemosa*), sedge (*Paspalum vaginatum*), white mangrove (*Avicennia africana*), some nypa palm (*Nypa fruticans*). Coconuts (*Cocos nucifera*) and mangoes (*Mangifera indica*) are found at the upper shore limits especially in the fishing settlements and camps along the creek. Rainfall is typical of a tropical rainforest. The creek is brackish water influenced by tidal fluxes and very important as the largest brackish water in the Local Government Area.

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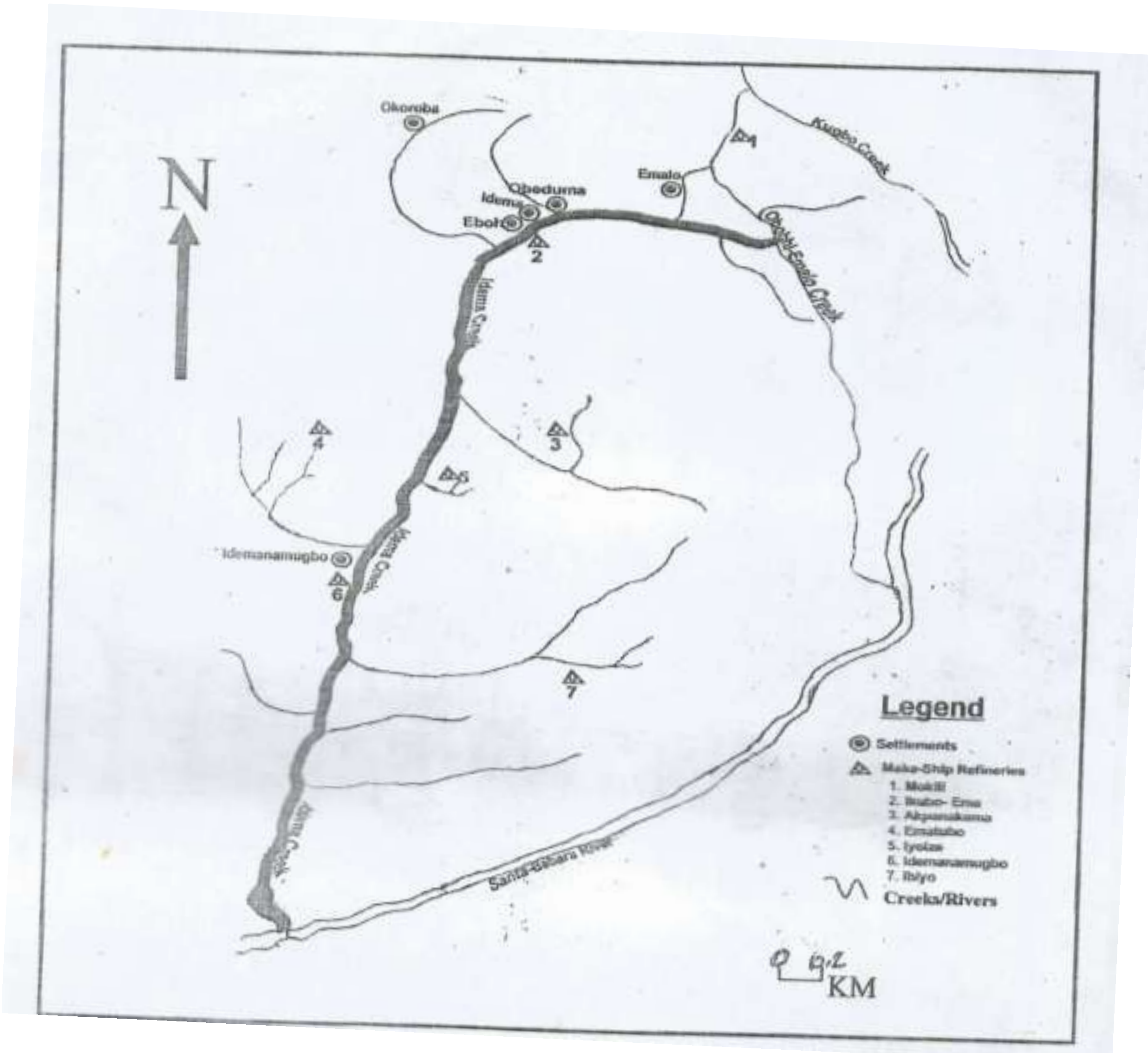

Idema Creek Basin Showing Sampled Locations

Figure 1. SOURCES: Cartograghic Unit, Dept of Geography and Environmental Management, Niger Delta University, Bayelsa state.

