POTENTIAL CARCINOGENIC RISK FROM POLYCYCLIC AROMATIC HYDROCARBONS IN SELECTED SMOKED FISH SPECIES FROM A TYPICAL RURAL MARKET IN WEST AFRICA

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ABSTRACT
The profile of polycyclic aromatic hydrocarbons (PAHs) in selected smoked fish species from a typical rural market in Nigeria, West Africa, was determined employing Gas Chromatographic technique, in order to evaluate potential carcinogenic risk on the unwary consuming public. The mean concentrations of the individual PAH congeners in specific fish species ranged from below quantification limit (BQL) for chrysene in *Scomber scombrus* and *Trichurus lepturus* to 8.822 µg/kg for indeno(1,2,3c-d)pyrene in *Trachurus trachurus.* Significant differences ($p<0.05$), were observed in the mean concentrations of all the PAH congeners in fish between months while no significant differences ($p>0.05$), were observed in the mean concentrations of pyrene, chrysene, benzo(k)fluoranthene, and benzo(a)anthracene, between fish species. The estimated daily intake (EDI) values for PAHs in mg/person/day, ranged from 0.00005 for benzo(k)fluoranthene to 0.278 for indeno(1,2,3c-d)pyrene while the hazard quotient (HQ) values ranged from 0.004 for benzo(k)fluoranthene in the fish species to 17.64 for indeno(1,2,3c-d)pyrene in *T. lepturus* . The toxic equivalency (TEQ) values were generally dominated by benzo(a)pyrene, which peaked at 6.206 in *T. lepturus.* The cancer risk factors revealed that indeno(1,2,3,c-d)pyrene, benzo(b)fluoranthene, benzo(a)pyrene and benzo(a)anthracene presented critical values as they exceeded the United States Environmental Protection Agency (USEPA) cancer risk guideline value of $1.0 \times 10^{-}6$. It was advocated that such fish should be consumed with caution in order to avert unwholesome carcinogenic health effects in the long run.

Key words: *Carcinogenic, Smoked fish, Polycyclic aromatic hydrocarbons.*

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) or polynuclear aromatic hydrocarbons are organic matter consisting of carbon and hydrogen atoms arranged in aromatic rings [1]. They consist of low molecular weight and high molecular weight congeners, the latter of which are stable and more toxic compared to the former [2]. The production of PAHs are linked to both natural and anthropogenic sources such as vehicular transport, industrial plants, refuse incineration, mining, burning of biomass, domestic cooking, household heating, spillage of petroleum products, urban runoff and agricultural activities [3]. The negative impact of PAHs on man and ecosystems has been attributed to their potential carcinogenic, tetratogenic and mutagenic effects [4]. According to the United States Environmental Protection Agency (USEPA), benzo(a)pyrene has been classified as the most carcinogenic and deleterious PAH [5]. In Nigeria, fish is either consumed fresh or cured. Curing entails the application of some form of processing such as smoking which serves primarily to extend the shelf-life of fish in addition to adding flavour. In Nigeria, it has been recognized that there is paucity of information on the PAH content in smoked fish [6, 7] although there are studies on the PAH profile of fresh fish obtained from the natural aquatic media [8, 9] . The present research, focused on selected smoked fish of the species *Scomber scombrus, Trachurus trachurus and Trichurus lepturus* from a typical rural market in Nigeria, where these products are readily found for onward patronage by the unwary consuming public. Levels of PAHs in the aforementioned smoked fish species have been compared to the World Health Organization (WHO) International standard limit for PAHs in fish and fishery products, with the ultimate goal of protecting public health.

2. MATERIALS AND METHODS

2.1 The study area

The focus of the study was Royal market, situated in Ekpoma, Edo state, Nigeria, gridlocked between Latitude 6º 45' N and Longitude 6º 08'E [10]. The major occupation of the people is farming and trading. The encountered smoked fish sellers were mainly women who obtain fresh fish from neighbouring states like Delta and Kogi states prior to processing. From personal observation and communication, the fishes are commonly smoked traditionally using rubber wood (*Hevea brassilensis*). Experimental fish samples were purchased from the aforementioned market between November 2015 and May 2016. A total of 45 fish samples were used for the study, consisting of 15 samples each of the three fish species of interest viz, *Scomber scombrus* (mean total length= 24.37 cm; mean weight= 95.43 g), *Trachurus trachurus* (mean total length= 25.62 cm; mean weight= 98.61 g), *and Trichurus lepturus* (mean total length= 23.94 cm; mean weight= 105.78 g).

