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Human Health Risk Assessment of PAHs in Fish and Shellfish from Amariaria Community, Bonny River, Nigeria

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ABSTRACT : The concentration of polycyclic aromatic hydrocarbons (PAHs) in Fish (Mullet fish-*Mugil cephalus)* and Shellfish (Tiger prawn-*Penaeus Monodon* and crab-*Uca tangeri)* samples from fishing areas in Amariaria Community, downstream of Bonny River, Southern Nigeria, were assessed to determine possible human health risk associated with consumption. Mean levels (mg/kg) of total PAHs ranged from 0.059 to 0.126 in fish, 0.015 to 0.106 in prawn and 0.057 to 0.063 in crab. A considerable predominance of the 3 and 4-rings PAHs in all the matrices was observed with benzo (a) anthracene dominating in all three species. Estimated daily intake (EDI) of PAHs through consumption of fish ranged from 0 to 0.0005 mg/kg/day, for prawn, 0 to 0.0002 mg/kg/day and for crab, 0 to 0.0002 mg/kg/day. EDI values were, however, lower than the reference dose (RfD) indicating low risk from consumption. Results of the estimated excess cancer risk (ECR) for Benzo (a) anthracene in fish, however, suggests that lifetime exposure to Benzo (a) anthracene through fish consumption would result in cancer risk.

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Aquatic organisms like fish and shellfish are vulnerably exposed to toxic chemicals released from industrial, agricultural and municipal sources (Copat *et al.,* 2013). Many of these chemicals, which most times are carcinogenic accumulate in fish and shellfish, binding to fatty tissues or muscle tissues (Copat *et al.,* 2013). Dietary exposure is, therefore, the predominant route of exposure of humans to these contaminants (Wu *et al.,* 2012).One of such contaminants is PAHs.

PAHs are persistent organic compounds (POPs) with a wide range of distribution in various environmental media (Wu *et al.,* 2012). They are important components of crude oil and have been reported in areas of crude oil spills (Awajiusuk, 2015). The Bonny River is one of such rivers affected by oil spills (Awajiusuk (2015)). Along the Bonny river is a mobile Nigeria National petroleum Corporation (NNPC) filling station, and gas flaring stations from three oil and gas companies (Exxon Mobil, Nigeria Liquefied Natural Gas company (NLNG), and SHELL Nigeria). Worse still are activities of illegal bunkering and refining of crude oil locally known as '*kpo'* fire which most times led to incessant spills (Awajiusuk, 2015). Amariaria community is one of the fishing settlement and landing site for fish catch along Bonny River that is affected by most of these spills. PAHs found in crude oil have the potential to accumulate in aquatic organisms and can consequently result in potential health risk through ingestion of contaminated seafood (Yender *et al.,* 2002). Fish, crustaceans, such as shrimp, prawn, and crab are especially likely to be contaminated (Law *et al.,* 2002).

PAHs have been reported in different environmental media including fish and shellfish in this region (Nkpaa *et al.,* 2013; Nwaichi and Ntorgbo, 2016*).*PAHs have received considerable attention in recent times because of their highly carcinogenic potentials (Wu *et al.,* 2012) therefore, be reasonable to comprehend that residual levels of PAHs in fish and shellfish, especially edible species could have a great effect to human health (Llobet *et al.,* 2006). Sadly, only very few studies have paid direct attention to the public health consequences of eating PAH contaminated aquatic species used as food.

The study was therefore carried out to evaluate the degree of contamination of fish (Mullet fish-*Mugil cephalus)* and Shellfish (Tiger prawn-*Penaeus Monodon* and crab-*Uca tangeri)* from Amariaria, a major fish landing site along the Bonny River, to

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assess the potential risk to human health from consumption.

MATERIALS AND METHODS

Study area: The Bonny River (4° 26′ 0″ N and 7° 10′ 0″ E) is an arm of the Niger River Delta in Rivers state, Southern Nigeria. The River is a terminal for crude oil export and along its coast are three oil and gas exploration companies (Shell Nigeria, Mobil producing and Nigeria Liquefied Natural Gas (NLNG)). There is also an awareness of illegal bunkering activities by militants. Amariaria Community (4° 24′ 10″ N and 7° 8′ 12″ E) is located in Finima town, Bonny Local Government Area, downstream of Bonny River. This community is on the East side of the Nigeria Liquefied Gas company export site. It is a fishing settlement and a landing site for fish catch (Figure 1).

