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Research Article

Polycyclic Aromatic Hydrocarbons in Sediment and Tissues of the Crab *Callinectes pallidus* from the Azuabie Creek of the Upper Bonny Estuary in the Niger Delta

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Abstract: The accumulation of Polycyclic Aromatic Hydrocarbons (PAH) in sediment and the flesh/carapace of the edible swimming crab *Callinectes pallidus* from Azuabie creek in the upper Bonny Estuary, Niger Delta and Nigeria were studied. Sediment and crab samples were collected from three sampling stations along the Azuabie creek in July (rainy season) and November (dry season) 2007. These stations were selected to reflect point of abattoir waste input (which includes ash from tyre used for roasting meat) into the creek as well as locations upstream and downstream of the point. Seasonal and spatial variations were observed in the concentrations of PAH. Significant differences in PAH ($p<0.01$) were observed between rainy season and dry season samples. The major classes of PAH found in sediment and *C. pallidus* were Naphthalene, Benzo (a) pyrene, Benzo (a) anthracene and Phenanthrene which are known to be carcinogenic. The PAHs were higher during the rainy season probably due to run-off of tyre ash from the nearby abattoir. The observations made in this study suggest that *C. pallidus* from the Azuabie creek might not be safe for human consumption because of the levels of PAHs found in the flesh of samples mostly in the rainy season. The abattoir appears to impact the creek because significantly higher levels of PAHs were recorded at the proximate sampling station.

Keywords: Bioaccumulation, Bonny estuary, Nigeria, polycyclic aromatic hydrocarbons, sediment

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are compounds that consist of fused aromatic rings and do not contain heteroatoms or carry substituent's (Fetzer, 2000). PAHs occur in oil, coal and tar deposits and are produced as a by-product of fuel burning. They are of concern as a pollutant because some of them are carcinogenic, mutagenic and teratogenic (causing malformation of an embryo). They are also found in foods like cereals, oils and fats with smaller amounts in vegetables and cooked meats (Larsson, 1983). According to Luch (2005) the PAHs known for their carcinogenic, mutagenic and teratogenic properties are benz (a) anthracene and chrysene, benzo (b) fluoranthene, benzo (j) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, benzo (ghi) perylene, coronene, dibenz (a, h) anthracene (C_2OH_{12}), indenol (1, 2, 3-Cd) pyrene ($C_{22}H_{12}$) and ovalene. Their toxicity is structurally dependent with isomers varying from being non-toxic to extremely toxic.

Burning tyres can have serious environmental impact as they produce vast amount of harmful emissions that will pollute the atmosphere and water courses through run-offs. Burning tyres can have a serious environmental impact and tyre fires are quite common in Nigeria. The use of tyres for roasting meat in abattoirs is also a common practice. This burning results in palls of black smoke that is visible from quite a distance. They produce vast quantities of harmful emissions that will pollute the atmosphere and water courses by input of potentially harmful compounds, including PAHs through run-offs.

Uptake of hydrocarbons, particularly PAHs compound by aquatic biota is very rapid and may accumulate high tissue concentrations (Eisler, 1987; Varanasi *et al*., 1989). The PAHs are relatively stable constituent of petroleum and many of these compounds are potentially toxic, carcinogenic and mutagenic (Ghauch *et al*., 2000; Aderemi *et al*., 2003). Various species of crabs have been used to monitor PAH exposure around the world: *Cancer magister* in British Columbia, Canada (Eickhoff *et al*., 2003), *Callinectes sapidus* from Chesapeake Bay, (Mothershead *et al*., 1991; Hale, 1988), *Cancer iroratus* from New York Bight (Humason and Gadbois, 1982; Pancirov and Brown, 1977); *Polybius henslowi* on the north coast of France and Spain (Baumard *et al*., 1998); *Scylla serrate* from Brisbane River estuary in Australia (Kayal and Connell, 1995); *Callinectes sapidus* from Ondo Coastal area in Nigeria (Oladele *et al*., 2008). In this study, we report the concentrations of PAHs in sediment and

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tissues of the edible crab *Callinectes pallidus* from Azuabie creek which receives run-off of effluents from tyre burning activities. Seasonal and spatial patterns of PAH levels in relation to the sources of contamination are discussed.