2.2 Laboratory procedures

The smoked fish samples were dried using a DHG 9202® thermostatic drying oven (Healthquip Medical, England) at a temperature of 85º C for 2 hours and thereafter completely milled using a Panasonic® MX-J210PN electric blender. Extraction of PAHs from milled samples was done in accordance with the method by Dean and Xiong [11]. Blanks were prepared following the same procedures without adding the dried and milled fish sample. All extracts were separated and activated copper was added to the combined extract for desulphurization. After subsequent drying over anhydrous sodium sulphate and concentrated to 1.0 ml using a rotary evaporator . An internal standard mixture solution was added to the extract. Final analysis for PAHs was executed using an Agilent Gas Chromatograph (HP 5890 series II)® with mass selective detector (MSD), after following conditioning procedures. All PAH values were expressed in µg/kg.

2.3 Calculation of Hazard quotient (HQ) for PAHs

The Hazard Quotient (HQ) expresses the possibility of a contaminant being an ecological risk or a contaminant of potential ecological concern [12]. The HQ is expressed as follows:

$$HQ = \frac{Measured\ concentration\ of\ contaminant}{Toxicity\ reference\ value\ or\ selected\ screening\ benchmark}$$

HQ ≥ 1 = Possibility of ecological risk indicated or a contaminant of potential ecological concern (COPEC).

2.4 Calculation of toxic equivalency (TEQ) for PAHs

According to the United States Environmental Protection Agency (USEPA), the toxic equivalency estimates the individual PAH potencies relative to that of benzo(a)pyrene, in order to obtain a benzo(a)pyrene equivalent [5].

$TEQ = \sum Ti * TEF$

Where: TEQ = Toxic Equivalency
Ti = PAH concentration in organism
TEF = Toxic Equivalency Factor.

Compounds having TEF values of zero are not important in calculating TEQ. Benzo(a)pyrene has a TEF value of 1 and it serves as an index PAH for other PAH compounds. Benzo(a)anthracene, benzo(k)flouranthene, benzo(b)flouranthene and indeno(123-cd)pyrene have TEF values of 0.1.

2.5 Estimation of daily intake of PAHs compounds by man

The daily intake of PAH compounds by man is estimated by the following equation [13].

40g/person/day * (CPAH) mg/kg/1000g/kg = X mg/person/day.
Where:
40g/person/day = Estimated average fish consumption in Niger Delta, Nigeria.
(CPAH) = PAH concentration in fish.

2.6 Calculation of cancer risk factor

The general equation for estimating human exposure to cancer through the consumption of fish is stated below [14].

ECR = EI*ED*CSF/BW*AT

Where, ECR= Excess Cancer Risk; EI=Estimated Intake; ED= Exposure Duration (30 years for adults); CSF= Oral Cancer Slope Factor; BW=Body Weight (assuming 60 kg weight); AT= Average Time for carcinogens (70 years for adults). Cancer Risk Guideline Value = 1.0×10^{-6}

2.7 Calculation of risk specific dose (RSD)

A risk specific dose is the concentration associated with a specified cancer risk on the assumptions of a 70-kg body weight, consumption of water 2 l/day over a lifetime (70 years), and a cancer potency estimate for the compound derived from carcinogenicity dose–response data using a linearized multistage model, which is a conservative model. The risk-specific dose at a risk level of 10^{-6} represents the concentration of a carcinogen in drinking water associated with an excess cancer risk of one in a million for a70-kg person drinking 2 l/day water for a lifetime, 70 years [15, 16].

RSD = (70 kg body weight) × (risk level) /(2 l/day) ×(q)

Where; Risk level = Usually specified e.g 10^{-6} or 1 in a million; q = Cancer potency factor.

For this study, the modified and applied RSD was;

RSD = (70 kg body weight) × (risk level) /(EDI) ×(q)
Where; EDI = Estimated daily intake of PAHs via fish
Risk level = Usually specified e.g 10^{-6} or 1 in a million; q = Cancer potency factor.

2.8 Statistical procedure

A GENSTAT® computer software (Version 12.1 for Windows) was used for statistical analysis.
Data generated from the study were subjected to Analysis of Variance (ANOVA) to determine significant differences between mean values of PAHs at 5% level of significance. Significant means were separated using Duncan Multiple Range Test while Microsoft Excel (for Windows 2010), was used for all graphical presentations.