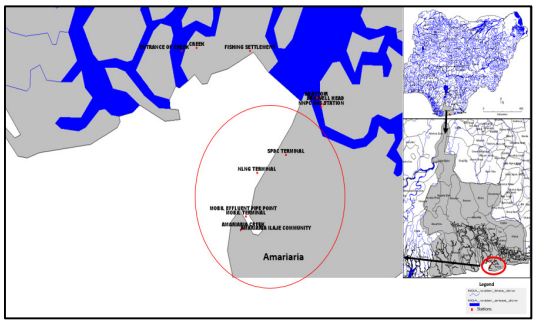

Fig 1: Map of Bonny River, showing Amariaria Community with Sampling Stations

Sample Collection: Mullet fish *(Mugil cephalus*), Tiger prawn (*Penaeus monodon*) and crab (*Uca tangeri*) samples were purchased from local fishermen at sampling locations. All samples were weighed (g), washed then wrapped in aluminum foil and transported immediately to the laboratory in polythene bags. They were refrigerated at 4 °C until extraction (Ezemonye *et al.* 2008).

Analytical procedures: The whole samples of biota were analyzed for PAHs. Analytical procedures for PAHs used in this study are described in detail previously (US EPA, 1986). Frozen composite whole-body tissue was inserted into a homogenizer cup and 100 ml of acetone was added. Samples were homogenized for 20 minutes at 100 rpm and mixed further with 5g of anhydrous sodium sulphate. Extraction was done using soxhlet extraction for approximately 5 hours using dichloromethane and n-hexane mixture. The resulting solvent was eluted with 50 ml n-hexane solvent, evaporated again until 1 - 3 ml. Determination of PAHs in the biota was carried out following standard procedures using Gas chromatography (GC, Hewlett-Packard HP-5890 Series II *with* flame ionization detection (*GC-FID*)).

Human Health Risk Assessment: Human health risk assessment was carried out to estimate the probability of adverse health effects in humans as a result of exposure to PAHs through consumption of contaminated fish. All calculations were done based on USEPA standards (USEPA, 1996). The assessment was carried out for adults (70kg) for both non-carcinogenic and carcinogenic health risk. The description and values of the parameters used for the various calculations are presented in Table I.

Estimated daily intake (EDI): The estimated daily intake (EDI) (mg/kg/day) of PAHs in fish, prawn and crab samples were estimated using Equation 1.

$$\text{Estimated Daily Intake (EDI)} = \frac{Cf\ X\ IFR}{BW} \quad 1$$

Assessment of non-carcinogenic and carcinogenic health risks: Assessment of non-carcinogenic and carcinogenic health risks was achieved by estimating the hazard quotient (HQ) and hazard index (HI), while the carcinogenic potency of individual PAHs and Excess Cancer Risk (ECR) were used specifically to further estimate carcinogenic health risk. The HQ for non-carcinogenic risks from exposure to PAHs was calculated by dividing the EDI by reference dose (RfD) (Equation 2), while the HQ for carcinogenic risks was estimated using Equation 3.

$$\text{Hazard Quotient (HQ}_{\text{Non-carcinogenic}}) = \frac{EDI}{RfD} \quad 2$$

$$\text{Hazard Quotient (HQ}_{\text{Carcinogenic}}) = EDI\ X\ SF \quad 3$$

The hazard index, which estimates the total risk from multiple contaminant pathways, was obtained by summing the HQ of the contaminant pathway (Equation 4). Risk was evaluated for both non-carcinogenic and carcinogenic risks. Values of HQ and HI of contaminants under one (1) are considered as safe (USEPA, 1986).

$$\text{HI} = \sum_{i=1}^{n} HQi \quad 4$$

The carcinogenic potency of individual PAHs was determined as the product of the concentration of individual PAH congeners and their toxicity equivalency factor (TEF) (Equation 5), while ECR was estimated using Equation 6.

$$\text{Carcinogenic potencies for PAHs (B(A)Pteq)} = PAHi\ X\ TEFi \quad 5$$

$$\text{Excess Cancer Risk (ECR)} = \frac{\sum Q\ X\ B(A)Pteq\ X\ IFR\ X\ ED}{BW\ X\ ATn} \quad 6$$