MATERIALS AND METHODS

Study sites: The Azuabie creek is located between latitude 7º3' N, longitude 4°48' E and latitude 7º 1' 30" N and longitude 4°52" in the upper Bonny Estuary,

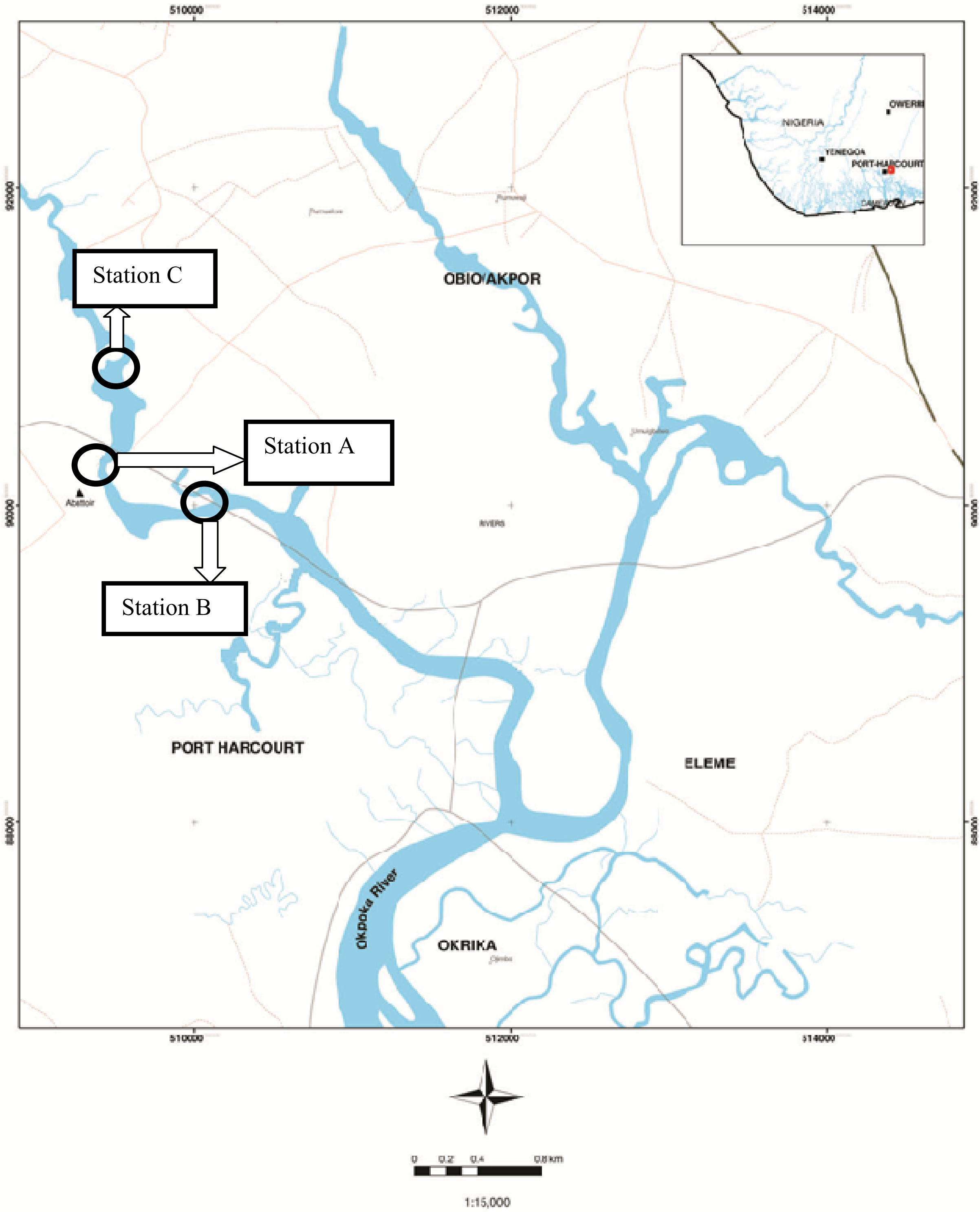

Fig. 1: Azuabie creek showing locations of sampling stations. Inset-the Niger delta showing location of the creek

Southern Nigeria (Daka *et al*., 2007). The hydrodynamics of the estuarine creek and the presence of the point source of effluent run-off from the coastal abattoir were key considerations for the location of study stations along the creek. Three stations were established (Fig. 1) as follows:

- **Station A:** Abattoir-Opposite abattoir representing point source of surface run-off from the tyre burning activity from the roasting of animal skins
- **Station B:** Downstream-*Ca*. 1 km downstream of the abattoir
- **Station C:** Upstream-*Ca*. 1 km upstream of the abattoir

Sampling and analyses: Samples were collected from each station during the wet season-July 2007; and dry season-November 2007. Sediment samples were collected from each station using an Eckman grab. The swimming crab (*Callinectes pallidus*) was collected using a baited crab trap. The trap was set for a while at midstream and trapped crabs were pulled up and placed in labelled containers. Sediment and crab samples were preserved in ice in the field and then taken to the laboratory where sediment was air-dried while the crabs were stored at -19ºC until analysis.

Sediment samples were air-dried, disaggregated and sieved. The crabs were dissected to separate the flesh (edible portion) from the carapace before the tissues were dried to constant weight in an oven and ground. Ten gram of dry sediment or ground tissue samples were mixed