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# DIAGNOSTIC RATIOS OF POLYAROMATIC HYDROCARBONS FOR THE IDENTIFICATION OF POLLUTION SOURCES IN CLAMS FROM OKWAGBE, DELTA STATE NIGERIA

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## ABSTRACT

Polyaromatic hydrocarbons (PAHs) are pollutants found in the environment. Their sources are anthropogenic and natural. Since clams are filter feeders, they can accumulate (PAHs) in their tissues providing vital tool for pollution source monitoring. This study investigated different pollution sources using diagnostic ratio of PAHs. Clams were collected from Okwagbe River, Delta State, Nigeria and analyzed for their polyaromatic hydrocarbon (PAH) contents. The sixteen (16) priority PAHs, including: 1-methylnaphthalene and 2-methylnaphthalene were determined in flesh and shells using gas chromatography. A mixture of acetone/dichloromethane/n-hexane was used for the Soxhlet extraction. The concentration of mean total PAHs was 2.495 µg/kg for flesh and 2.156 µg/kg for shell. The benzo(a)pyrene (B[a]P) of flesh was 0.210 µg/kg and 0.312 µg/kg for shells. These values did not exceed the permissible limit of 6.0 µg/kg for benzo(a)pyrene and 35.0 µg/kg for PAHs. The isomeric ratio was used to find the sources of PAHs in flesh and shells. It showed the sources were petrogenic, pyrogenic and wood burning. Clams can be a tool for tracking pollution source identification and improve understanding of human activities on ecosystems.

**Keywords:** PAHs, Clams, Isomer Ratios, River, Sources, Pollution.

## INTRODUCTION

Polyaromatic or polycyclic aromatic hydrocarbons (PAHs) are organic compounds with two to six fused aromatic rings with different structural arrangements (Umudi and Umudi, 2021). They are usually found in the environment through activities such as volcanic eruptions, forest fire, oil spills, vehicular traffic fossil fuels, industrial emissions and biomass combustion. Their capacity for long-term migration and deposition is possible through absorption onto particulate matter (Li *et al.*, 2023). They are detrimental to health and environment since they are carcinogenic, mutagenic, teratogenic etc. Sixteen (16) PAHs members have been documented as priority contaminants according to the United States Environmental Protection Agencies (Adekunle *et al.*, 2018; Iwegbue *et al.*, 2020; Ambade *et al.*, 2023; Akinnusotu *et al.*, 2023). They are mostly generated by heat decomposition – pyrolysis and recombination i.e. pyrosynthesis for their production. We have two categories considering the number of benzene rings present in them. Low molecular weight (LMW) and high molecular weight (HMW) (Roy *et al.*, 2022; He *et al.*, 2023). They are soluble in organic solvents with fused benzene since they are lipophilic each with distinct UV spectrum (Gao *et al.*, 2018). PAHs can cause cancers, mutation, abnormalities in fetus and disrupt endocrine and

immune systems (Iwegbue *et al.*, 2020). They usually reside in air, soil, water, biota, sediments and cosmetics making them ubiquitous chemicals (Sharma *et al.*, 2021; Ambade *et al.*, 2023). Their exposure route are thermal absorption, inhalation and ingestion with the environment as the primary receiver and sediment, the reservoir sink. This causes the food chain to be contaminated bringing a threat to man and his environment (Cui *et al.*, 2023). Fish and bottom filters are aquatic animals for PAH detection. There have been reports for other studies in water and other invertebrates from Okwagbe River, but there has been no report on studies of PAHs for Okwagbe River on clams which is one of the delicacies in this area. Okwagbe is a commercial center involving fishing, lumbering, transportation, local gin marketing, speed boat repairs. Clams from these areas are usually boiled for soup, smoked or fried for consumption with tapioca (kpokpo-gari) spiced with grinded dried pepper and onions.