Table 1: Parameters used for estimating exposure assessment through Fish Consumption

Parameters	Unit	Value	Reference
Mean concentration of PAHs	mg/kg-fish, Prawn, and Crab	Table 2	Table 2
Reference Dose (?????)	mg/kg/day	Table 2	USEPA, 1993
Fish/Crustacean ingestion rate *(IFR)*	Kg/capita/day	0.85(Marine Fish)0.33 (Crustaceans)	FAO, 2014
Exposure Duration (*ED*)	years	70	Qu *et al.* 2015
Exposure Frequency (*EF*)	Days/year	365	Qu *et al.* 2015
Adult body weight (*BW*)	kg	70	Tongo *et al.*, 2017
Average life span *(ATn)*	days	25550	Papadakis *et al.* 2015
Oral Slope Factor *(SF)*	mg/kg/day	US EPA 2005	US EPA 2005
Toxicity equivalence factor (*TEFi*)	No Unit	Nisbet and LaGoy, 1992	Nisbet and LaGoy, 1992

RESULTS AND DISCUSSION

*PAHs levels in Fish, Prawn, and Crab***:** Quantitative results of PAH congeners in fish and shellfish samples from Bonny River, Southern Nigeria is presented in Table 2.

Table 2: Mean concentration of PAHs in Fish and Shellfish from Amariaria Community, Bonny River, Nigeria

		Fish	Prawn	Crab
PAHs (mg/kg)	Code	Mean±SD	Mean±SD	Mean±SD
Naphthalene	NaP	0±0	0±0.000	0.001±0.002
Acenaphthylene	AcPY	0±0	0.004±0.007	0.004±0.006
Acenaphthene	AcP	0.001±0.002	0.022±0.042	0.009±0.014
Fluorene	Flu	0±0	0.008±0.015	0.006±0.010
Phenanthrene	Phe	0.003±0.006	0.017±0.033	0.015±0.031
Anthracene	Ant	0.004±0.008	0.005±0.006	0.005±0.005
Fluoranthene	FL	0.003±0.005	0.002±0.003	0±0
Pyrene	Pyr	0±0	0.001±0.001	0±0
Benzo(a)anthracene	BaA	0.049±0.048	0.047±0.042	0.013±0.018
Chrysene	Chr	0.002±0.004	0±0	0.001±0.002
Benzo(k)fluoranthene	BkFL	0±0	0±0	0±0
Benzo(a)pyrene	BaP	0.004±0.00	0.002±0.003	0.004±0.008
Benzo(b)fluoranthene	BbFL	0±0	0±0	0±0
Indeno(1,2,3)pyrene	Ind	0±0	0±0	0±0
Dibenzo(a,h)anthracene	DBA	0±0	0±0	0±0
Benzo(g,h,i,)perylene	BP	0±0	0±0	0±0
TOTAL PAH	∑PAH	0.065±0.061	0.106±0.141	0.057±0.088
Total Carcinogenic PAHs	∑CPAH	0.055±0.049	0.048±0.044	0.018±0.025

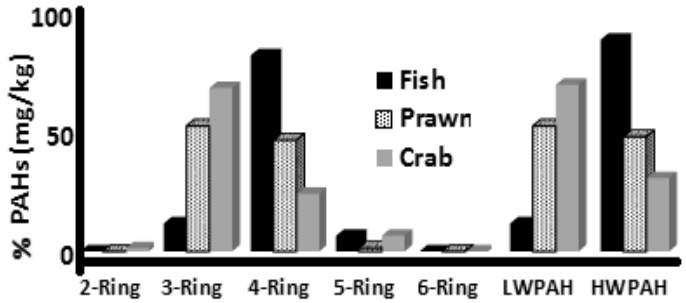

Fig 2: Mean percentage composition of PAHs by ring-type in biota from Amariaria Community, Bonny River, Nigeria