with 60 mL of xylene in a Sohxlet apparatus. Four mL of cyclohexane was further added to the mixture for extraction. The extracts were treated with Silica gel and Centrifuged before determination of PAH with Unicam PROGC gas chromatography. PAH was measured in ng/µL and converted to µg/g.

Statistical analyses: Two-way Analysis of Variance (ANOVA) was used to test for significant differences between environmental matrices (sediment, tissues of *Callinectes*-flesh/carapace). One-way ANOVA (and Tukey tests for mean separation where ANOVA gave significant differences) were performed to determine spatial differences for each environmental matrix as two-way ANOVA showed no significant interaction between season and location for sediment/tissue. Data were log (x+1) transformed before input into the ANOAVA model. Statistical analyses were performed using MINITAB software.

RESULTS AND DISCUSSION

The concentrations of PAH were higher in sediment than crab tissues, with values in the flesh being higher than the levels in carapace (Fig. 2). The overall mean concentrations were: sediment (0.973±0.631 µg/g wet season, 0.020±0.014 µg/g dry season), flesh (0.627±0.311 µg/g wet season, 0.019±0.008 µg/g dry season), carapace (0.155±0.082 µg/g wet season, 0.007±0.003 µg/g dry season). There was significant difference in the PAH values between the seasons ($p \leq 0.01$), but no significant difference was observable between sediment and the crab tissues, neither was there any significant interaction between environmental matrix and season (Table 1).

PAH levels in sediment were higher at station A; followed by station B with the least being station C in both rainy season dry seasons (Fig. 2). Mean concentrations ranged from 0.18 µg/g at Stn C to