3. RESULTS AND DISCUSSION

As shown in Table 1, the mean concentrations of the individual PAHs in fish across the sampled months ranged from below quantification limit (BQL) for chrysene in the months of December, and January; benzo(k)fluoranthene, in the months of December, January, April and May to 10.581 µg/kg for indeno(1,2,3c-d)pyrene in the month of April. Significant differences ($p<0.05$), were observed in the mean concentrations of all the PAHs in fish between months. As shown in Table 2, the mean concentrations of the individual PAHs in specific fish species ranged from below quantification limit (BQL) for chrysene in *Scomber scombrus* and *Trichurus lepturus*; benzo(k)fluoranthene in *Trachurus trachurus* and *Trichurus lepturus* to 8.822 µg/kg for indeno(1,2,3c-d)pyrene in *Trachurus trachurus*. No Significant differences ($p>0.05$), were observed in the mean concentrations of pyrene, chrysene, benzo(k)fluoranthene, and benzo(a)anthracene, between fish species. As shown in Figure 1, the hazard quotient (HQ) values of PAHs in fish species, ranged from 0.004 for benzo(k)fluoranthene in the fish species to 17.64 for indeno(1,2,3c-d)pyrene in *Trichurus lepturus* while the estimated daily intake (EDI) values for PAHs in mg/person/day, ranged from 0.00005 for benzo(k)fluoranthene to 0.278 for indeno(1,2,3c-d)pyrene as presented in Figure 2. As shown in Figure 3, the TEQ values were generally dominated by benzo(a)pyrene, which peaked with a value of 6.206 in *Trichurus lepturus.* As presented in Table 3, the cancer risk factors ranged from 3.0 x 10^{-7} for benzo(a)anthracene to 1.10 x 10^{-3} for benzo(b)fluoranthene while the risk specific dose (RSD) values for PAHs ranged from 0.19 mg/person/day for benzo(a)anthracene to 0.45 mg/person/day for benzo(b)fluoranthene, as shown in Figure 4. The total PAH content(µg/kg) in fish species ranged from 18.42 in *Scomber scombrus* to 33.55 in *Trachurus trachurus* as shown in Figure 5.

Table 1: Mean concentrations (µg/kg) of PAHs in fish across various months

PAH congener	Nov	Dec	Jan	April	May
Pyrene	0.035^c	0.123^b	0.263^a	0.130^b	0.277^a
Phenathrene	0.104^c	0.257^b	0.523^a	0.100^c	0.203^b
Naphthalene	0.933^b	2.210^b	4.557^a	3.857^a	3.863^a
Indeno(1,2,3c-d)pyrene	0.001^c	4.977^b	10.268^a	10.581^a	10.267^a
Fluoranthene	0.033^b	0.077^b	0.157^b	0.297^a	0.410^a
Fluorene	0.173^b	0.403^b	0.837^b	1.497^a	2.241^a
Chrysene	0.001^a	0.000^b	0.000^b	0.033^a	0.043^a
Benzo(k)fluoranthene	0.003^a	0.000^b	0.000^b	0.000^b	0.000^b
Benzo(b)fluoranthene	0.001^c	4.320^b	8.903^a	7.837^a	5.273^b
Benzo(a)pyrene	0.001^c	3.527^b	7.280^a	6.237^a	6.940^a
Benzo(a)anthracene	0.010^b	0.051^b	0.103^a	0.067^a	0.133^a
Anthracene	0.016^b	0.123^b	0.263^a	0.253^a	0.201^a
Acenaphthylene	0.521^c	0.217^c	2.513^a	1.587^b	2.853^a
Acenaphthene	0.597^c	1.397^b	2.881^a	2.057^b	2.791^a

Mean values with the same superscripts are not significantly different ($P>0.05$).Horizontal comparison only.

Table 2: Mean concentrations (µg/kg) of PAHs in specific experimental fish species

PAH congener	*Scomber scombrus*	*Trachurus trachurus*	*Trichurus lepturus*	*WHO Threshold
Pyrene	0.135[a]	0.156[a]	0.206[a]	0.5
Phenathrene	0.175[b]	0.286[a]	0.251[a]	0.5
Naphthalene	1.969[b]	3.958[a]	3.325[a]	0.5
Indeno(1,2,3c-d)pyrene	5.056[b]	8.822[a]	6.920[a]	0.5
Fluoranthene	0.146[b]	0.250[a]	0.188[a]	0.5
Fluorene	0.483[b]	1.966[a]	0.640[b]	0.5
Chrysene	0.000[a]	0.046[a]	0.000[a]	0.5
Benzo(k)fluoranthene	0.002[a]	0.000[a]	0.000[a]	0.5
Benzo(b)fluoranthene	3.824[b]	7.156[a]	4.820[b]	0.5
Benzo(a)pyrene	3.426[b]	6.206[a]	4.758[b]	0.5
Benzo(a)anthracene	0.043[a]	0.087[a]	0.088[a]	0.5
Anthracene	0.096[b]	0.218[a]	0.200[a]	0.5
Acenaphthylene	1.551[b]	2.024[a]	1.639[b]	0.5
Acenaphthene	1.506[b]	2.379[a]	1.947[a]	0.5

Mean values with the same superscripts are not significantly different (P>0.05).Horizontal comparison only. *World Health Organisation [17].