Mean concentrations for total carcinogenic PAHs (sum of BaA, Chr, BkFL, BaP, BbFL, Ind, DBA, BP) accounted for 85%, 45% and 31% respectively in fish, prawn and crab of the total PAHs (Table 2). Total mean carcinogenic PAH concentrations were higher in fish (0.05 mg/kg) than prawn and crab, but differences in concentrations were not statistically significant between the species ($p>0.05$, $F= 0.26$). Total mean PAH concentrations were higher in prawn (0.12 mg/kg) than fish and crab, however, concentrations were not significantly different between the species ($p>0.05$, $F= 0.40$). For individual concentrations of PAHs, benzo(a)anthracene was the most dominant congener in fish and prawn samples (Table 2) and concentrations were significantly higher ($p<0.05$) than the other congeners, with mean concentrations of 0.049±0.048 and 0.047±0.042 mg/kg, accounting for 75% and 44% of the total PAHs in fish and prawn respectively. Phenanthrene was the most dominant congener in crab with a mean concentration of 0.015±0.031 mg/kg and a percentage contribution of 27%. However, Phenanthrene concentrations in crab were not significantly higher than the other congeners ($p>0.05$).

Table 3: Estimated daily intake, Non-Carcinogenic and Carcinogenic Risk of PAHs for adult (70-kg body weight) from consumption of fish and shellfish

	Prawn				
PAHs	EDI	HQ(Non-carcinogenic)	HQ Carcinogenic	B(A)Pteq	ECR
NaP	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
AcPY	1.7E-05	4.1E-03	NA	3.5E-06	3.3E-10
AcP	1.0E-04	NA	NA	2.2E-05	2.1E-09
Flu	3.9E-05	6.5E-04	NA	8.3E-06	7.8E-10
Phe	7.9E-05	2.0E-03	NA	1.7E-05	1.6E-09
Ant	2.5E-05	NA	NA	5.3E-05	5.0E-09
FL	9.4E-06	3.1E-05	NA	2.0E-06	1.9E-10
Pyr	2.4E-06	5.9E-05	NA	5.0E-07	4.7E-11
BaA	2.2E-04	7.3E-03	NA	4.7E-03	4.4E-07
Chr	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BkFL	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BaP	7.1E-06	NA	5.2E-08	1.5E-03	1.4E-07
BbFL	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
Ind	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
DBA	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BP	0.0E+00	NA	0.0E+00	0.0E+00	NA
		HI= 1.4E-02	**HI=5.2E-08**		
	Fish				
PAHs	EDI	HQ(Non-carcinogenic)	HQ(Carcinogenic)	B(A)Pteq	ECR
NaP	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
AcPY	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
AcP	9.1E-06	NA	NA	7.5E-07	1.8E-10
Flu	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
Phe	3.3E-05	8.3E-04	NA	2.8E-06	6.7E-10
Ant	5.1E-05	NA	NA	4.2E-05	1.0E-08
FL	3.3E-05	1.1E-04	NA	2.7E-06	6.5E-10
Pyr	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
BaA	6.0E-04	2.0E-02	NA	4.9E-03	1.2E-06
Chr	2.1E-05	NA	1.6E-05	1.8E-05	4.3E-09
BkFL	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BaP	5.2E-05	NA	3.8E-07	4.3E-03	1.0E-06
BbFL	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
Ind	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
DBA	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BP	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
		HI=2.1E-02	**HI=1.6E-05**		
	Crab				
PAHs	EDI	HQ(Non-carcinogenic)	HQ(Carcinogenic)	B(A)Pteq	ECR
NaP	3.5E-06	1.8E-04	NA	7.5E-07	7.1E-11
AcPY	1.9E-05	4.7E-03	NA	4.0E-06	3.8E-10
AcP	4.0E-05	NA	NA	8.5E-06	8.0E-10
Flu	2.9E-05	4.9E-04	NA	6.3E-06	5.9E-10
Phe	7.2E-05	1.8E-03	NA	1.5E-05	1.4E-09
Ant	2.4E-05	NA	NA	5.0E-05	4.7E-09
FL	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
Pyr	0.0E+00	0.0E+00	NA	0.0E+00	0.0E+00
BaA	6.0E-05	2.0E-03	NA	1.3E-03	1.2E-07
Chr	4.7E-06	NA	3.4E-06	1.0E-05	9.4E-10
BkFL	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BaP	1.8E-05	NA	1.3E-07	3.8E-03	3.5E-07
BbFL	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
Ind	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
DBA	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
BP	0.0E+00	NA	0.0E+00	0.0E+00	0.0E+00
		HI=9.2E-03	**HI=3.6E-06**		