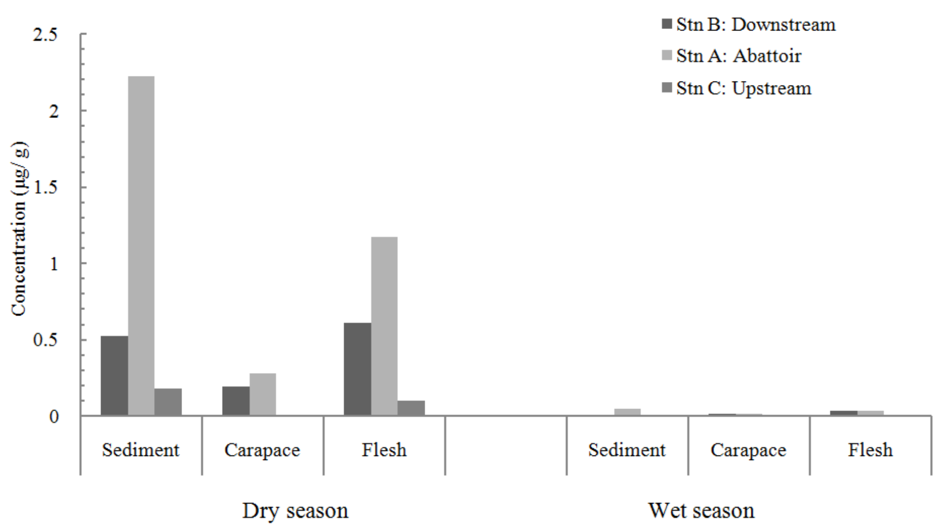

Fig. 2: Spatial and seasonal differences in the concentrations of PAH in sediment and tissues (carapace and flesh) of *Callinectes pallidus* during the study period

Table 1: Two-way analysis of variance to test environmental matrix and season

Source	DF	Seq SS	Adj SS	Adj MS	F	p≤
Matrix	2	0.03127	0.03127	0.01564	1.23	0.328
Season	1	0.11972	0.11972	0.11972	9.38	0.010
Matrix*season	2	0.02782	0.02782	0.01391	01.09	0.367
Error	12	0.15315	0.15315	0.01276		
Total	17	0.33197				

Table 2: Summary of one-way analysis of variance and pair-wise comparisons with Tukey tests for PAH in sediment

A: summary of ANOVA

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	p≤
Site	2	0.23352	0.23352	0.11676	132.06	0.001
Error	3	0.00265	0.00265	0.00088		
Total	5	0.23618				

B: Tukey tests-t-values; probability values in parenthesis

	Stn A: abattoir	Stn B: downstream	Stn C: upstream
Stn A: abattoir	-		
Stn B: downstream	10.965 (0.0033)	-	
Stn C: upstream	15.870 (0.0011)	4.906 (0.0327)	-

Table 3: Summary of one-way analysis of variance and pair-wise comparisons with Tukey tests for PAH in carapace of *Callinectes pallidus*

A: summary of ANOVA

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	p≤
Site	2	0.0119694	0.0119694	0.0059847	28.20	0.011
Error	3	0.0006366	0.0006366	0.0002122		
Total	5	0.0126061				

B: Tukey tests-t-values; probability values in parenthesis

	Stn A: abattoir	Stn B: downstream	Stn C: upstream
Stn A: abattoir	-		
Stn B: downstream	2.279 (0.2028)	-	
Stn C: upstream	7.337 (0.0107)	5.058 (0.0301)	-

Table 4: Summary of one-way analysis of variance and pair-wise comparisons with Tukey tests for PAH in flesh of *Callinectes pallidus*

A: summary of ANOVA

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	p≤
Site	2	0.087809	0.087809	0.043904	216.04	0.001
Error	3	0.000610	0.000610	0.000203		
Total	50.088418					

B: Tukey tests-t-values; probability values in parenthesis

	Stn A: abattoir	Stn B: downstream	Stn C: upstream
Stn A: abattoir	-		
Stn B: downstream	9.24 (0.0055)	-	
Stn C: upstream	20.75 (0.0005)	11.51 (0.0029)	-

