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Polycyclic Aromatic Hydrocarbons in Fresh and Smoked Fish Samples from Three Nigerian Cities

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Nigeria is a major producer of crude oil in sub-Saharan Africa. In-shore and off-shore wells are located in richly watered creeks in the southern part of the country. Although published data on environmental impact assessment of the petroleum industry in Nigeria are lacking, there is a growing concern about the possible contamination of estuarine and coastal waters and of marine species by polycyclic aromatic hydrocarbon (PAHs).

PAHs are ubiquitous priority pollutants that occur naturally in crude oil (Vazquez et al.,1991), automobile exhaust emissions and smoke condensates from incomplete combustion of carbonaceous materials (Asita et al.,1991). PAHs with high molecular weight are less readily biodegraded by indigenous microorganisms in some regions (Mueller et al.,1991), and given their marked hydrophobic characteristics, may persist in the aqueous environment, thus contaminating the food chain by bioaccumulating in aquatic species like fish and mussels (Van-der-Oost, 1991).

Major Nigerian oil wells are located in the vicinity of breeding and harvesting sites serving the fresh-water fishing industry. Large hauls of fresh fish are normally consumed cooked in soups or smoke cured in handcrafted traditional ovens using freshly cut red mangrove (Rhizophora racemosa) wood as fuel. Though smoke curing is economical and may ensure longer conservation of fish (Osuji, F.N.C.,1976), it undoubtedly increases the burden of PAHs in finished products as a result of partial charring and from smoke condensates of mangroves that also contain PAHs in measurable quantities as reported by Asita et al.(1991). Apart from PAHs analyzed by Emerole (1980) in smoked food samples from Ibadan using simple analytical methods, those from industrial and other anthropogenic sources have rarely been analyzed in Nigeria. We tried therefore to update the data and address this discrepancy.

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MATERIALS AND METHODS

Fresh and smoked samples of two species of fish (Heterotis niloticus, Pseudotolithus elongatus) commonly consumed by the local population in Calabar and Warri were bought as supplied in the respective local markets, while all samples from Lagos (Carnax hippos, Heterotis niloticus, Pomadasys peroteti, Pseudotolithus elongatus) were procured fresh from peasant fishermen on the lagoon near the Marina. Five pairs of these fish species from Lagos were smoked where caught for 5 h in a makeshift oven fueled with logs of wood normally used by the fishermen. All samples were sealed in Domopak plastic bags, labelled per site and stored at -20°C until analysis, which was completed within four months. We also determined the levels of PAHs in the waters of the bay at the Marina, Calabar, the lagoon at the Marina , Lagos and at the bay near Ogboijaw Market (Warri), using the blue rayon method described by Sakamoto and Hayatsu (1990), with a few modifications to suit our case.

Briefly, portions of 0.5 g of blue rayon obtained from Funakoshi Chemicals (Kanda Surugadai 2-3,Chiyoda-ku, Tokyo 101) were placed in plastic mesh, hung on a floating board, (two boards per site, ten meshes per board and two sites for each city). These floating boards with their blue rayon load were suspended in the bays of the three cities for 24 hours. All suspended portions of blue rayon were recovered, gently squeezed to remove excess water and placed in aluminum bags each labelled per site and kept at -20°C until analysis. Combined portions of blue rayon from each board were thawed, washed with bidistilled water and dried with filter paper. The blue rayon was spiked with 60 ng of 3-methylcholanthrene as internal standard, then eluted twice with methanol:concentrated ammonia (50:1 v:v, 75 ml/0.5 g blue rayon) by gently shaking on a Clinoshaker for 30 min using an aliquot of 37.5 ml per run. The combined eluent was filtered through filter paper and concentrated to 2 ml with a rotary evaporator at 40°C. The concentrated extract was flushed to dryness in nitrogen stream, dissolved in 400 µl of acetonitrile: water (80:20 v:v) and 20 µl were analyzed by HPLC as described below.