Designation of Sampling Stations

Three sampling stations were designated along the Idema Creek basin for the analysis of Polycyclic Aromatic Hydrocarbons (PAHS) in the tissues of T.fuscatuss (fig 1.).Samples were collected tri-monthly from April,207 to March,208

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Station 1 (Ikubo-Ema)

This sampling location is situated at Ikubo-Ema along Idema Creek (fig 1). This station is also influenced by the inland waters and fresh water from Emalo Creek and Iyobia Creek especially during the rainy season. Vegetation is dominated by *Rhizophara racemosa* (red mangrove) and *Avicenia Africana* (white mangrove). Oil palms, coconut and Nypa palms (*Nypa fruticans*) are also found here. The creek is regularly contaminated by crude oil waste from various local refineries outlets adjacent to the creek. This station is significant because, it inhabits three major Abureni towns such as Idema, Obeduma and Eboh town all in Ogbia Local Government Area, Bayelsa State.

Pictures showing sampling station 1 (Ikubo-Ema)

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Station 2 (Iyoize)

Station 2 is located at the apex of a small creek known as Iyoize which is a tributary of Idema creek. Two local refineries of different sizes are established at this station, including a newly constructed one. The vegetation here is also dominated by red and black mangrove. The intertidal flat and surface water is covered by crude oil waste from the makeshift refineries.

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Pictures showing sampling station 2 **(Iyoize kpo-fire site)**

Station 3 (Idemanamugbo)

This station is located at a former fishing settlement known as Idemanamugbo, along Idema creek. Two large local refineries (Kpo-fire) is situated at the end of a small creek (tributary of Idema creek) (fig 1) (picture above). The surface water and sediments in this location is inundated with petroleum products owing to its bunkering activities.

The mangrove and some macrofauna in this location is destroyed. This is exemplified by the numerous empty shells of Tympanotonus fuscatus found in station 3

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Pictures showing sampling station 3 **(Idemanamugbo kpo-fire site)**

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Field sampling

Tympanotonus fuscatus (periwinkle) were collected by hand picking at their respective stations. The macrofauna collected were stored in ice packed coolers and transported to the laboratory where they were stored at 20oc after extraction of the tissues which were washed, packed in Sterile aluminum foil until further analysis.

Laboratory Analysis of polycyclic Aromatic Hydrocarbons Profiles in the tissue of *Typanotonus fuscatus*

Tissues of *T. fuscatus* were oven dried , grounded to powder and kept in air tight containers prior to the extraction process.1g each of the dried and powdered samples where weighed in sample vials, and 10ml aliquot of Dichlorometane (DCM) were measured into the vials. The vials and their contents were left on the bench and hand shaken at intervals. They were kept tightly closed for 72 hours. They were then decanted and filtered. The filtrate was then made up to the 10ml mark in a 10 ml volumetric flask. The samples were then run on the Gas chromatography using Flame Ionization Detector (FID). Transferred extracts were concentrated to 2 μl for clean up/separation in gas chromatographic analysis (HP 5890 series 11, GC apparatus, coupled with flame ionization detector (FID) HP Wilmington, DE, USA. The concentrated aliphatic fraction was transferred into labeled glass vials with leflon or rubber crimp caps for GC analysis.1µl of the concentrated sample was injected by means of hypodermic syringe through a rubber septum into the column. Separation occurred as the vapour constituent partitioned between the gas and liquid phases. The sample was automatically detected as it emerged from the column (at a constant flow rate) by the FID detector whose response was dependent upon the composition of the vapour.

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Typanotonus fuscatus
(Periwinlke)

STATISTICAL ANALYSIS

Data for PAHS parameters were analyzed by SPSS package to calculate means and standard deviations and one- way analysis of variance (ANOVA) was used to estimate the significant difference between stations. Duncan mean separation technique was also performed to separate the means.

RESULTS

The results for PAHs profile or bioaccumulation in the Periwinkle (*T. fuscatus*) are presented in table 1, 2, figure 2. Fifteen (15) Polycyclnic aromatic hydrocarbons were identified in the tissues of *T.fusctus*).The mean individual PAHs distribution in the tissues of *T. fuscatus* is also found in table 2. The monthly mean total PAH (∑PAH) in *T. fuscatus* are presented in table 1.

