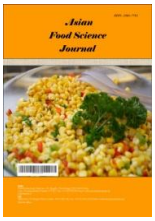

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Polycyclic Aromatic Hydrocarbons in Two Commonly Consumed Fresh and Smoked Fishes in Ekiti State and their Effects on Human Health

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSA designed the study, performed the statistical analysis, author AJA wrote the protocol, and wrote the first draft of the manuscript. Authors A. A. Adebawore and A. A. Araromi managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objective: Polycyclic aromatic hydrocarbons have received substantial consideration as an environmental organic pollutant in many continents such as Africa, Europe, and Asia as well as parts of America. Many polycyclic aromatic hydrocarbon compounds have been proven, identified and quantified in nearly all segments of the environment due to their carcinogenicity, mutagenicity, and cytotoxicity even at very low concentrations. The objective of the study was to look at the levels of polycyclic aromatic hydrocarbons in fresh and smoked *Scomber scombrus* (Atlantic mackerel or Titus) and *Trachurus trachurus* (horse mackerel or kote in southwestern Nigeria) sold in Ado-Ekiti major markets, Nigeria and also assess the risks involved in their exposure and consumption.
Materials and Methods: Fresh and smoked samples of two selected fishes (Kote and Titus) were taken for this study. They were cleaned and wrapped in aluminium foils, then refrigerated and the

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homogenized samples were extracted simultaneously by solvent-solid and Soxhlet extraction. The extracts were analyzed for sixteen polycyclic aromatic hydrocarbons using the Agilent 6890N GC-FID/MS. One and 2-way ANOVA and SPSS were employed for the statistical analysis. **Results:** The mean total polycyclic aromatic hydrocarbons levels in the fish samples ranged from 0.028 and 0.145 µg/kg. High molecular weight PAHs (HMW-PAHs) were generally predominant compared to low molecular weight PAHs (LMW-PAHs). The LMWPAH/HMW-PAH ratios were < 1 for both samples, indicating anthropogenic, mainly pyrogenic, the origin of PAHs in the sourced environment. Risk assessment conducted using benzo(a)pyrene carcinogenic and mutagenic toxicity equivalency factors (TEF and MEF, respectively) showed low risk (8.69e-08 – 5.93e-07 and 1.02e-07 – 1.83e-07 µg/kg, respectively for carcinogenicity and mutagenicity) associated with consuming both smoked and fresh fish samples were below USEPA guideline (1.0e-05) for potential cancer risk. The mean hazard indexes ranged from 6.77e-08 – 4.61e-07 and were below 1 in line with an acceptable cumulative threshold. Correlation is significant at the 0.01 < P > 0.05 levels (2-tailed).
Conclusion: This study showed that there are no adverse health effects of PAHs content on consumers of these two fish samples, however, levels of PAHs present in smoked fish may pose elevated cancer risks if consumed at high consumption rates over a long period.

Keywords: Fresh fish; smoked fish; polycyclic aromatic hydrocarbons; carcinogenicity; mutagenicity; human health; hazard index.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) compound are widespread environmental contaminants representing an important group of potent carcinogens that are present in the environment; traces of these substance has been found in various food products [1,2,3]. PAHs are formed by incomplete combustion processes which occur whenever wood, coal or oil are burnt. Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants containing two or more fused benzene rings known to be ubiquitous in both marine and terrestrial environments [4]. Foods can be contaminated by PAH that is present in the air (by deposition), soil (by transfer) or water (deposition and transfer).

The occurrence of PAH in foods is influenced by the same physicochemical characteristics that determine their absorption and distribution in man. These are their relative solubility in water and organic solvents, which determine their capacities for transport and distribution between different environmental compartments and their uptake and accumulation by living organisms. The transportation of PAH in the atmosphere is influenced by their volatility. The chemical reactivity of PAH influence adsorption to organic material or degradation in the environment. All these factors determine the persistence and capacity of PAH to bio-accumulate in the food chain. PAHs are common to the marine environment and originate from different emission sources, some of the natural, but mostly related to anthropogenic activities such as fuel combustion, offshore production and oil spills [5,6].

PAHs have received much attention due to their potential adverse human health and ecosystem impacts. The possible sources of PAHs in food are environmental contamination, as well as thermal treatment of varying severity which is used in the preparation and manufacturing of foods [7], the absorption and deposition of particulates during food processing such as grilling, boiling, smoking and toasting, the pyrolysis of fats and the incomplete combustion of charcoal [8,9,10].