For PAH determination in fresh fish, we used the method described by Dennis et al.(1983) with some modifications. Ten grams of edible tissue from the mid-portion of fish were rinsed with 75 ml of distilled water, dried on filter paper and crushed into a fine paste in a porcelain mortar, transferred into a 500 ml round-bottom flask and spiked with 60 ng of 3-methylcholanthrene as internal standard. The preparation was then extracted in a reflux funnel for 2 h in 75 ml of methanol:water (9:1 v:v) saponified with 2 g of KOH and we added 7 g of anhydrous sodium sulphate. All glass and porcelain ware were washed with chromic acid solution and rinsed thoroughly with bidistilled water, to eliminate extraneous PAHs. Precautions were taken to protect PAHs from photodegradation. The methanolic extract

was filtered through glass wool which was rinsed with 25 ml of the same methanol:water solution. The combined methanolic phase was extracted twice with 70 ml of isooctane, pooled and washed with 70 ml of methanol:water (1:1 v:v). We then extracted the isooctane phase with N,N-dimethylformamide:water (9:1 v:v) discarding the isooctane phase. The N,N-dimethylformamide:water phase was diluted with 70 ml of water and re-extracted with the same volume of isooctane divided in two aliquots. These final extracts were pooled, dehydrated on 2 g of anhydrous sodium sulphate (desiccated to a constant weight in an electric oven at 120 °C) in a glass column and concentrated to about 2 ml with a rotary evaporator at 40°C. The concentrated extract was passed through a silica gel column previously conditioned with 20 ml of chloroform and isooctane in sequence, eluted with 20 ml isooctane, evaporated to dryness using a stream of nitrogen. The residue was dissolved in 400 µl of acetonitrile:water (80:20 v:v), a 40 µl aliquot was analyzed with a HPLC apparatus equipped with a C18 reverse phase column (Vydac, i.d. 0.46 cm, length 25 cm; particle size 5 µm). The eluting solution was acetonitrile:water (45:55 v:v) with a flow rate of 1.6 ml/min. Resolution was obtained with a gradient in which the acetonitrile concentration increased from 45% to 88% in 28 min followed by a non-linear gradient increasing from 88% to 100% of acetonitrile in 7 min and 10 min to reestablish the initial conditions. A fluorometric detector (excitation wavelength: 290 nm; emission wavelength 430 nm) and a Shimadzu integrator were used for the determination of peak areas, while peaks were identified by comparing the retention times with those of standards. The percent recovery was 39.8+/- 9.6 (SD) using the internal standard method. We determined the following PAHs: acenaphthene (ACEN), fluorene (FLUO), phenanthrene (PHEN), fluoranthene (FLUOR), pyrene (PY), benzo(a)anthracene (B(a)A), chrysene (CHRY), benzo(b)fluoranthene (B(b)F), benzo(k)fluoranthene (B(k)F), benzo(a)pyrene (B(a)P), dibenzo(a,h)anthracene (DBA) and benzo(g,h,i)perylene (B(g,h,i)P). Each was expressed as µg/kg dry weight of fresh fish or as µg/g of lipids, and calculated using the area ratio of each peak relative to the internal standard. The same procedure was adopted in quantifying PAHs in smoked samples, with the only exception being that weighed whole fish was extracted and the volume of the extraction medium was corrected accordingly for increased weight, with 120 ng of 3-methylcholanthrene being used in this case to reflect the increased burden of PAHs in smoked samples. All samples, fresh or smoked, were analyzed in duplicate and all values are averages of two extractions per species of fish.