The occurrence of pollutants in fish and shellfish depends largely on environmental concentrations of these compounds and on the physiology and ecological characteristics of the species *(*Meador *et al., 1995).* Crustaceans are especially likely to be contaminated because of reduced rates of biological clearance of PAHs in these species (Law *et al.,* 2002). This could explain the reason for the higher concentrations of PAHs in prawn compared to fish and crab. Concentrations reported in this study for PAHs for prawn were higher than that reported by Nkpaa *et al.,* 2013 from Ogoniland, Rivers State, Nigeria, and Llobet *et al.,* 2006 from Catalonia, Spain. The PAH composition pattern by ring type showed a considerable predominance of the three-ring and four-ring type PAHs (Fig. 3). The mean percentage concentration of the lower molecular weight PAHs (LWPAHs) (two to three rings) was higher than the higher molecular weight PAHs (HWPAHs) (four to six rings) in prawn and crab accounting for 52% and 69% respectively of the total PAH, while for fish the mean percentage concentration of the HWPAHs was higher than the LWPAH accounting for 88% of the total PAHs in fish (Figure 2). Differences in concentrations between the HWPAH and LWPAH PAHs among the species were however not statistically significant ($p>0.05$).

Human Health Risk Assessment of PAHs levels in Fish, Prawn, and Crab: Toxicological risk connected to PAHs was assessed by comparison with legal limits and through estimation of dietary intake, non-carcinogenic and carcinogenic risks (Tongo *et al.,* 2017). Benzo (a)pyrene (B(a)P) is usually used as a marker for the occurrence and effect of carcinogenic PAHs in food (Lee and Shim, 2007). Consequently, Benzo (a) pyrene (BaP) concentrations in fish and shellfish were compared to the existing EU recommended limit. Concentrations of B (a) P in fish and shellfish were observed to have exceeded the safe limit of 0.002mg/kg for human fish consumption and 0.0005 mg/kg for consumption of crustaceans (shellfish). The high Benzo (a) pyrene (BaP) concentrations in fish and shellfish exceeding the EU recommended safe limit thus calls for serious health concerns. (Table 2).

For risk assessment, dietary exposure to PAHs, the non-carcinogenic and carcinogenic risks were estimated. Daily dietary intake of PAHs (mg/kg body weight/day) through fish and shellfish consumption for adult (70kg) is shown in Table 3. Consumption of fish contributed to the highest intake of PAHs with Carcinogenic PAHs accounting for 45%, 84% and 31% in prawn, fish, and crab respectively. The estimated daily intake of PAHs in all the species analysed were however observed to be lower than the reference dose (RfD) indicating low risk through consumption. The average HQs and HIs for PAHs in fish and shellfish samples for non-carcinogenic and carcinogenic health risk also showed no potential negative health effect on consumers as values were below 1. The potency of PAHs in fish and shellfish to cause carcinogenic health risk was evaluated using individual carcinogenic potencies for PAHs. Benzo(a)anthracene had the highest carcinogenic potency (mg/kg) in prawn (0.0047) and fish (0.0049) while Benzo(a)pyrene had the highest carcinogenic potency (mg/kg) in crab (0.0038)(Table 3). Results for individual carcinogenic potencies for benzo(a)anthracene and Benzo(a)pyrene in fish and shellfish showed values exceeding the guideline screening value of 0.67 ng/g (wet wt) (USEPA 2000), for human consumption indicating high potential carcinogenic risk. In addition results of the estimated excess cancer risk (ECR) from lifetime exposure to PAHs through fish and shellfish consumption was calculated and compared to the acceptable guideline value of 1×10^{-6} set by USEPA (Ding *et al.,* 2012). The ECR for Benzo(a)anthracene in fish (Table 3) suggests that lifetime exposure to Benzo(a)anthracene through fish consumption would result in cancer risk.

Conclusion: The present study showed varying levels of PAHs in Fish and Shellfish from in Amariaria Community, downstream of Bonny River, Southern Nigeria and also revealed high potential for carcinogenic risk in humans from fish consumption. The study therefore provides reasonable evidence on the need to fully evaluate the risks of PAHs in fish and shellfish to safeguard the health of consumers.

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