The lipophilic character of PAHs is responsible for the accumulation in the fat of animals which eat contaminated plants accordingly [10]. No matter how little, non-processed fish contains low PAHs concentration because fishes rapidly metabolize PAHs, resulting in low steady-state level in the tissue [11,12,13]. Various anthropogenic activities have contributed to PAH contamination of coastal and marine environments. These activities include the use of creosote-treated wood in mussel aquaculture [14,15], combustion of organic matter on the lithosphere, offshore oil production and conveyance, and discharge of industrial effluents into the coastal and marine environments [16,17].

PAHs occur as a potent contaminant in diverse food categories and beverages including various set of liquid substances such as water [18,19],

fruit, cereals, oils [20,21], smoked meat [22,23] and smoked fish [24,25,26,27]. The accumulations of these in the body have resulted in growth retardation, low birth weight, small head circumference, low IQ, damaged DNA in unborn children and the disruption of endocrine systems, such as estrogens, thyroid, and steroids [28]. Skin changes such as thickening, darkening, pimples, and reproductive-related effects including early menopause due to destruction of ova have been identified with PAHs [28,29].

It is well defined in mammalian cells that PAHs undergo metabolic activation to diol, and epoxides that bind covalently to cellular macromolecules, including DNA, thereby promoting damages to DNA replication and mutations that initiate the carcinogenic process [28,30,31,32]. Polymorphisms causing glutathione transferase deficiencies (GSTM1) may also occur which could result in elevated breast cancer, lung cancer and other forms of human cancer risk from PAHs [33,34]. Because of their potent mutagenic and carcinogenic effects, PAHs have been included in several priority pollutant lists of the Agency of Toxic Substances and Disease Register (ATSDR), the International Agency for Research on Cancer (IARC), the European Community (EC) and the Environmental Protection Agency (USEPA). Numerous studies have been carried out to determine the tendency levels of exposure of humans to PAHs [35].

Smoking is one of the hoariest food preservation technologies used to achieve the characteristic taste, colour and aroma in most of these special food products (meat and meat products, fish and fish products) [36].

Thus, PAHs have been lengthily and widely studied in the coastal and marine environments quite a several times in many parts of the world [18,37,38,39], as well as the impost on human health risk assessment [37,40]. Although studies conducted on PAHs in the aquatic environment in Nigeria have focused mainly on lagoons and seawater [41]. PAHs are a persistent organic pollutant that belongs to such as low molecular weight compounds consisting of fewer than four rings and high molecular weight compounds of four or more rings [42].

Generally, the sources of PAHs in the coastal environment are described as either petrogenic (if the source is derived from petroleum, e.g. natural oils seepage and oil spills) or pyrogenic/diagenetic (if the source is derived from the incomplete combustion of organic matter and fossil fuel [42,43,44,45].

The ratio of high molecular weight PAHs (HMW-PAHs) to low molecular weight PAHs (LMW-PAHs) has been used to characterize the origin of PAHs in the environment [15]. Petrogenic sources of PAHs show a characteristically higher proportion of LMW-PAHs such as naphthalene and acenaphthenes while pyrogenic PAHs have a characteristically higher proportion of HMW-PAHs such as pyrene and benzo [a] pyrene [46,47]. Thus, petrogenic sources of PAHs exhibit LMW/HMW ratios > 1 whereas pyrogenic sources of PAHs exhibit LMW/HMW ratios < 1 [47].

In addition to the LMW/HMW ratios, isomeric ratios of PAHs have been widely used as indices for the identification of PAH sources of the environment (e.g. Yunker, et al. 2002). For instance, a Benzo [a] anthracene/(Benzo [a] anthacene + Chrysene) (BaA/(BaA + Chry) ratio > 0.35 indicates pyrogenic or combustion sources while a ratio < 0.20 has been attributed to pyrogenic sources although these sources are indistinguishable from ratios of the range 0.20–0.35 [15,48].

This study seeks to determine the effects of the smoking process on PAHs content in smoked fish samples (catfish and sole fish) in Nigeria. The data from the results will be used to determine the levels of PAHs in two species, to identify the sources of the PAHs, and to assess the associated carcinogenic health risks.

2. MATERIALS AND METHODS

2.1 Study Area

The study covered majorly Ado-Ekiti and Iworoko-Ekiti metropolis. These locations were chosen because the fishes consumed in other parts of the state were usually gotten from there and also for population.