The highest ∑PAH value of 1.95755 µg/g d.wt in *T.fuscatus* was analyzed for the month of April, 2017 in station (2) and the lowest ∑PAH value of 0.010431µg/g d.wt in *T.fuscatus* was recorded for the month of February, 2018 in station 2 (Ikabo-Ema) Table 1 and figure 2.

The mean distribution of the identified PAHs in *T.fuscatus* in the studied area as follows: Benzo (b)

Fluoranthene – 0.01003± 0.008µg/g dry weight d.wt Benzo (k) Fluoranthene – 0.00060± 0.00µg/g d.wt.Benzo (a) Pyrene – 0.03392±0.0121µg/g d.wt. Indeno (1,2,3-cd) Pyrene – 0.00038±00µg/g d.wt. Dibenz (a,h) anthracene – 0.00166±0.00µg/g dwt. Acenaphthene – 0.00105±0.00µg/g dwt. Acenaphthylene – 0.00248±0.00µg/g d.wt. Naphthalene – 0.00838±0.00µg/g dwt.2-methyl naphthalene – 0.01139±0.01µg/g dwt. Fluorene – 0.00160±0.00µg/g d.wt. Anthracene – 0.00300±0.00µg/g dwt. Flouranthene – 0.00172±0.00µg/g dwt. Pyrene – 0.00105±0.00µg/g d.wt. Benz (a) anthracene – 0.0032±0.00µg/g dwt (Table 2)

Benzo (a) Pyrene recorded the highest mean value of 0.34±0.02µg/g dry weight and Indo (1,2,3-cd) Pyrene recorded the lowest mean value of 0.00038±0.00µg/g dry weight (Table 2). The highest monthly mean total PAH of 0.727± 0.617 µg/g d.wt in *T. fuscatus* was recorded for the month of April, 2017 and the lowest PAH mean value of 0.019±0.007µg/g d.wt was analysed for the month of February, 2018 (Table 2 , figure 2). There was no significant difference (P > 0.05) of PAHs occurrence across months and stations (Table 1 and 2)

Table 1: Total PAH in All the Sampling Months of Idema Creek Basin April 2017 – March 2018

		ΣPAH (µg/g)			
	Station	**Apr-17**	**Aug-17**	**Nov-17**	**Feb-18**
T. fuscatus					
	1	1.95775	0.020476	0.0216	0.010431
	2	0.17189	0.225377	0.025628	0.032721
	3	0.05053	0.096142	0.020223	0.012763
	Mean	0.726723	0.113998	0.022484	0.018638
	STD	1.067826	0.103611	0.002809	0.012252
	S.E	0.61651	0.05982	0.001622	0.007073

P >0.05

Table 2: Mean Distribution of PAH per Species of Idema Creek Basin April 2017 – March 2018

		Benzo (b) Fluoranthene	**Benzo (K) Fluoranthene**	**Benzo (a) Pyrene**	**Indeno (1,2,3 – cd) Pyrene**	**Dibenz (a, h) anthrancene**	**Acenaphthene**	**Naphthalene**	**2-Methyl Naphthalene**	**Acenaphthylene**	**Fluorene**	**Phenanthrene**	**Anthracene**	**Flouranthene**	**Pyrene**	**Benz (a) anthracene**	**Chrysene**
Tympanotonus fuscatus	Mean (µg/g)	0.01003	0.00060	0.03392	0.00038	0.00166	0.00105	0.00838	0.01139	0.00248	0.00160	0.00174	0.00300	0.00172	0.00105	0.00323	0.34387
	S.E	0.00838	0.00017	0.01212	0.00009	0.00086	0.00053	0.00156	0.00577	0.00068	0.00065	0.00071	0.00118	0.00048	0.00045	0.00098	0.21248