2.22 μg/g at Stn A (wet season) and 0.002 to 0.048 μg/g (dry season). Values were significantly higher during the rainy season (p<0.01). There were significant differences between sampling stations (p<0.001, Table 2) and pair-wise comparisons showed significant differences between means in the order: Stn A>Stn B>Stn C (Table 2). Similarly the highest values of PAH in crab tissues (flesh and carapace) followed the order Stn A>Stn B>Stn C (Fig. 2) with much higher values during the wet season (the only exception was the putatively higher dry season values in carapace as wet season was <0.001 μg/g). The mean values ranged overall from <0.001 μg/g (below detectable limit) to 0.280 μg/g in carapace and 0.001 to 1.175 μg/g in flesh. ANOVA showed significant differences between stations for both carapace (p<0.01, Table 3) and flesh (p<0.001, Table 4). Tukey test multiple comparisons for mean PAH in carapace showed significance where Stn A = Stn B>Stn C, while the pattern for flesh was Stn A>Stn B>Stn C.

The PAH classes found in the samples include Phenanthrene, Benzo (a) anthracene, Benzo (a) pyrene, Naphthalene, Fluoranthrene, Fluorene, Anthracene (Fig. 3 to 5). All classes were found in sediment and crab tissues but anthracene was detected only the in sediment during the dry season. In sediment Polycyclic aromatic hydrocarbon concentrations were significantly higher during the rainy season in all compartments (sediment; flesh and carapace of *Callinectes sapidus*). The higher wet season values show that run-off from