2.2 Collection of Test Samples

Fresh and smoked samples of two selected important fishes (*Scomber scombrus* (Atlantic mackerel or Titus) and *Trachurus trachurus* (horse mackerel or kote in southwestern Nigeria) were bought from Oja-Oba Market, Ado-Ekiti in Ado Local Government Areas (LGA) of Ekiti

State, Nigeria. The two fish samples were cleaned and wrapped in aluminium foils, then kept frozen in an ice chest before transported to the laboratory for analysis.

2.3 Reagents

All reagents used in this study were of analytical grades.

2.4 Determination of Polycyclic Aromatic Hydrocarbons Levels in Fish

2.4.1 Processing of fish

The two selected fish (smoked and fresh) samples were dried in the oven for 144h. They were then grounded with a blender (National, MX 795N, Japan) and kept in airtight containers before the extraction process. The sample identities were: smoked Titus (TS), fresh Titus (TF), smoked kote (KS) and fresh kote (KF)

2.4.2 Extraction

Two grams (2 g) of the sample was weighed into a clean extraction container (50 ml beaker) and 10ml of extraction solvent (dichloromethane) was added into the sample and mixed thoroughly and allowed to settle. The sample was carefully filtered into clean solvent rinsed extraction bottles, using filter paper fitted into Buchner funnels. The extract was concentrated to 2 ml and then transferred for cleanup/ separation.

2.4.3 Cleanup/separation

1 cm of moderately packed glass wool was placed at the bottom of 10mm ID*250 mm Loup chromatographic column. A slurry of 2 g activated silica in 10 ml methylene chloride was prepared and placed into the chromatographic column. To the top of the column was added 0.5 cm of sodium sulphate. The column was rinsed with additional 10 ml methylene chloride and pre-eluted with 20 ml of dichloromethane. This was allowed to flow through the column at a rate of about 2 minutes until the liquid in the column was just above the sulphate layer. Immediately 1ml of the extracted samples was transferred into the column. The extraction bottle was rinsed with 1ml of dichloromethane and added to the column as well. The stop clock of the column was opened and the element was collected with a 10 ml graduated cylinders. Just before exposure of the sodium sulphate layer to the air, dichloromethane was added to the column in 1-2 increments. An accurately measured volume of 10 ml of the eluent was collected and labelled.

2.4.4 Gas chromatography analysis

The concentrated aliphatic fractions were transferred into labelled grass vials with rubber clip cap for gas chromatography analysis. 1µl of the concentrated sample was injected utilizing hypodermic syringe through a rubber septum into the column. Separation occurred at the vapour constituent partition between the gas and liquid phase. The sample was automatically detected as it emerges from the column (at constant flow rate) by the FID detector whose response is dependent upon the composition of the vapour.

2.4.5 Chromatographic conditions

The gas chromatography was Hewlett Packed 5890 series II, gas chromatography apparatus, coupled with Flame Ionization Detector (FID) (Hewlett Packard, Wilmington, DE, USA), powered with HP Chemstation Rev. A 09:01 (10206) software to identify and quantify compounds. The GC operating conditions were as follows: fused silica column [30 m*0.25 µmfilm of HP-5(thickness)]; the inlet and injection temperature was set at 275ºC to 310ºC. The split injection was adopted with a split ratio of 8:1. Using rubber septum and volume injected was 1ul. The column temperature was programmed as follows; hold at 65ºC for 2 min; 65-260ºC at 12ºC /min; 260-320ºC at 15ºC /min and maintained at 310ºC for 8minutes and oven temperature was set at 650C. Nitrogen was used as a carrier gas. The hydrogen and compressed air pressure were 30psi. The oven programmed was: the initial temperature at 65ºC. Verification of peaks was carried out based on retention times compared to those of external PAHs. Procedural blank and solvent blanks were analyzed and quantified, but no PAHs were found in these blanks.

2.5 Human Health Risk Assessment of Polycyclic Aromatic Hydrocarbons

The USEPA guideline, as described by Cheung, et al. [39] was followed in determining the carcinogenic risk of exposure to PAHs in fish. By this method, Benzo [a] Pyrene is used as a marker for the occurrence and effect of carcinogenic PAHs in foods and, therefore, the overall carcinogenic health risk from the measured PAHs was estimated based on toxic equivalency factors (TEFs) derived from the

cancer potencies of individual PAH compounds relative to the cancer potency of Benzo [a] Pyrene [15]. The product of the PAH concentration (µg/g) and its TEF gives a Benzo [a] Pyrene equivalent concentration (BaPeq) for each PAH. All the individual Benzo [a] Pyrene equivalent was then summed up to give a carcinogenic potency equivalent concentration (PEC) of all the PAHs according to equation (3) [49].