The lipid content in all species of fresh fish was determined following the procedure described below. For this purpose, 10 g of tissue from the mid-portion of the fish were dried in an electric oven at 105-110°C, crushed to a fine fish flour in a mortar and the solids were extracted with an adequate volume of ethyl ether for 8 h

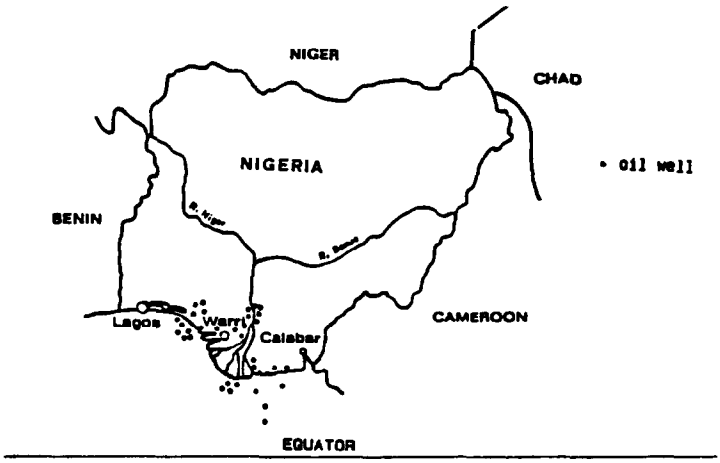

Figure 1. The geographical location of the three cities sampled.

in a Soxhlet apparatus. The weighed residue obtained after evaporating the solvent gave the lipid content .

RESULTS AND DISCUSSION

Figure 1 illustrates the sampled cities which include: Lagos, a mayor industrial town; Warri, located in the oil belt with important petroleum installations; and Calabar, a small urban center. The levels of individual PAHs present in fresh fish obtained from the sampling sites are reported in Table 1.

All species of fish from the three sites contained levels of acenaphthene below the detection limit of our method, thus confirming the findings earlier reported by DouAbul et al.(1987) for the Arabian Gulf. Fluorene was the most representative PAH with 1526 µg/kg dry weight in Heterotis niloticus from Lagos.

Among established carcinogenic PAHs, chrysene (Hecht et al., 1974), was the most representative PAH measured in samples from all species of fish from Lagos and Warri, while samples from Calabar had undetectable levels of this PAH. Benzo(a)pyrene, the prototypical carcinogen within this class (Dipple et al., 1984) was identified in all samples from the three cities.

Samples from Lagos had the highest concentration of this PAH measured in Pseudotolithus elongatus, followed by Warri, whereas samples from Calabar had significantly lower levels of benzo(a)pyrene.

There was a good correlation between carcinogenic PAHs and lipids in fresh fish as illustrated in Fig.2. This agrees with previous observations that marine organisms including fish have the ability to bioaccumulate PAHs in their fatty tissues (Rahman et al.,1986).

Table 1. Concentrations of 12 PAHs (μg/kg dry weight) in fresh fish samples from 3 Nigerian cities.

Sites	Species	ACEN	FLUO	FEN	FLUOR	PY	B(a)A*
LAGOS	P.E.	N.D.	506	N.D..	18.8	83	0.38
LAGOS	P.P	N.D.	926	N.D.	11.5	53	1.4
LAGOS	H.N.	N.D.	1526	55	22	136	28
LAGOS	C.H.	N.D.	637	N.D.	6.5	35	0.55
WARRI	H.N.	N.D.	344	N.D	61	239	8.3
WARRI	P.E.	N.D.	330	N.D.	13.6	89	0.98
CAL.	P.E.	N.D.	494	14.7	1.9	2.0	0.21
CAL.	H.N.	N.D.	700	8.5	10	47	0.09

Sites	Species	CHRY*	B(b)F*	B(K)F*	B(a)P*	DBA*	BGP
LAGOS	P.E.	175	11.5	0.33	44	4.9	149
LAGOS	P.P.	104	5.5	0.23	25	2.9	150
LAGOS	H.N.	263	0.57	0.22	11	N.D.	18
LAGOS	C.H.	84	3.4	0.08	19.4	1.9	104
WARRI	H.N.	191	6.8	0.3	19.4	1.9	104
WARRI	P.E.	123	7.0	0.14	25	N.D.	72
CAL.	P.E.	N.D.	4.5	0.03	7.5	N.D.	6.6
CAL.	H.N.	N.D.	5.0	0.02	5.4	0.9	7.0