Clam is a common name for edible bivalve molluscs that live as infauna, and spend half their life span embedded in the sands of seafloors or riverbeds. Clams have two shells of equal size connected by two adductor muscles with powerful burrowing foot. Some are ovoid or triangular, all clams have two calcareous shells (Zhang *et al.*, 2023) joined near a hinge with a flexible ligament which are filter feeders and sources of food for many animals (Joaquim *et al.*, 2016). The shells are also used as sources of calcium in bird feeds and in making ceramics (Roszbach *et al.*, 2019; Roszbach *et al.*, 2020). The large number of clam fossils in the British Isles in middle mounds around occupied sites point to the fact that it is a staple food within that region. They are also used in the manufacture of “Wampum” a piece of sacred jewelry and in making shell money.

Water quality is affected by build-up of generated organic compounds resulting in their presence in water sediments and biota. The metabolites of PAHs being more toxic than their parent compound. Studies showed that PAHs accumulate in clams and their soft tissues highlighting their ability to be absorbed and stored within clam organisms. This occurs as clams filter feed, ingesting water and particles from their surrounding leading to the uptake of contaminants like PAHs present in the marine environment (Roma *et al.*, 2017; Moslen *et al.*, 2019). Compositional analysis has been carried out (Ukwo *et al.*, 2022; Tongo *et al.*, 2018). This work aims to report PAHs content and their sources in clams.

## MATERIALS AND METHOD

### Sample Area

Okwagbe is a common town along Forcados River in Ughelli South Local Government Area of Delta State. It lies between latitude 5°31'43" N to 5°47'23" E with distinct rainy and dry seasons in May – October and November – April respectively. With palm trees of raffia, mangrove forest and timber trees, it is known for its commercial purposes.

### Sample collection

Clams were collected from fishermen along the bank of the river and from different sellers to reconstitute composite and transported in cartons. They were washed with distilled water and cracked to remove the muscular (fleshy) parts, which were rewashed, pulverized and stored at -10 °C before extraction and analysis.

### Clams Extraction

The PAHs were extracted from clams using Soxhlet extraction following USEPA method 3540 (USEPA, 1996). Using a mincer, they were reduced to small sizes, after which 5.0 g of the minced clam was thoroughly homogenized with 10g of anhydrous Na<sub>2</sub>SO<sub>4</sub> by grinding with a pestle and spike with 200 g deuterated PAH standard. 100 ml of acetone and n-hexane was added, homogenized and centrifuged for 20 mins at 100 rpm. A mixture of dichloromethane and n-hexane was used for the extraction using a Soxhlet extraction apparatus for about 5 hrs. and evaporated to dryness. The resultant extract was reconstituted using 50 ml n-hexane and later 1 ml under a flow of nitrogen gas.

### Sample analysis

The concentrations of PAHs were determined in the clams using an Agilent 7980 A gas chromatography, following the method described by Iwegbue *et al.* (2022). A pulsed splitters mode injected one microliter (1 µL) of sample concentration into the column. Separation was achieved with a DB – 5 capillary column of 30 m, internal diameter of 0.25 mm and thickness of 0.25 µm. The oven temperature was maintained at 60 °C for 3 mins then increased to 180 °C at 10 °C min<sup>-1</sup> and increased to 300 °C at 3 min intervals. The temperature of the source ion was at 230 °C while the injector maintained at 280 °C. A comparison of the PAHs retention times in the sample with those of the original standards and the quantification and confirmation ions were utilized to identify the PAHs in the samples.

### Quality assurance/control

Standard PAH comprising the prioritized PAHs purchased from chemical scientific stores was used for the calibration. The PAH quantification was achieved by using an external calibration method. The square of the correlation coefficient for the PAH calibration curve varied from 0.9995 to 0.9999 software for PAH calibration and quantification was employed. The analysis was carried out in triplicates with precision measured by the relative standard deviation (RSD) which varied from 0.9 % to 5.0 %. The limits of detection (LODs) for the PAHs were obtained at the lowest concentration that yielded a signal to noise ratio of 3:1 of the blank, while limit of quantification was measured at 3 times the LODs. The LODs and LOQs of PAHs ranged from 0.01 to 0.06 µg/kg and 0.03 to 0.18 µg/kg<sup>-1</sup> respectively (Iwegbue, *et al.*, 2022).