2.6 Calculation of BaP-equivalent Concentrations

BaP-TEQ (carcinogenic equivalents, ng/m³) and BaP-MEQ (mutagenic equivalents, ng/m³) were calculated by multiplying the concentrations of each PAH compound with its TEF for cancer potency relative to BaP [50] and MEF relative to BaP [51,52], respectively. BaP-TEQ and BaP-MEQ levels for the sum of nonvolatile PAH (∑8PAH; MW≥228) were calculated as follows:

- $(BaP\text{-}TEQ)_{\sum 8PAH}$ = [BaA] x 0.1 + [Chry] x 0.01 + [BbFA] x 0.1 + [BkFA] x 0.1 + [BaP] x 1 + [IP] x 0.1 + [DahA] x 5 + [BghiP] x 0.01 (1)

- $(BaP\text{-}MEQ)_{\sum 8PAH}$= [BaA] x 0.082 + [Chry] x 0.017 + [BbFA] x 0.25 + [BkFA] x 0.11 + [BaP] x 1 + [IP] x 0.31 + [DahA] x 0.29 + [BghiP] x 0.19. (2)

The product of the PAH concentration (µg/g) and its TEF gives a BaP equivalent concentration (BaPeq) for each PAH. All the individual BaPeq were then summed up to give a carcinogenic potency equivalent concentration (PEC) of all the PAHs according to equation (3) [49].

$$PEC = \text{total} \sum(TEF \times Concentration) \quad (3)$$

Potency equivalent concentration values were then compared with a screening value for carcinogenic PAHs. The screening value was calculated from Equation (4) [53].

$$SV = [(RL/SF) \times BW]/CR \quad (4)$$

Where;

SV = screening value (µg/g)
RL=maximum acceptable risk level (dimensionless)
SF = USEPA oral slope factor (µg/g day)
BW = body weight (g)
CR = consumption rate (g/day).

Screening value (SV) is the threshold concentration of total PAHs in fish tissue that is of potential public health concern; BW is the average body weight (g) and was set to 70000 g (i.e. 70 kg) for the adult population [54]; CR is the consumption rate (g/day). Fish consumption rate was set at 68.5 g/day from the annual per capita fish consumption of 25 kg for Nigeria, similar to 68.5 g/day set for the annual per capita fish consumption of 25 kg for Nigeria [54]. RL is the maximum acceptable risk level (dimensionless), which is set to 10-5 [55] so that the maximum risk would be one additional cancer death per 100,000 persons, if an adult weighing 60 kg consumed 68.5 g of fish daily with the same measured concentrations of PAHs for 70 years; SF is the USEPA oral slope factor for PAHs, used to estimate an upper-bound probability of an individual developing cancer as a result of a lifetime (70 years) exposure to carcinogenic PAHs and has a value of 7.30 µg/g day [56]. For safety reasons, a consumption rate of 1 g/day was used to estimate the minimum level that a consumer may be protected from the carcinogenic effects of PAHs detected in these fishes [15].

Table 1. PAHs and their toxic equivalency factors (TEF) relative to the cancer potency of BaP [50]

PAH compound	TEF
BaP	1
Nap	0.001
Acy	0.001
Ace	0.001
Fluo	0.001
Phe	0.001
A	0.01
Fl	0.001
Py	0.001
BaA	0.1
Chry	0.01
BbF	0.1
BkF	0.1
IP	0.1
DahA	5

2.7 Dietary Exposure to PAHs

Human dietary exposure doses express as (mg kg^{-1} BW day^{-1}) occurring over a lifetime were determined.

$$\text{Average daily dose} = \frac{\text{TEQ or MEQ x IR x CF}}{\text{BW}} \quad (5)$$

Where IR is the ingestion or intake rate of carcinogenic (mutagenic) PAHs based on

average fish consumption rate set at 68.5 g day^{-1} per person from the annual per capita fish consumption of 25 kg for Nigeria [54]. CF is the conversion factor (0.001 mg kg^{-1}) and BW is the bodyweight which is set at 70 kg.

2.8 Quality Control

Each air monitoring result was assessed and flagged if there are any issues of sampling conditions such as tube disconnection from the pump, late-takedown, pump failure, switch error, and any other human errors. Once flagged, air monitoring data was given a quality assurance (QA) scores of 1 (0: highest quality) and further examined for an additional score for the erroneous length of sampling time, the erroneous flow rate of the pump, and missing documentation. If a final QA score is ≥3, the data were excluded, sampling was redone. Flagged data were included for analysis if they passed a quality control test (QA 2), as described [57]. Five failed the quality control test.