Table 3: Calculated cancer risk factors for polycyclic aromatic hydrocarbons

PAH congener	EI	ED	CSF (USEPA values)	BW	AT	ECR
Pyrene	0.00664	30	NA	60	70	NA
Phenathrene	0.00948	30	NA	60	70	NA
Naphthalene	0.136	30	NA	60	70	NA
Indeno(1,2,3c-d)pyrene	0.278	30	0.73	60	70	*1.45×10^{-3}
Fluoranthene	0.0078	30	NA	60	70	NA
Fluorene	0.0412	30	NA	60	70	NA
Chrysene	0.0006	30	0.0073	60	70	0
Benzo(k)fluoranthene	0.00005	30	0.073	60	70	0
Benzo(b)fluoranthene	0.21068	30	0.73	60	70	*1.10×10^{-3}
Benzo(a)pyrene	0.00292	30	0.73	60	70	*1.52×10^{-5}
Benzo(a)anthracene	0.00005	30	0.73	60	70	3.0×10^{-7}
Anthracene	0.00684	30	NA	60	70	NA
Acenaphthylene	0.06952	30	NA	60	70	NA
Acenaphthene	0.078	30	NA	60	70	NA

USEPA cancer risk guideline value = 1.0×10^{-6} NA= Not available *Critical value