(P.E.) Pseudotolithus Elongatus; (P.P) Pomadasys Peroteti;
(H.N.) Heterotis Niloticus; (C.H.) Carnax Hippos.
(N.D.) Not detectable
* carginogenic PAHs

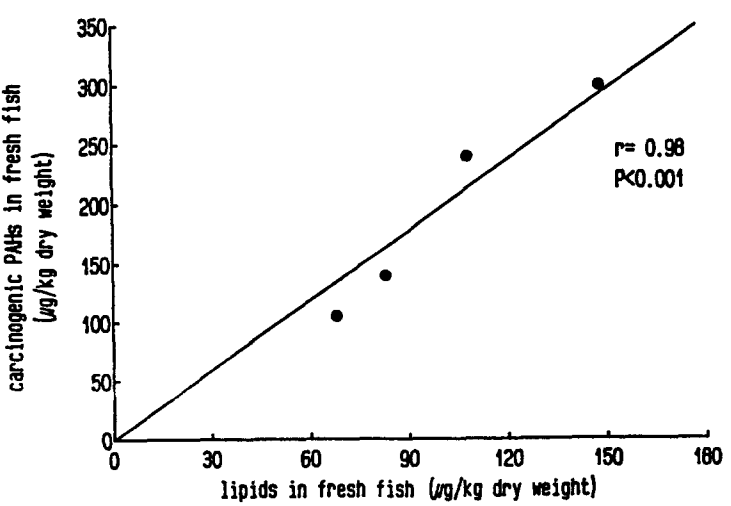

Figure 2. Correlation between 5 carcinogenic PAHs (marked with an asterisk as shown in Tab. 1) and lipids in four different species of fresh fish from Lagos.

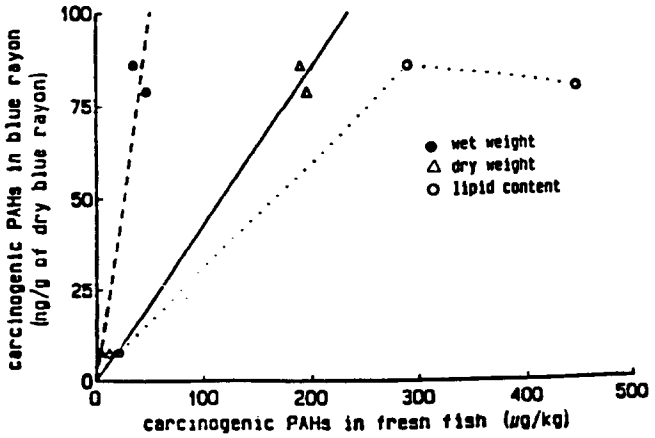

Figure 3. Correlation between 6 carcinogenic PAHs in blue rayon (ng/g of dry blue rayon) and carcinogenic PAHs in two species of fresh fish available in the three cities sampled, expressed as: ● µg/kg wet weight, Δ µg/kg dry weight, O µg/kg of lipid content.

Table 2. Concentrations of 12 PAHs (µg/kg dry weight) in smoked fish samples from 3 Nigerian cities.

Sites	Species	ACEN	FLUO	FEN	FLUOR	PY	B(a)A*
LAGOS	P.E.	3030	565	61.8	20.7	117	4.5
LAGOS	P.P.	3330	661	33	28.4	74	5.6
LAGOS	H.N.	4054	2102	114	59	200	10.8
LAGOS	C.H.	1241	1172	40	20.6	35	5.1
WARRI	P.E.	820	878	43	352	99	49
WARRI	H.N.	650	687	29	97	135	25
CAL.	P.E.	4372	1204	23	178	207	29
CAL.	H.N.	2959	1336	35	270	285	51