Mean recovery of deuterated surrogate standards was 97.9% (17% Standard deviation, SD) and 102.6% (15%, SD) for d10 anthracene and d14-p-terphenyl, respectively in all batches except for one. In one batch of measures, the mean recovery efficiency exceeded 130% in some samples (attributed to evaporation during storage) and adjustment was made downward by the multiplier 100/ (mean recovery) and included for the data analysis. The limit of detection (LODs) for 8 individual PAH was 0.03 ng/m^3.

2.9 Statistical Analysis

One-way analysis of variance (ANOVA) was employed for between and within-group comparison while the spearman correlation coefficient was used for paired comparison. 95% and 99% levels of significance ($0.01>p<0.05$) were used for the statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Results

The concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) (µg/kg), Total mean PAH concentrations (µg/kg), LMW-PAH/HMW-PAH in two different fish samples are shown in Table 2. A total of 16 PAHs were analyzed for in smoked and fresh Kote as well as smoked and fresh Titus fish denoted by KS, KF, TS, and TF respectively. The mean concentrations of these PAHs were ranged from 0.028 to 0.145. The highest concentration of 0.937 µg/kg wet wt. was recorded for Py. I-cdP was not detected in all the samples. Ace was only found in (FK). DahA and BghiP were also present in (FT and FK). The calculated Potency Equivalent Concentration (PEC) values were ranged from (0.046 to 1.001) in (FT, ST, FK, and SK) respectively. LMW-PAH/HMW-PAH ratios in fish samples were 0.084, 0.161, 0.345 and 0.059 respectively. From the results, the LMW-PAH/HMW-PAH ratios in the fish samples were < 1.

The PAH ratios of selected compounds are generally considered to be a good indicator of the pollution and the mechanism of PAH distribution of foods. Yunker, et al. [15] have summarized the literature on PAH ratios (Table 3). The ratio of (Ant/(Ant + Phe)) in this study ranged from 0.00 to 0.52 with a mean value of 0.25 indicating a predominance of wood combustion as a source (> 0.10) in the smoked fish. Also, Fla/(Fla + Py) ranged from 0.03 to 0.12 with a mean value of 0.05 indicating the source of petroleum which could either be from the source of collection or lightening of the wood. BaA/(BaA + Chr) in this study ranged from 0.05 to 0.33 with a mean value of 0.43. Benzo [a] Pyrene concentrations on the Fish samples analyzed were below the European Union (EU) limit of 2 µg/kg wet wt. The ratio of BaP/(BghiP) in this study ranged from 0.00 to 2.36 with a mean value of 0.81 indicating a predominance of wood combustion as a source (> 0.10) in the smoked fish. The ratio of IcdP/(IcdP + BghiP) was not found in the study.

Tables 4 and 5 showed the results computed based on carcinogenic and mutagenic risk respectively which ranged from ND to 0.1770. From the results, it was showed that it is possible for 4 or 5 out of 10,000,000 people ingesting fish daily to suffer from cancer diseases.

Table 6 showed the values of non-carcinogenic equivalency which ranged from ND to 0.001 and the levels of hazard index pose on people. The corresponding EQ-BaP daily doses were calculated for carcinogenic, mutagenic and non-carcinogenic risk in the life-time of 70 year's ingestion of smoked and fresh fish products as shown in Tables 4, 5 and 6 respectively. Form the results, it shows that all the fish samples pose no adverse effect according to the standard value released by USEPA [55,56] cancer risk of 1.0×10^{-05} threshold. Also, the non-carcinogenic PAHs produced hazard indexes less than 1 (<1)

as described by EPA which pose no appreciable risk for the development of non-cancer health effects through ingestion.

3.2 Discussion

According to the U.S Environmental Protection Agency [58], seven PAHs have been classified as potent carcinogens: BaA, BaP, BbF, BkF, Chr, DahP and I-cdP. Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) to fish involves bio-concentration from water across their gills and skin [59] and after consumed PAH-contaminated particulate substance along with food, it adsorbs onto the particulate organic matter in the form of soil sediments [60,61]. The resulting biochemical disruption and cell damage can lead to mutations, tumours, and cancer [62]. The lipophilic nature of PAH makes it very easy to accumulate in the fatty tissues of fishes during their uptake [4]. Smoked Kote (KS) fish showed a high amount of PAHs as compared to Fresh Kote (KF) and other samples of Fresh Titus (TF) and Smoked Titus (TS). The reason has been that they feed on debris disposed on the water bank and most species have unusually muscular stomachs and pharynx that help in digestion [63]. These may be the reasons why some of these fish samples have a significant difference between ($P < 0.05$) and ($P<0.01$) concentration of PAHs, most especially in the following sets of PAHs (BaA & Nap; Fl & Ace; Ant & Ace; Ant & Fl; Py & Fl; Ant & Phe; BkF & Chr; BghiP & DahA) respectively. High molecular weight PAHs (HMW-PAHs) were generally predominant compared to low molecular weight PAHs (LMW-PAHs).