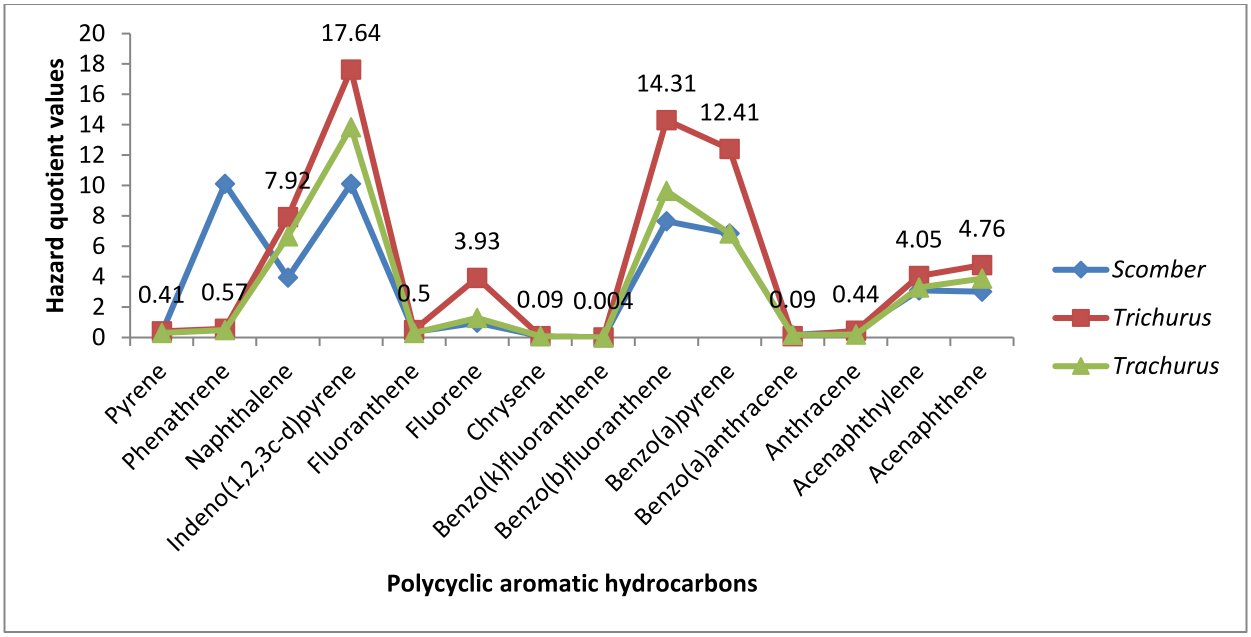

Fig.1: Hazard quotient (HQ) values for PAHs in fish species

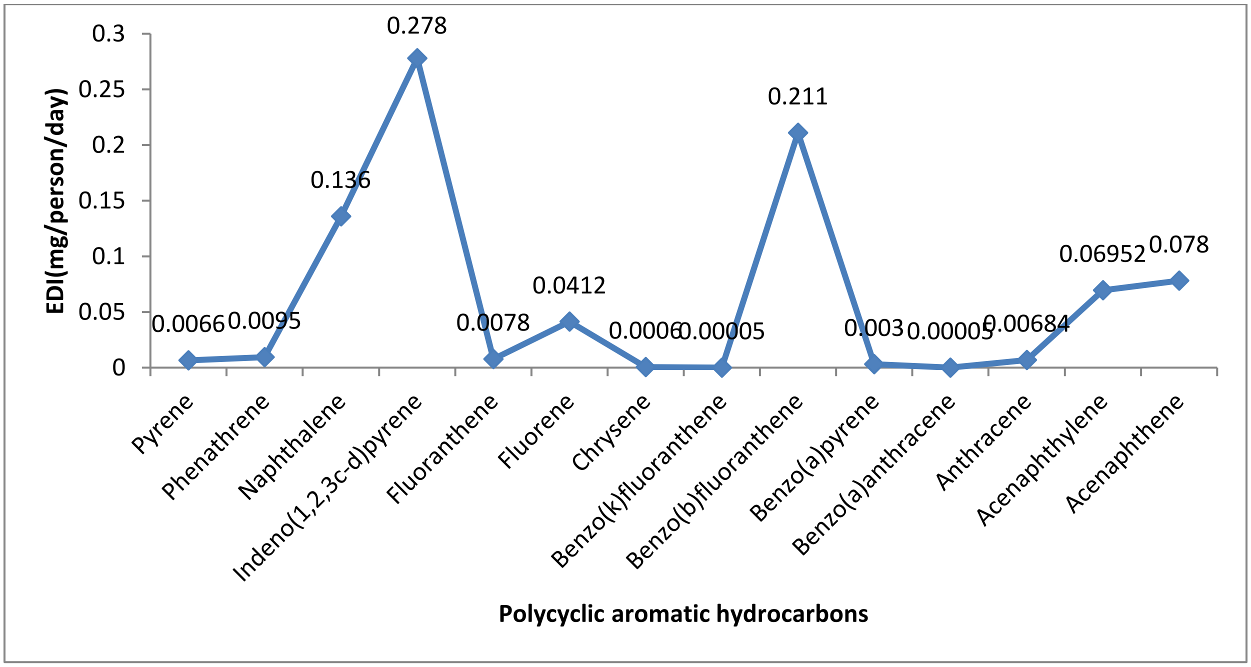

Fig. 2: Estimated daily intake (EDI) values for PAHs

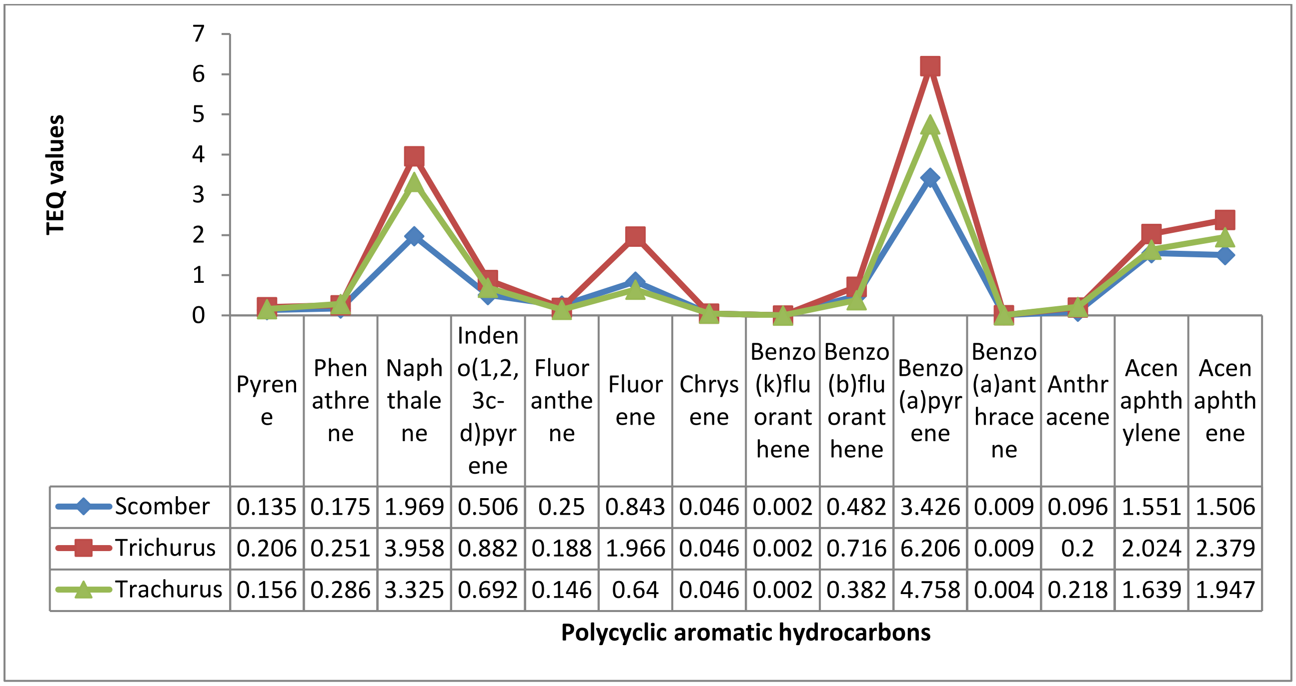

Fig. 3: Toxic equivalency (TEQ) values for PAHs in fish species

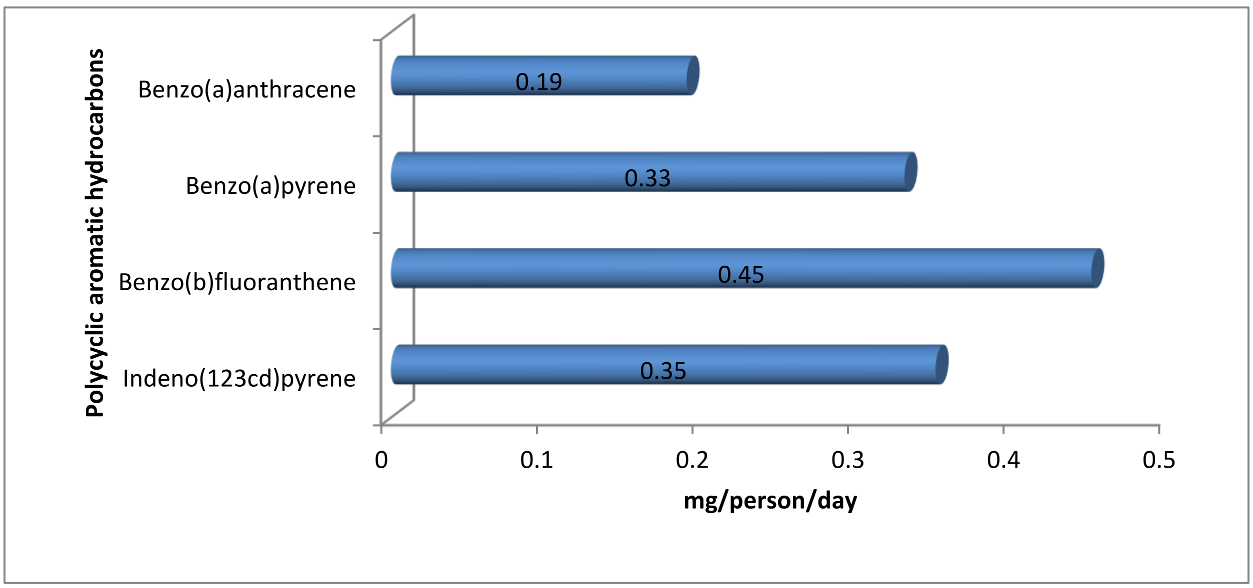

Fig. 4: Risk specific dose (RSD) values for PAHs

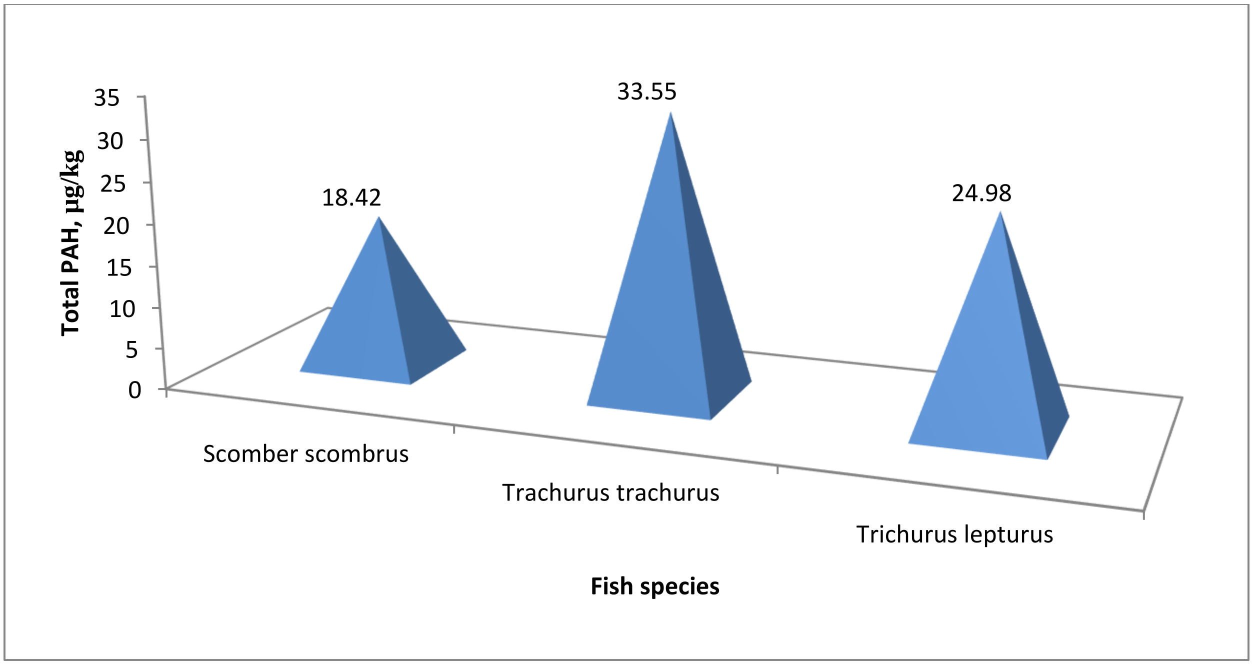

Fig. 5: Total PAH content in experimental fish species

The traditional fuels used in smoking fresh fish on the Africa continent such as charcoal, saw dust and fire wood, have been reported to contribute to the PAH content of fish [6, 7]. In addition, PAHs occur in curing smoke which may eventually migate into the superstructure of such fish [18]. Polycyclic aromatic hydrocarbons are noted for their lipophilicity, semi-volatility and persistency, which altogether make them easily bioavailable [19]. Time-wise, the mean concentrations of PAHs in fish varied across the sampled months with some PAHs such as chrysene and benzo(k)fluoranthene appearing below quantification limits (BQL) in December, January, April and May while the highest mean concentration was recorded for indeno(1,2,3 c-d)pyrene in April. Significant differences ($P<0.05$) were observed in the mean concentrations of PAHs in fish between months. This finding may be attributed to seasonal variations in the input of PAHs into fish. It could also mean that the cured fish species may have come from different sources or different handlers across the various months. Species-wise, there were also variations in PAH content, an indication that the uptake and retention of PAHs by fish varied from one species to another, probably due to their different lipid content. As earlier noted, PAHs are lipophilic substances and this attribute may influence their ultimate concentrations in fish. The mean concentrations of PAH congeners in fish species were BQL for chrysene in *Scomber scombrus* and *Trichurus lepturus*; benzo(k)fluoranthene in *Trachurus trachurus* and *Trichurus lepturus* while peaking for