### Diagnostic ratio for source appointment

One of the techniques for determining the approach of the origin of PAHs is the diagnostic ratio using isomer ratios (Davis *et al.*, 2019). It is on the basis that PAHs possess analogous chemical characteristic behaviour in natural states similar in terms of transportation and degradation. Isomer ratios remain the same from emissions to the time of measurement, to differentiate between pyrogenic sources and petrogenic sources. Usually ratios of Ant/(Ant + Phe) and Fla/(Fla + Cry). Petroleum source is indicated by a ratio Ant/(Ant + Phe) < 0.1, that of combustion is indicated by a ratio of Ant/(Ant + Phe) > 0.1, Fla/(Fla + Pyr) > 0.5 indicates a combination from biomass or coal sources; those of 0.4 and 0.5 indicates combustion from petroleum sources (Tobiszewski *et al.*, 2012). The ratio of low molecular weight (LMW) to high molecular weight (HMW) PAHs < 1 shows a pyrogenic source while > 1 indicates a petrogenic source. In accordance, ratios of BaA/(BaA + Chr) below 0.2 imply a petroleum source, but values in the range of 0.2 – 0.3 suggested petroleum sources especially liquid fossil fuel and crude oil. While values above 0.35 indicate the combustion of coal, grass and wood (Wang *et al.*, 2013).

## RESULTS

### Concentration of PAHs and distribution in shell and fish samples.

The concentration of PAHs in the shell and flesh of clams are presented in Table 1.

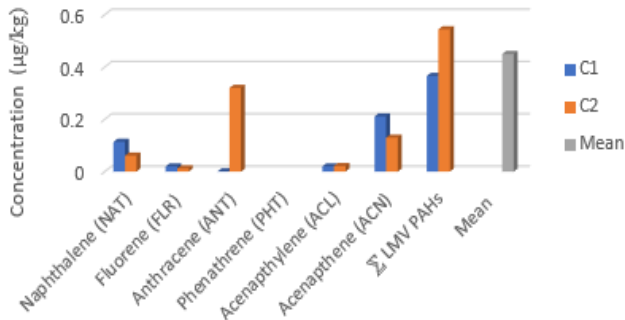
**Table 1:** PAHs in the flesh (C1) and shell (C2) clam samples.

Components of LMW PAHs	Flesh (C1)	Shell (C2)
Naphthalene (NAT)	0.114	0.061
Fluorene (FLR)	0.020	0.012
Anthracene (ANT)	0.001	0.320
Phenathrene (PHT)	-	-
Acenaphylene (ACL)	0.020	0.021
Acenaphthene (ACN)	0.211	0.130
Total LMW PAHs	<b>0.366</b>	<b>0.544</b>
Components of HMW PAHs		
Pyrene (P)	0.210	0.020
Fluorathene (Fit)	-	-
Chrysene	0.121	0.111
Benzo(a)anthracene (B[a]A)	-	-
Benzo(b)fluoranthene (B[b]F)	0.180	0.133
Benzo(k)fluorathene (B[k]F)	-	-
Benzo(a)pyrene (B[a]P)	0.210	0.312
Indeno(1,2,3-cd)pyrene (IndP)	0.580	0.420
Dibenzo(a,b)anthracene (D[ab]A)	0.310	0.301
Benzo(g,h,i)perylene (B[ghi]P)	0.510	0.282
Total HMW	<b>2.121</b>	<b>1.579</b>
1-methyl naphthalene	0.001	0.021
2-methyl naphthalene	0.004	0.012
Total 1-methyl naphthalene and 2-methyl naphthalene	<b>0.005</b>	<b>0.033</b>
Total PAHs (µg/Kg)	<b>2.492</b>	<b>2.156</b>

Table 1 shows the concentration of PAHs in the flesh and shell of the clam samples, coded C1 and C2 respectively, with varying concentrations. Phenathrene was below detectable.