The reports on the results analyzed indicated that fresh and smoked fish concentrations were below the detection limit for most of the different PAHs and in a few cases, quantifiable but low levels were found and conclusively the report on PAHs concentration in marine life as recommended by WHO should not exceeds 20 µg/kg (human) body weights maximum. Theoretically, assuming a human body weight of 75 kg and the concentrations of PAH's in smoked fish establishes in the present study, a person could eat up to half-a-kilo of smoked fish per day and still be below the WHO recommended maximum daily intake. Thus, fish consumption bought in the markets, including smoked fish, was shown not to pose a health risk of the community. The possible presence of hydrocarbons in the fish samples could be traced to the source of wooden materials used for smoking. This investigation showed that the accumulation of hydrocarbons in fish tissue does not pose serious health risks in Ekiti State and its environs. The observed differences in PAH bioaccumulation in both samples may also be attributed to differences in feeding preferences and general behaviour [64], as well as the mode of feeding in these species. Smoking is one of the oldest food preservation technologies and can be used to achieve the characteristic taste, colour and aroma for food [36]. However, foods are nowadays smoked for sensory quality rather than for the preservative effect. Yanar, et al. [65] reported that the acceptance of smoked fish in developed countries is based primarily on the sensory characteristic it imparts to the product while Akintola, et al. [66] confirmed the nutritional values and qualities and the adequacy. The LMW- PAH/HMW-PAH ratios indicate that the HMW-PAHs were generally predominant compared to the LMW-PAHs. The predominance of HMW-PAHs maybe because LMW-PAHs are preferentially degraded during PAH transport and burial into sediments [67].

The levels of concentrations of contaminants such as Polycyclic Aromatic Hydrocarbons in fish reflect the state of contamination of the environment [68] and, therefore, the observed levels of total PAHs in fish in this study indicate high levels of PAH contamination in all the fish samples. The LMW-PAH/HMW-PAH ratios observed in both species were < 1, indicating that the sources of these PAHs in the fish analyzed are mainly pyrogenic [47], and are a clear indication of anthropogenic pollution of PAHs in the coastal marine environment. The observed BaA/(BaA + Chry) ratios in both species were > 0.35 which also indicated pyrogenic sources of PAHs contamination. This discovery also confirms the finding of Gilbert, et al. [40] and Adeyeye, et al. [19]. The potential risk of PAH exposure based on TEQ or MEQ may be underestimated if the interaction of some PAHs is synergistic rather than additives. Possible anthropogenic sources include combustion of petroleum, automobile tire, and wood and vehicle emission. According to Lipiatou and Saliot [69], PAHs may be transported from their point of release to the coastal environment via surface runoff and atmospheric deposition. It was observed that in all the fish samples study in this research, the BaP concentrations do not exceed the European Union (EU) limits of 2 µg/kg wet wt. for fish, the safe level for human consumption.

The estimated fish consumption rate of 68.5 g/day for people in Nigeria at large is less than the USEPA fish consumption rate of 142.2 g/day for subsistence consumers (USEPA, 2000). Fish constitutes a major source of animal protein in the diet. Therefore the coastal people that tend to consume larger quantities of fish could be at a greater risk. A consumption rate of 1 g/day, however, appears to be protective towards the carcinogenic effects of the current PAH levels [15]. It is also important to note that the BaP equivalent-based approaches according to Chen and Liao [70] used for carcinogenic risk assessment is restricted to a few PAHs that have been examined in ambient air, and does not account for the toxicity of all PAHs to which the general population are exposed. From the literature, PAHs are also known to cause growth decline [53], endocrine alteration [61], distortions of embryo and larvae [71,72] and DNA impairment [73] in fish, as well as human health effects such as cancer, mutations and birth defects [74,75,19] and it, may also pose adversarial impacts on marine life [15]. However, these working environments may include other potent pollutants than PAHs which may be carcinogenic. Animal studies showed effects of