indeno(1,2,3,c-d)pyrene in *Trachurus trachurus*. No Significant differences ($P>0.05$), were observed in the mean concentrations of pyrene, chrysene, benzo(k)fluoranthene, and benzo(a)anthracene, between fish species, indicating a conceivably uniform input of these PAHs into fish during the study period. It has been observed that smoking of fish introduces PAHs where they were not detected originally and may increase their levels where they already occur [20]. In addition, the status of PAHs in fish can be influenced by several factors including duration of exposure, lipid profile of fish and exposure to other xenobiotics [21]. The hazard quotient (HQ) values in this study, were all above unity for naphthalene, indeno(1,2,3,c-d)pyrene, benzo(b)fluoranthene, benzo(a)pyrene, acenaphthylene and acenaphthene in all the experimental fish species, indicating a risk or hazard to potential consumers of smoked fish. The aforementioned PAHs are thus contaminants of potential ecological concern especially with regard to indeno(1,2,3 c-d)pyrene, which had the highest HQ value in this study. In a study on the PAH content of a freshwater cichlid, *Hemichromis fasciatus*, the calculated HQ value were generally below unity and the workers opined that the PAHs present in the fish species did not present an immediate ecological risk and that the possibility of them being contaminants of ecological concern was low [9]. The estimated daily intake (EDI) values for PAHs in mg/person/day, ranged from 0.00005 for benzo(k)fluoranthene to 0.278 for indeno(1,2,3 c-d)pyrene. This finding is not surprising as generally, the mean concentrations of benzo(k)fluoranthene and indeno(1,2,3 c-d)pyrene in fish were the lowest and highest respectively. The concentrations of PAHs in fish would therefore determine the direction of the EDI ranking. In calculating the EDI values, the average fish consumption level in the Niger Delta of 40 g/person/day, was adopted, as the study area, is within the Niger Delta zone. However, the National figure for Nigeria is 68.5 g/person/day, based on the annual per capita fish consumption