P >0.05

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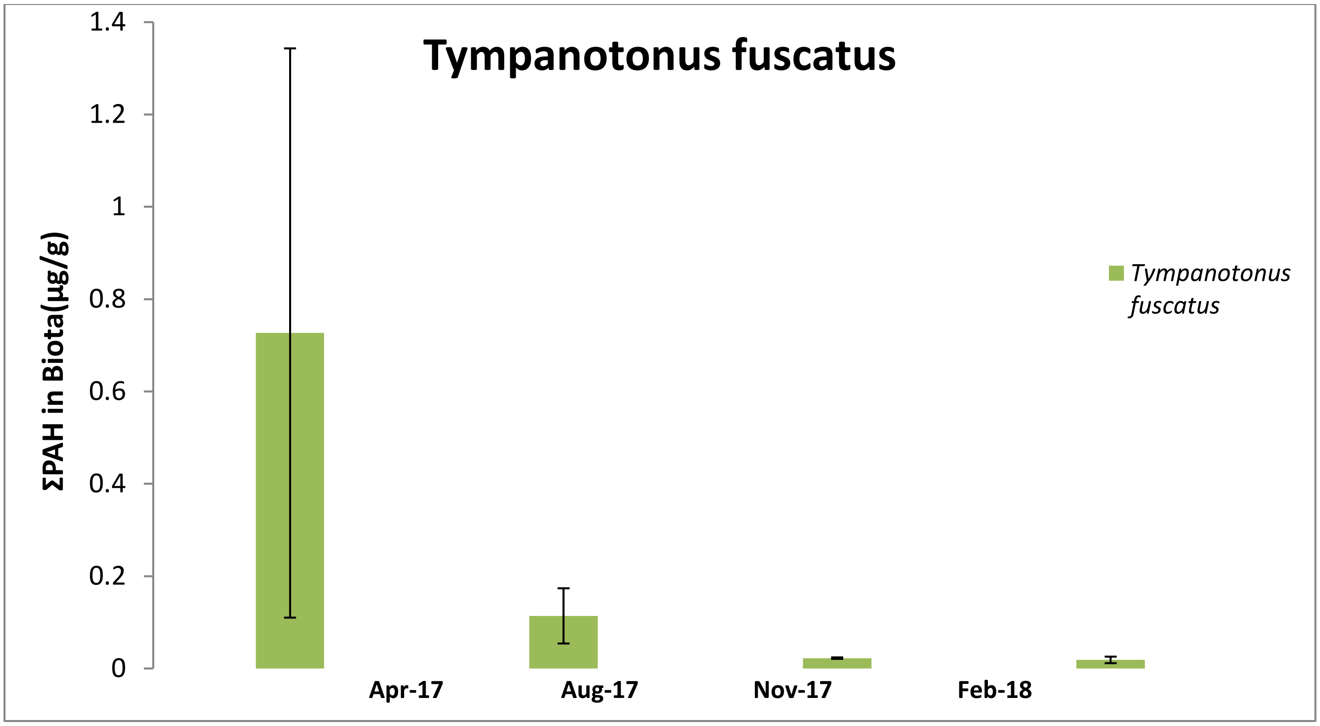

Figure 2: Monthly Mean ΣPAH *in T.fuscatus in Idema* Creek Basin

DISCUSSION

The results from the analysis, identified fifteen Polycyclic Aromatic Hydrocarbons in the tissue of T.fuscatus in Idema creek basin :

Seven Low Molecular Weight PAHs (L MW-PAHs) such as:

Naphthalene, 2-Methyl Naphalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene and Anthracene were identified in the tissues of the studied marcrofauna in Idema creek basin. While eight High Molecular Weight PAHs (HMW-PAHs) which include: Fluoranthene, Pyrene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Benzo (a) anthracene, Dibenz (a,h) anthracene and Ideno 1,2,3-cd pyrene were found in the tissues of T. fuscatus

The above findings indicate that; PAHs Identified in the tissues of these aquatic fauna may have originated from incomplete combustion of fossil fuel (anthropogenic activities) and crude oil spills occasioned by the various local refineries (kpo-fire) in the study area. Fifteen (15) of the identified PAHS are among the 16, listed PAHs by USEPA as priority pollutants in aquatic and terrestrial ecosystems (Keith and Telliard 1979). Seven of these PAHs may cause cancer in humans (USEPA 1993) and Benzo (a) Pyrene is considered the highest cancer risk among the 16 PAHS (USEPA 1993, Lagoy and Quirk, 1994). Polycyclic

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aromatic hydrocarbons is also ranked 9th on the 2015 Agency for Toxic substances and Diseases Registry (ATSDR) Priority list of hazardous substances (PLHS) as a result of their toxicity, frequency of occurrence at USEPA National priorities list sites and potential for human exposure (ATSDR, 2015).