Table 2. PAH concentrations, mean concentration (µg/kg), PEC value, LMW-PAH/HMW-PAH and BaA/ (BaA + Chry) ratios in fish samples

PAHs	TF	TS	KF	KS
Nap	0.028	0.087	0.072	0.039
Acy	0.011	0.041	0.017	0.028
Ace	ND	ND	0.006	ND
Fl	0.014	0.042	0.015	0.034
Phe	0.006	0.029	0.003	0.014
Ant	ND	0.031	ND	0.014
Flu	0.012	0.027	0.005	0.036
Py	0.092	0.937	0.183	0.891
BaA	0.019	0.067	0.041	0.025
Chr	0.039	0.139	ND	0.479
BbF	0.161	0.092	0.026	0.18
BkF	ND	0.06	0.037	0.245
BaP	0.177	0.105	0.035	0.075
DahA	0.127	ND	ND	0.175
I-cdP	ND	ND	ND	ND
BghiP	0.075	ND	ND	0.087
Total PAHs	0.761	1.657	0.440	2.322
Mean Concentration	0.048	0.104	0.028	0.145
Carcinogenic PAHs	0.523	0.463	0.139	1.179
Non-Carcinogenic	0.238	1.194	0.301	1.143
LMW-PAH/HMW-PAH	0.084	0.161	0.345	0.059

Naphthalene, Acy: Acenaphthylene, Ace: Acenaphthene, Fl: Fluorene, Phe: Phenanthrene, Ant: Anthracene, Flu: Fluoranthene, Py: Pyrene, BaA: Benzo(a)Anthracene, Chr: Chrysene, BbF: Benzo(b)Fluoranthene, BkF: Benzo(k)Fluoranthene, BaP: Benzo(a)Pyrene, DahP: Dibenz(a,h)Anthracene, I-cdP: Indeno(1,2,3-cd)Pyrene, BghiP: Benzo(g,h,i)Perylene, TEF: Toxic Equivalency Factors for cancer potency relative to BaP [50]; MEF: Mutagenic Potency Factor relative to BaP [51,52]; BaP-TEQ: Carcinogenic Equivalents calculated from the cancer potency relative to BaP (TEF) multiplied by the concentration of PAH in a sample. BaP-MEQ: Mutagenic Equivalents calculated from the mutagenic potency relative to BaP (MEF) multiplied by the concentration of PAH in a sample

Table 3. Source characterization and assessment

PAHs Sources	TF	TS	KF	KS	Petroleum	Wood
Ant/(Ant + Phe)	0.00	0.52	0.00	0.50	<0.10	>0.10
Fla/(Fla + Py)	0.12	0.03	0.03	0.04	<0.40	>0.50
BaA/(BaA + Chr)	0.33	0.33	1.00	0.05	<0.20	>0.20 - 5.00
BaP/(BghiP)	2.36	0.00	0.00	0.86	<0.60	>0.60 - 5.00
IcdP/(IcdP + BghiP)	0.00	0.00	0.00	0.00	<0.50	>0.50

certain PAHs on hematopoietic andimmune systems producing reproductive, neurologic and developmental effects [76,77,19]. Thus, the values reported in this study may need to be considered as the lower limit of estimated potential PAH health risk resulting from fish samples. Further investigations are needed to ascertain whether BaP-equivalent levels are associated with any observed health outcomes of the cohort.

Table 4. Risk assessment based on carcinogenic equivalency and the average daily dose

Carcinogenic equivalency	TF	TS	KF	KS
Benzo(a)Anthracene	0.00190	0.00670	0.00410	0.00250
Chrysene	0.00004	0.00014	ND	0.00048
Benzo(b)Fluoranthene	0.01610	0.00920	0.00260	0.01800
Benzo(k)Fluoranthene	ND	0.00060	0.00037	0.00245
Benzo(a)Pyrene	0.17700	0.10500	0.03500	0.07500
Dibenz(a,h)Anthracene	0.635	ND	ND	0.875
Indeno(1,2,3-cd)Pyrene	ND	ND	ND	ND
Total BaP-TEQ	0.83004	0.12164	0.04207	0.97343
BaPEQ daily dose (mg/kg/day)	8.12E-07	1.19E-07	4.12E-08	9.53E-07
LECR	5.93E-07	8.69E-08	3.01E-08	6.95E-07

LECR: Lifetime Excess Carcinogenic Risk, ND: Not Detected

Table 5. Risk assessment based on mutagenic equivalency and the average daily dose