of 25 kg [22]. The TEQ values calculated in this study, were generally dominated by benzo(a)pyrene, a high molecular weight PAH, which further confirms that this congener has potential for ecological hazard as corroborated by Purchase [12]. The cancer risk factors calculated in this study revealed that indeno(1,2,3,c-d)pyrene, benzo(b)fluoranthene, benzo(a)pyrene and benzo(a)anthracene presented critical values because they exceeded the USEPA cancer risk guideline value of 1.0 x 10^{-6} (USEPA, 1989). The estimated excess cancer risk values (3.12 x 10^{-8} to 3.96 x 10^{-6}) computed in a study for PAHs in a Siluriformid fish species, also exceeded the USEPA cancer risk guideline value [8]. Estimated cancer risk values of 2.37 x 10^{-7} to 1.43 x 10^{-6} for fish species in the Mumbai Harbour, India, which also exceeded the USEPA guideline value have been reported [23]. The implication of this observation is that the aforementioned PAHs are the potential carcinogenic congeners in smoked fish, should the consumption of such fish be sustained over time. The carcinogenicity of these PAHs has been further confirmed by the United States Environmental Protection Agency (USEPA) who classified indeno(1,2,3,c-d)pyrene, benzo(b)fluoranthene, benzo(a)pyrene and benzo(a)anthracene as probable human carcinogens [24]. The risk specific dose (RSD) values for PAHs reached a zenith for benzo(b)fluoranthene and was the least for benzo(b)anthracene. This pattern was clearly dictated by the cancer risk factors calculated in this study, which followed a similar trend. It would thus appear that benzo(b)fluoranthene is a major PAH congener to reckon with in terms of presentation of risk to potential consumers of smoked fish. The RSD values are generally the estimated concentrations of PAHs that will be associated with an actual cancer risk over time. The RSD formula applied in this research was modified to accommodate the estimated daily intake (EDI) of PAHs via fish consumption while retaining all other major components for uniformity and conformity. This modification was warranted as the study dwelt