Benzo (a) Pyrene recorded the highest mean value of 0.34+0.02µg/g dry weight and Indo (1,2,3-cd) Pyrene recorded the lowest mean value of 0.00038±0.00µg/g dry weight. The highest monthly mean total PAH of 0.727± 0.617 µg/g d.wt in T. fuscatus was recorded for the month of April, 2017 and the lowest PAH mean value of 0.019±0.007µg/g d.wt was analysed for the month of February, 2018 . There was no significant difference ($P > 0.05$) of PAHs occurrence across stations and months. Olalade et al., (2011) in their study of occurrence and dynamics of hydrocarbon in periwinkles (Littorina littorea) within oil polluted coastal area of Ondo State found PAHs to ranged from not detectable (nd) to 56.23 µg/g dw in the dry season and nd to 79.43 µg/g dw during wet season, above the recommended permissible limit of 2.0µg/g in sea food by European Union (2011) and USEPA (1993).

Ogeleka and Tudararo – Aherobo (2011) in their determination of sixteen PAHs in the tissues of T. fuscatus at a test termination of. 28 days identified only three components of PAHs at relatively low concentrations which ranged; Benzo (b) Fluoranthene (0.0017-0.0039 ppm), Phenanthrene (0.0021-0.00449 ppm) and pyrene (0.0035-0.0081ppm). Other studies in the Niger Delta identified Total hydrocarbon content (THC) and Total Petroleum hydrocarbon (TPH) in the tissues of T-fuscatus. Ogamba et al., (2016) recorded THC levels in T. fuscatus to range from 0.520 to 5.667 Mg/kg from the coastal region of Bayelsa State. THC concentrations in tissues of T.fuscatus and pachymelania aurita from upper Bonny estuary varied from 764.31 µg/g to (Ideriah et al., 2006). The high THC levels recorded was attributed to the level of anthropogenic activities at each station. Nwabueze et al., (2011) also recorded mean total hydrocarbon in the tissues of T. fuscatus from Warri river that ranged from 0.0045 – 0.093Mg/g but the concentrations were below the recommended tolerable levels.Benson and Essien, (2009) found the mean TPH in the tissues of T. fuscatus from Qua Iboe mangrove ecosystem to range from 9.40±1.0µg/g dw to .Nwaichi and Ntorgbo (2016) in their assessment of PAHs and seafood from different coastal waters in the Niger Delta recorded mean PAHs levels in samples which ranged from below detection limit (BD) of analytical instruments to 22.400 ± µg/g wet weight in Littorina littorea. The study demonstrated evidence of PAHs bioaccumulation in the tissues of T. fuscatus from the study area, although levels were within and below the permissible limits allowed in seafood established by EU (2011)/USEPA (1993).

CONCLUSIONS

The major objective of this study was to determine the level of polycyclic aromatic hydrocarbon in the tissues of T.fuscatus, in Idema Creek basin, Bayelsa state. Niger Delta, Nigeria. Fifteen (15) PAHs were identified in the tissues of the above studied macrofauna. Seven low molecular weight PAHs and eight high molecular weight PAHs were identified, indicating pyrogenic and petrogenic origin or source, may be as a result of the activities of crude oil bunkering and local refining in the study area. This research demonstrated evidences of PAHs bioaccumulation in the tissues of T.fuscatus, although levels were within and below the permissible limits of 2µg/g d.wt allowed in seafood established by EU (2011) /USEPA (1993).

The fifteen (15) of the identified PAHs in the tissues of the studied aquatic macrofauna are among the 16 listed PAHs by USEPA as priority pollutants in aquatic and terrestrial ecosystems and seven of these PAHs may cause cancer in humans (USEPA 1993) and benzo (a) pyrene is considered the highest cancer risk among the 16 PAHs. Polycyclic aromatic hydrocarbons, is also ranked 9th on the 2015 Agency for Toxic substances and Diseases Registry (ATSDR) priority list of hazardous substances (PLHS) base on their toxicity, frequency of occurrence at USEPA National priority list sites and potential for human exposure (ATSDR, 2015). There may be metabolites in the studied macrofauna that are capable of causing cancer and other health challenges in human that depends on this important aquatic macrofauna as source of proteins, especially the inhabitants of the studied area. Accumulation of these toxic polluntants (PAHs) will continue to increase in the tissues of aquatic species in the studied estuary if the activities of crude oil bunkering and local refining continue unabated.

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