Mutagenic equivalency	TF	TS	KF	KS
Benzo(a)Anthracene	0.0016	0.0055	0.0034	0.0021
Chrysene	0.0007	0.0024	ND	0.0081
Benzo(b)Fluoranthene	0.0403	0.0230	0.0065	0.0450
Benzo(k)Fluoranthene	ND	0.0066	0.0041	0.0270
Benzo(a)Pyrene	0.1770	0.1050	0.0350	0.0750
Dibenz(a,h)Anthracene	0.0368	ND	ND	ND
Indeno(1,2,3-cd)Pyrene	ND	ND	ND	ND
Total BaP-TEQ	0.2563	0.1425	0.0489	0.2079
BaPEQ daily dose (mg/kg/day)	2.51E-07	1.39E-07	4.79E-08	2.03E-07
*LECR	1.83E-07	1.02E-07	3.50E-08	1.48E-07

LECR: Lifetime Excess Carcinogenic Risk, ND: No Detected

Table 6. Risk assessment based on a non-carcinogenic equivalency, average daily dose and hazard index

Non-carcinogenic equivalency	TF	TS	KF	KS
Naphthalene	0.0000280	0.0000870	0.0000720	0.0000390
Acenaphthylene	0.0000110	0.0000410	0.0000170	0.0000280
Acenaphthene	ND	ND	0.0000060	ND
Fluorene	0.0000140	0.0000420	0.0000150	0.0000340
Phenanthrene	0.0000060	0.0000290	0.0000030	0.0000140
Anthracene	ND	0.0003100	ND	0.0001400
Fluoranthene	0.0000120	0.0000270	0.0000050	0.0000360
Pyrene	0.0000920	0.0009370	0.0001830	0.0008910
Benzo(g,h,i)Perylene	0.0007500	ND	ND	0.0008700
Total BaP-TEQ	0.0009	0.0015	0.0003	0.0021
BaPEQ daily dose (mg/kg/day)	8.93E-10	1.44E-09	2.95E-10	2.01E-09
Hazard Index	2.05E-07	3.31E-07	6.77E-08	4.61E-07

Table 7. Statistical analysis from the various distributions of PAHs in fish samples

	Nap	Acy	Ace	Fl	Phe	Ant	Flu	Py	BaA	Chr	BbF	BkF	Bap	DahA	Bghip
Nap	1.00														
Acy	0.650	1.00													
Ace	0.374	-0.366	1.00												
Fl	0.449	0.971*	-0.537	1.00											
Phe	0.534	0.949	-0.573	0.940	1.00										
Ant	0.574	0.980*	-0.509	0.967*	0.992**	1.00									
Flu	-0.061	0.703	-0.711	0.850	0.680	0.709	1.00								
Py	0.354	0.925	-0.508	0.980*	0.853	0.898	0.912*	1.00							
BaA	0.957*	0.785	0.093	0.618	0.740	0.757	0.113	0.497	1.00						
Chr	-0.291	0.418	-0.503	0.594	0.320	0.376	0.905	0.731	-0.210	1.00					
BbF	-0.787	-0.038	-0.843	0.197	0.108	0.067	0.623	0.262	-0.587	0.651	1.00				
BkF	-0.208	0.383	-0.296	0.531	0.219	0.299	0.821	0.688	-0.190	0.974*	0.493	1.00			
BaP	-0.526	-0.235	-0.700	-0.130	0.079	-0.040	-0.002	-0.225	-0.308	-0.178	0.600	-0.384	1.00		
DahA	-0.902	-0.290	-0.563	-0.503	-0.229	-0.240	0.473	0.073	-0.815	0.671	0.919	0.583	0.374	1.00	
BghiP	-0.939	-0.358	-0.574	-0.124	-0.267	-0.291	0.399	-0.012	-0.845	0.585	0.923	0.485	0.464	0.993**	1.00

** Correlation is significant at the 0.05 level (2-tailed) and ** Correlation is significant at the 0.01 level (2-tailed)*

4. CONCLUSION

This study has proven the fact that Polycyclic Aromatic Hydrocarbons levels detected in all the fish samples were below detection limits and, thus, consumption of these fishes may not pose a serious significant health risk to the populace that consumes this fish species. High molecular weight PAHs were predominant over low molecular weight PAHs, indicating that PAH contamination in this study is mainly from pyrogenic. The community should take a proactive and public stand against individuals or groups who use gasoline to preserves their fish. These activities predominantly result in a vast environmental footprint and pose a serious health hazard on people consuming the fish species.

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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