on smoked fish and not a liquid medium such as water as earlier espoused [15,16]. It is also pertinent to note at this point that the calculated EDI values in this study are specific to the research location only and thus cannot be applied to areas outside the zone of the study. The total PAH content in fish was the least in *Scomber scombrus* in this study, suggesting that prospective buyers of smoked fish from this market should opt for this particular fish species owing to its relatively lower total PAH load. The research revealed that the mean concentrations of indeno(1,2,3 c-d)pyrene, benzo(b)fluoranthene, benzo(a)pyrene, naphthalene, fluorene and acenaphthene in all the experimental fish species exceeded the World Health Organization [17] maximum threshold of 0.5 µg/kg for PAHs in smoked fish, indicating that such fish must be consumed with caution in order to avert unwholesome health effects in the long run. The adverse health effects of PAHs are usually tied to the trio of carcinogenicity, mutagenicity and teratogenicity. However, some of the other negative impacts on human health associated with PAHs are cutaneous problems, occular degeneration and impairment of the male and female reproductive systems [20].

4. OVERALL CONCLUSIONS

Arising from results from the study, the potential treat by PAHs to consumers of the aforesaid smoked fish species is a harsh reality. Potential and actual consumers of these smoked products are thus enjoined to exercise caution in their consumption in order to avoid carcinogenic health effects in the long run. It is suggested that other protein sources be included in the diet of consumers so as to alleviate the dependence on smoked fish. Food processors must be enlightened on the need to process fish with low heat in order to reduce the PAH content of fish. It is advocated that relevant Agencies such as the Ministry of Health, carry out enlightenment programmes in order to brief the public on the actual and potential hazards associated with the prolonged consumption of smoked fish products.

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