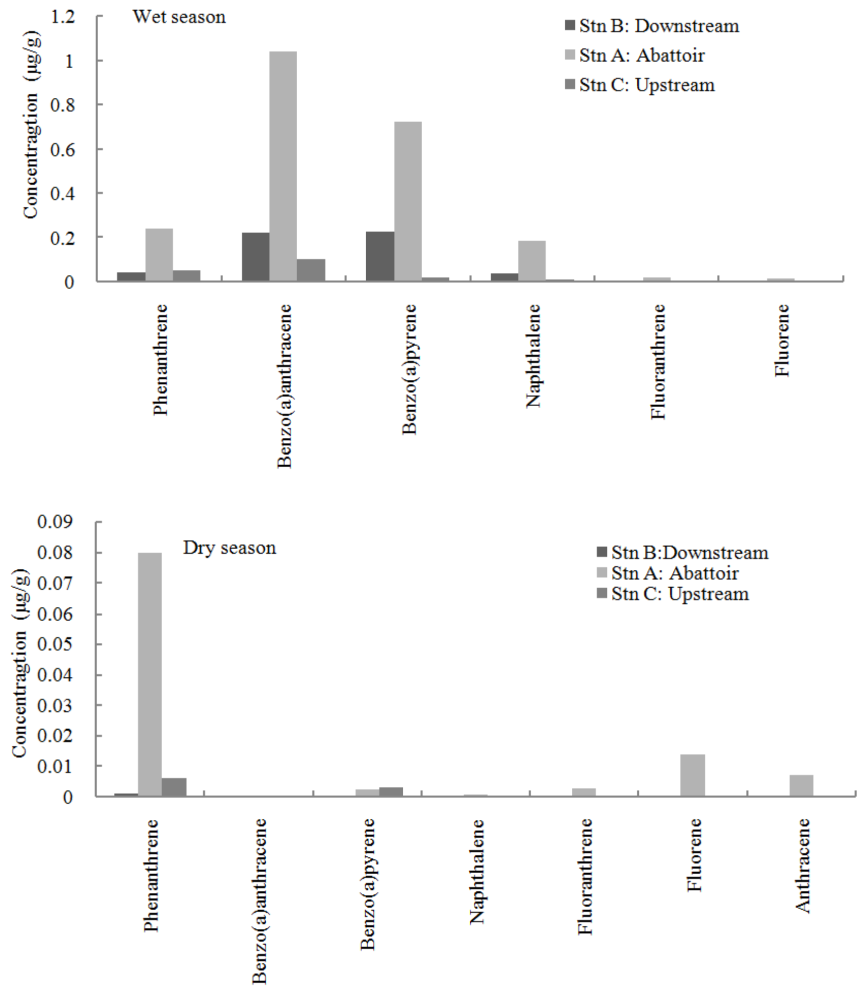

Fig. 3: Spatial and seasonal differences in the classes of PAH in sediment

the ash of tyres used for smoking animals slaughtered at the abattoir was the main source of PAH. More so, PAHs in this study were highest at station A which receives the run-off from tyre ash. The consistency in the reduction for all matrices suggests a flux within the estuary tending towards attenuation during the dry season. Sediments were expected to act as sink that could have led to accumulation over time; however, frequent dredging in the area may have precluded the build-up in sediment. PAHs are formed by incomplete combustion of carbon containing fuels likes diesel, fat, tobacco and tyres. The predominant PAHs found in this study were Benzo (a) pyrene, Benzo (a) anthracene, naphthalene and Phenanthrene. They are known for their carcinogenic, mutagenic and teratogenic properties. Variations in concentration of PAHs can be attributed to variations in the content of soot particles of tyre burning which enter the Azuabie creek during periods of elevated surface run-offs (rainy season). The heterogeneity in the BSAF of PAHs has been attributed to variations in the content of soot particles which enter the aquatic system during periods of elevated surface run-offs and which tend to accumulate preferentially with other fine materials in the high inter tidal zone (Capuzzo, 1987). However, all samples in this study were however collected subtidal. PAHs have been reported to bioaccumulate differentially in benthic invertebrates (Maruya *et al*., 1997), varying over almost three orders of magnitude. Maruya *et al*. (1997) also reported that Biota-Sediment Accumulation Factor (BSAF) of PAHs varied with season and along an inter tidal gradient in a costal marsh in San Francisco Bay.

PAHs can enter the human body by many different ways. People near hazardous sites can breathe air containing PAHs. Drinking water and swallowing food is another route. Though absorption is slow; they can enter the body if the skin comes in contact with soil that contains a high level of PAH (ATSDR, 2008). The rate of entry of PAH into the body also depends on the presence of other compounds that one is exposed to at the same time. PAHs enter all the tissues of the body that contain fat and can be changed into various substances which could be harmful. Mice fed high levels of benzo (a) pyrene during pregnancy had difficulty reproducing and so did their offspring

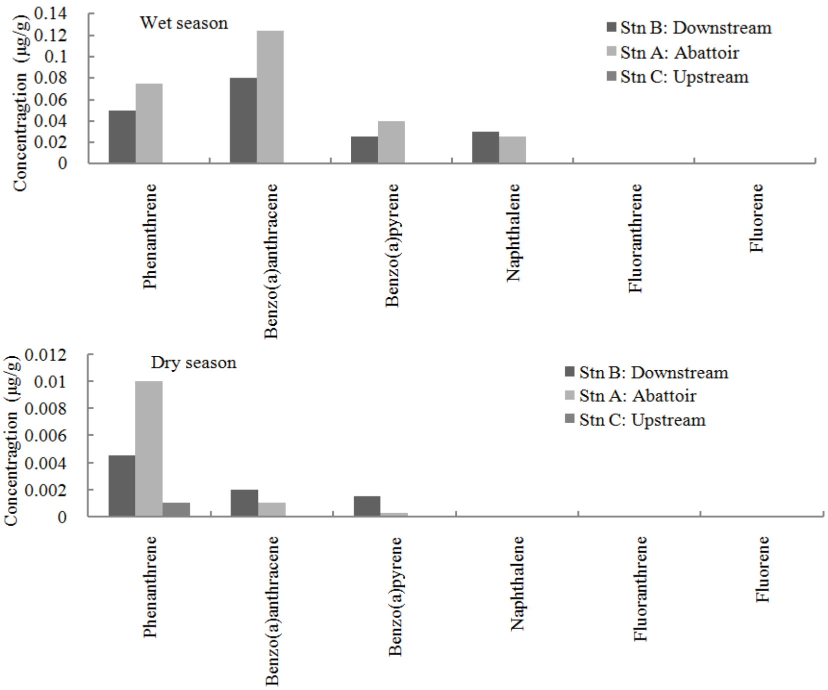

Fig. 4: Spatial and seasonal differences in the classes of PAH in carapace *Callinectes pallidus*