**DISCUSSION**

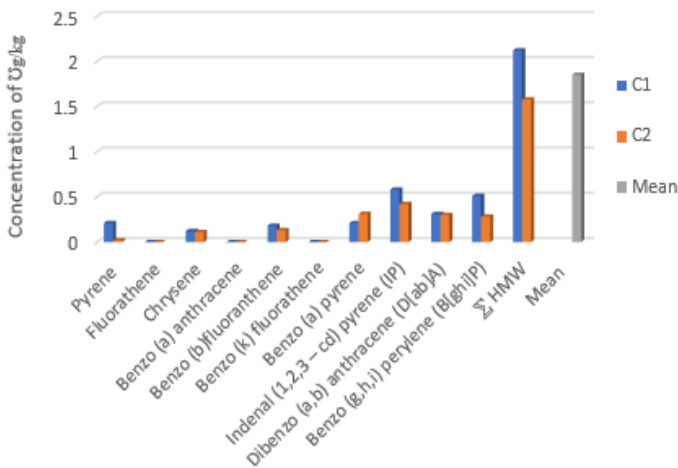
Limits for both flesh and shell. Naphthalene, fluorecene, anthracene, acenaphthalene and acenaphene in shell ranges from 0.001 – 0.211 µg/kg while for flesh 0.012 – 0.32 µg/kg. The concentration of LMW is higher in shells (0.54 µg/kg) than in flesh (0.360 µg/kg), with its distribution shown in Figure 1.



**Figure 1:** Concentration and total LMW PAHs (Σ LMW PAHs) and mean in shell and flesh of clams.

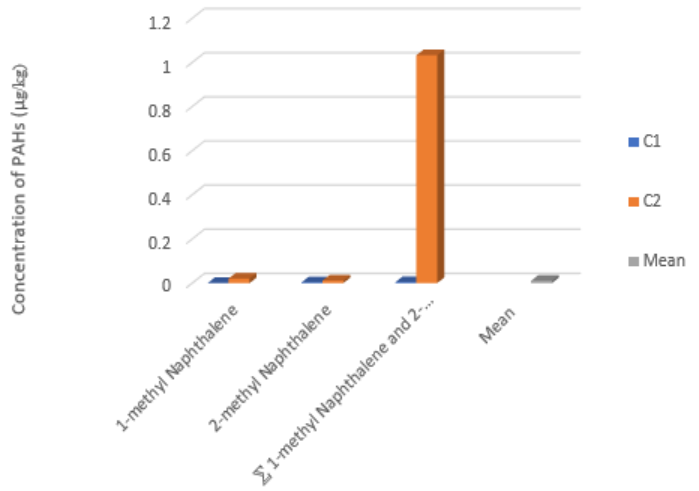
A study carried out by Onozato *et. al.* (2016) on bivalves on the Pacific coast of Japan obtained a concentration of 21-144 µg/kg which was higher than this present study.

Figure 2 shows the distribution for higher molecular weight (HMW) hydrocarbons. Fluoranthene, benzo(a)anthracene and benzo(k)fluoranthene were below detectable limits for flesh and shell. The concentration of pyrene, chrysene, benzo(b)anthracene (B[b]A), pyrene, benzo(b)fluoranthene (B[a]F), benzo pyrene (B[a]P), indeno (1,2,3-ed)pyrene (IndP), dibenzo(a,b)anthracene (B[ab]A) and benzo(g,h,i)perylene (B[ghi]P) with concentrations 0.210 - 0.510 µg/kg for flesh and 0.020 - 0.420 µg/kg respectively.