Sites	Species	CHRY*	B(b)F*	B(k)F*	B(a)P*	DBA*	BGP
LAGOS	P.E.	290	30	1.0	48	8.0	201
LAGOS	P.P.	220	29.5	1.1	60	21.8	177
LAGOS	H.N.	324	4.0	1.6	88	10	65
LAGOS	C.H.	139	8.6	1.5	35.5	35	206
WARRI	P.E.	386	75	2.9	77	1.4	132
WARRI	H.N.	300	48	1.6	94	2.0	140
CAL.	P.E.	525	41.4	1.6	60	0.27	16
CAL.	H.N.	83	88	3.2	139	1.5	27

The correlation reported in Fig. 3 demonstrated that, despite varying lipid contents in the species sampled, the levels of carcinogenic PAHs in fresh fish reflected the degree of contamination of the harvesting site measured by the value of carcinogenic PAHs in blue rayon.

The concentrations of the 12 PAHs (µg/kg dry weight) in smoked fish samples from the three cities are shown in Table 2. All PAHs evaluated had detectable levels; acenaphthene, that was absent in fresh fish samples, was the most prevalent PAH in all smoked samples we analyzed and detectable levels of chrysene were also obtained in smoked samples from Calabar.

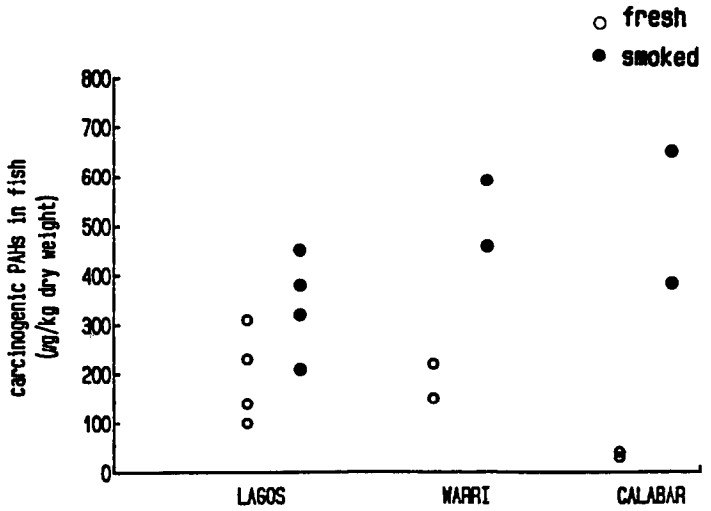

Figure 4. Levels of carcinogenic PAHs in fresh and smoked fish from three cities sampled.

Figure 4 illustrates the cumulative effect of smoke curing on the overall dietary burden of carcinogenic PAHs.

In Calabar and Warri, where basal levels were quite different, we measured similar values in smoked samples, probably indicating a longer duration of smoking. Smoked samples from Lagos on the other hand, had values of PAHs comparable to those in fresh fish possibly reflecting the shorter smoking time.

From these data, and on the basis of the current dietary practices in Nigeria, we calculated that an approximate daily consumption of 80 g of fresh fish per meal would correspond to a daily intake of about 0.5 µg of B(a)P for samples from Calabar, while the intake in the cases of Lagos and Warri would amount to about 2.0 µg of B(a)P. On the other hand, the consumption of 80 g of smoked fish per day would correspond to a daily intake of about 6 µg of B(a)P. These values are higher than the range reported by Grimmer (1983) for food consumed by European populations (0.94-3.3 µg of benzo(a)pyrene per day).

Recent studies of environmental and food carcinogens in Nigeria have centered on aflatoxins, nitrosamines and viruses as possible aetiological factors accounting for the reported incidence of some neoplasms in the Nigerian population (Emerole et al.,1982). PAHs, aflatoxins and nitrosamines are known initiators and promoters of some human cancers (Phillipson and Ioannides, 1989) and they may play a synergistic role in the genesis of cancers in countries like Nigeria, where these risk factors appear to coexist (Emerole et al., 1982).

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