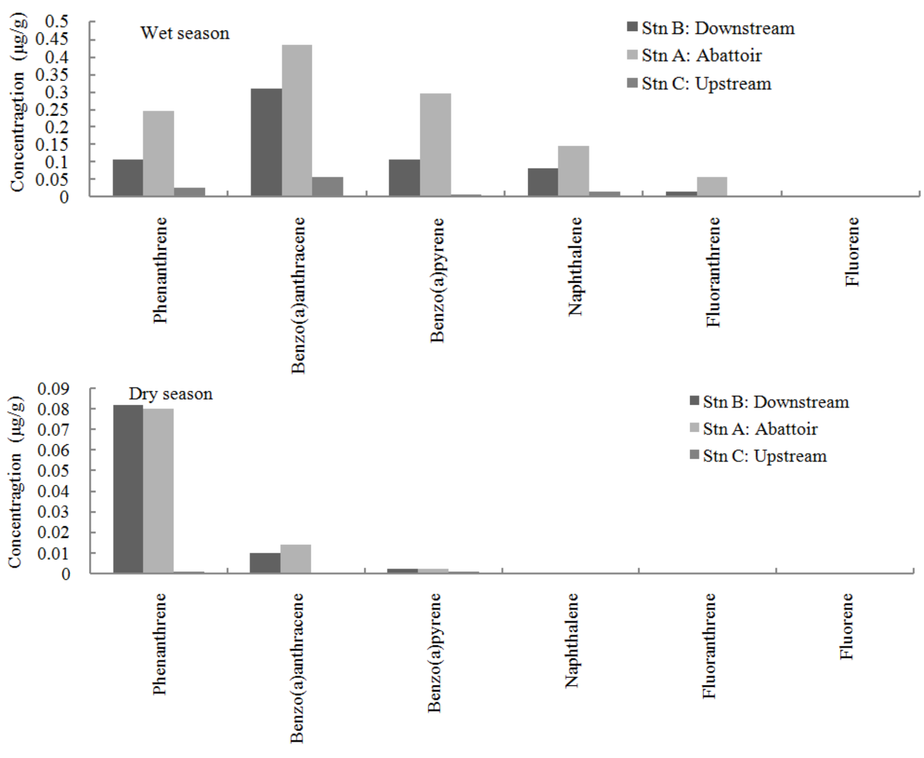

Fig. 5: Spatial and seasonal differences in the classes of PAH in flesh of *Callinectes pallidus*

(ATSDR, 2008). A meal of *C. pallidus* from the Azuabie could introduce high levels of PAH (especially during the wet season), however, the human body eliminates PAHs through urine and feaces (ATSDR, 2008).

In conclusion, the highest values of PAH were found in sediments and the tissues of *Calllinectes pallidus* from stn A which receives run-off of tyre burning effluent. Significantly higher values in the rainy season also confirm the run-off as the major source of PAH in the estuarine creek. The major classes of PAHs found in the sediments and crab were naphthalene, benzo (a) pyrene, benzo (a) anthracene and phenanthrene, which are potential carcinogens, thus crabs and indeed other fish products from the estuary may constitute a potential health hazard for consumers.

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REFERENCES

Aderemi, O.O., I.A. Olatunde and S.F. Olalekan, 2003. Isolation and estimation of PAHs in surface run off and sediments. Water Air Soil Pollut., 147: 245-261.