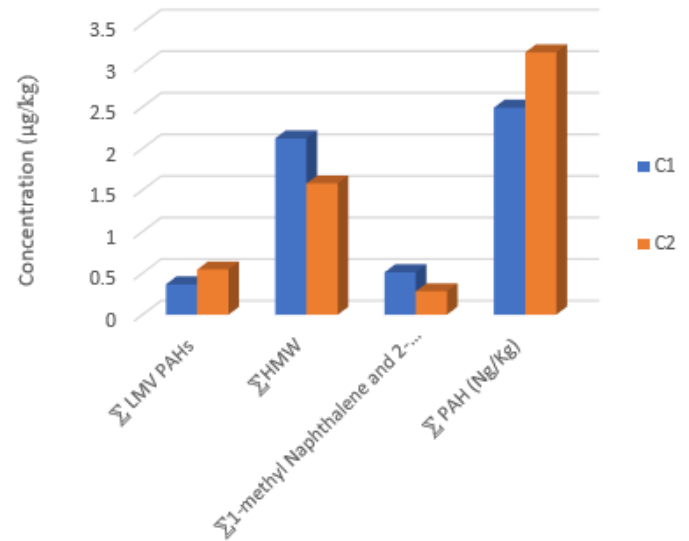


**Figure 2:** Concentration and total HMW (ΣHMW) PAHs and mean in shell and flesh of clams

Figure 3 shows the concentrations of 1-methyl naphthalene and 2-methyl naphthalene in both shell and flesh of clams as 0.001 and 0.004 µg/kg for flesh and 0.021 and 0.012 µg/kg for shell. The summation of shell was higher than that of flesh.



**Figure 3:** Concentration and mean total 1-methyl naphthalene and 2-methyl naphthalene in clam shell and flesh



**Figure 4:** Summation and total concentration of all PAHs in clam sample

The concentration of PAHs in the clam samples are below limits recommended by the European Commission (2015).

**Table 2:** Diagnostic ratios of PAHs in clams

	IndP/IndP- BghiP	Ant/Ant- Pbe	BaA/BaA - Cry	Fit/Fit- Pyr	LMW/HMW
Flesh (C1)	0.5	1	0.1	0.2	0.2
Shell (C2)	0.6	1	0.1	0.02	0.4

The results of diagnostic ratios of clams from Okwagbe river Delta State are shown in Table 2. The ratio of Ant/(Ant + Phe) is 1 > 0.1 which signifies that PAHs are of pyrogenic origin. The BaA/(BaA + Cry) ratio 0.1 < 0.2 suggests petroleum sources as indicated above. An IndP/(IndP + BghiP) ratio between 0.2 and 0.5 depicts

inputs from the combustion of liquid/fossil fuels and above depicts coal/biomass combustion sources (Abayi *et al.*, 2021; Tavokoly-Sany *et al.*, 2014; and Iwegbue *et al.*, 2022). The ratio of LMW/HMW PAHs was  $< 1$  which suggests pyrogenic sources. Studies on source identification and health risk assessments of polyaromatic hydrocarbons (PAHs) in Molluscos (Usoro, *et al.*, 2023) showed that PAHs molecular diagnostic ratios used for source identification were less than one, indicating pyrogenic. (Traves, 2014; Usoro, 2023 Flt/Flt + Pye) 0.2 for flesh suggest pyrogenic sources while that of the shell  $0.02 < 0.1$  meaning petrogenic source. Flesh is found to be dominated by HMW PAHs, while the shell was dominated by LMW PAHs. Petrogenic PAHs are characterized by two to three rings LMW while pyrogenic PAHs are characterized by a high proportion of above 4 rings (HMW) (Wang *et al.*, 2019). Degradation caused by microbes accounts for the resistance of HMW PAHs leading to LMW/HMW ratio, low volatility and solubility of LMW. The value of the total index (TI) is the sum of normalized relative isomer ratios. Ant/(Ant + Phe), BaA/BaA + Chy), Flt/Flt + Pyr) and Indp/IndP + Pghip. A TI value of  $C1 < 4$  indicates the sources of PAHs are linked to low-temperature combustion processes, while TI values  $> 4$  indicate that PAHs originated from high-temperature processes. The values sum up in flesh and shell is  $< 4$  indicating the prevalence of low-temperature processes as sources of PAHs in clams (Grigoriou *et al.*, 2021).

### Conclusion

The result shows that HMWs are more prominent in the flesh than in the shells of clams, LMWs are more in the shells than the flesh of clams from the Okwagbe River. Total PAHs were more in flesh and their concentrations were lower than the limit of European Commission regulations. Most of the sources were petrogenic, pyrogenic and low-temperature processes. Constant monitoring of PAHs in the studied area should be carried out. More work should be done on water and sediments and risk assessment carried out.

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