ATSDR, 2008. Public Health Statement for Polycyclic Aromatic Hydrocarbons (PAHs). Department of Health and Human Services, Public Health Service. Atlanta, GA, US, pp: 8.

Baumard, P., H. Budzinski, P. Garrigues, J.C. Sorbe, T. Burgeot and J. Bellocq, 1998. Concentrations of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level. Mar. Pollut. Bull., 36: 951-960.

Capuzzo, J.M., 1987. Biological Effects of Petroleum Hydrocarbons: Assessment from Experimental Results. In: Boesch, D.F. and N.N. Rabelais (Eds.), Long Term Environmental Effects of Off-shore Oil and Gas Development. Elsevier, New York, USA, pp: 343-410.

Daka, E.R., M. Moslen, C.A. Ekeh and I.K.E. Ekweozor, 2007. Sediment status of two creeks in the upper bonny estuary, Niger Delta, in relation to urban/industrial activities. Bull. Environ. Contam. Toxicol., 78: 151-521.

Eickhoff, C.V., S. He, F.A.P.C. Gobas and F.C.P. Law, 2003. Determination of polycyclic aromatic hydrocarbons in dungeness crabs (*Cancer magister*) near an aluminum smelter in kitimat arm, British Columbia, Canada. Environ. Toxicol. Chem., 22(1): 50-58.

Eisler, R., 1987. Polycyclic aromatic hydrocarbon hazards to fish, wildlife and invertebrates. A synoptic review. Biological Report, 85(1.11), Patuxent Wildlife Research Centre. U.S Fish and Wildlife Service. Laural, Md., pp: 81.

Fetzer, J.C., 2000. The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons. Wiley, New York.

Ghauch, A., J. Rima, C. Fadchingeo, J. Suptil and M. Martin Bowger, 2000. Temperature phosphorescence analysis of PAHs using an imaging sensing system combined with a bifurcated optical filter and a cooled charge coupled device detector. Talanta, 51: 807-816.

Hale, R.C., 1988. Disposition of polycyclic aromatic hydrocarbons in blue crabs, *Callinectes sapidus*, from the southern Chesapeake Bay. Estuaries, 11: 255-263.

Humason, A.W. and D.F. Gadbois, 1982. Determination of poly-nuclear aromatic hydrocarbons in the New York Bight area. Bull. Environ. Contam. Toxicol., 29: 645-650.

Kayal, S. and D.W. Connell, 1995. Polycyclic aromatic hydrocarbons in biota from the Brisbane River estuary, Australia. Estuar. Coast Shelf Sci., 40: 475-493.

Larsson, B.K., 1983. Polycyclic aromatic hydrocarbons in grilled food. J. Agric. Food Chem., 31: 867-873.

Luch, A., 2005. The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons. Imperial College Press, London, ISBN: 1-86094-417-5.

Maruya, K.A., R.W. Risebrough and A.J. Horne, 1997. The bioaccumulation of polynuclear aromatic hydrocarbons by benthic invertebrates in an intertidal marsh. Environ. Toxicol. Chem., 16: 1087-1097.

Mothershead, R.F., R.C. Hale and J. Graves, 1991. Xenobiotic compounds in blue crabs from a highly contaminated urban subestuary. Environ. Toxicol. Chem., 10: 1341-1349.

Oladele, I.A., L. Adele and I.A. Amoo, 2008. Occurrence and toxicity of hydrocarbon residues in crab (*Callinectes sapidus*) from contaminated site. J. Appl. Sci. Environ. Manage., 12: 19-23.

Pancirov, R.J. and R.A. Brown, 1977. Polynuclear aromatic hydrocar-bons in marine tissues. Environ. Sci. Technol., 11: 989-991.

Varanasi, U., J.E. Stein and M. Nishimoto, 1989. Biotranformation and Disposition of PAHs in Fish. In: Varanasi, U. (Ed.), Metabolism of PAHs in the Aquatic Environment. CRC Press Inc., Boca Raton FL, pp: 93-150.