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CONCENTRATION AND POTENTIAL HEALTH RISK ASSOCIATED WITH DIETARY INTAKE OF SMOKED FISH FROM LAGOS LAGOON

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ABSTRACT

This study assessed the concentration and potential health risk associated with dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in smoked fish products from four fishing communities along Lagos Lagoon, Nigeria. Sixty smoked fish samples obtained from two fish species (*Chrysichthys nigrodigitatus* and *Elopslacerta*) which were processed individually with three firewood (*Cola nitida*, *Funtumia elastica* and *Alchornea cordifolia*) were collected from fish processors between October and December, 2018. Samples were subjected to polycyclic aromatic hydrocarbons (PAHs) analysis using gas chromatography-mass spectrometry (GC-MS) and human health risk model analysis. The PAH congeners varied in smoked fish while Pyrene was the most dominant congener in all the fish samples, accounting for more than 70 % of the total PAHs. The highest total PAHs levels (2431.85 mg kg⁻¹) was observed in *E. lacerta* smoked with *C. nitida*. The Dietary Daily Intake (DDI) values for total and carcinogenic PAHs were higher in *E. lacerta* smoked with *C. nitida* and *C. nigrodigitatus* smoked with *A. cordifolia*, respectively. Carcinogenic Toxic Equivalent (TEQ) values were lower than the estimated Screening Value (SV) of 12.83 indicating low risk of developing cancer through consumption of assessed smoked fish products. Excess Cancer Risk estimated exceeded the permissible limit (1.0×10^{-6}) set by USEPA. Positive correlations ($p < 0.01$) existed between TEQ and total PAHs, noncarcinogenic PAHs, PAH₄, and DDI. This study provides insights into the variation in PAHs level and appropriateness of different fuelwood for smoking similar or dissimilar fish species.

Key words: *Chrysichthys nigrodigitatus*, dietary daily intake, *Elopslacerta*, fish smoking, food safety, health risk, non-carcinogenic

INTRODUCTION

Fish smoking, particularly hot smoking, remains the most preferred and commonly used methods of fish processing that is still currently being employed by the small-scale fish processors in Nigeria and other developing countries (Adebowale *et al.*, 2012; George *et al.*,

2014). Smoked fish have come to stay in the markets due to its nutritional value, organoleptic properties and affordability (Olaoye *et al.*, 2015). Fish smoking requires a large quantity of wood as heat source and majority of smoked fish processors had preference for hardwood owing to the intensity of heat generated, higher flame temperature and maximum thermal efficiency when compared with other heat sources (Oyewole *et al.*, 2006). However, the choice of wood species for smoking depends on availability at each locality (Obodai *et al.*, 2009; Njai, 2000; George *et al.*, 2014).

Polycyclic Aromatic Hydrocarbons (PAHs) are often generated when there is incomplete combustion or pyrolysis of organic matter whenever wood, coal or oil are burnt during fish smoking (Tongo *et al.*, 2017; Ezike and Ohen, 2018). PAHs are a class of chemical compounds with high affinity for fats, oils/lipids and non-polar solvents, some of which have been described as environmental pollutants (Essumang *et al.*, 2014), potent carcinogens and mutagens in human (Silva *et al.*, 2011; Ezike and Ohen, 2018). Dietary ingestion of PAHs-contaminated foods remains the most common route of absorption and bioaccumulation of PAHs in man. Notable among them is smoked fish because incomplete combustion of fuelwood produces smokes, which then come into direct contact with the fish flesh that are arranged on mesh or trays during smoking, thereby resulting to its contamination with PAHs, especially if the process is not adequately controlled (Stumpe -Viksna *et al.*, 2008; Silva *et al.*, 2011). The actual levels of PAHs contamination in the smoked fish depend on several variables including the type of smoke generator, combustion temperature, degree of smoking as well as composition of the smoke (Njai, 2000).

Majority of the small-scale fish processors still employ the use of traditional smoking ovens, hot smoking techniques, firewood, charcoal and/or saw dust as heat source (Davies *et al.*, 2008; George *et al.*, 2014; Odediran and Ojebiyi, 2017). Ubwa *et al.* (2015) investigated the effects of firewood smoke, saw dust smoke and charcoal smoke on the concentration of PAHs in five fish species (*Arius heudeloti*, *Cynoglossus senegalensis*, *Clarias gariepinus*, *Blunt hawke* and *Mud minnow*). They reported that fish samples processed with saw dust smoke recorded the highest concentrations of total PAHs, followed by firewood smoked samples while charcoal smoked samples had the least. Tongo *et al.* (2017) also observed that the concentrations of PAHs obtained in smoked *Clarias gariepinus*, *Tilapia zilli*, *Ethmalosa fimbriata*, and *Scomberscombrus*, using traditional smoking methods, were above the recommended limits set by the European Union for PAHs in smoked fish and smoked fishery products. This implies that consumption of such smoked products could pose potential health effects to humans. Due to the public health concerns arising from consumption of PAHs-contaminated foods, it becomes imperative to investigate the PAHs concentration and safety level of smoked fish processed using three different wood species by fish processors in Lagos State, Nigeria. Therefore, this study was conducted to assess the PAHs levels and the possible risks of smoked fish offered for sale by small-scale fish processors in fishing communities along Lagos Lagoon, Lagos State.

MATERIALS AND METHODS

Sample collection

Samples of smoked fish were collected from four fishing communities along the Lagos lagoon [Epe (6°36'53"N; 3°57'49"E), Ejinrin (6°39'52"N; 3°52'50"E), Agbowo-Ikosi (6°39'52"N; 3°42'52"E), and Bayeku (6°32'54"N; 3°34'53"E)] based on high intensity of smoked fish processors (Figure 1). Lagos Lagoon is a tropical, coastal estuary located in the heart of Lagos metropolis and is the largest of the four lagoon systems in the State (Olaniyi *et al.*, 2017). It is well-known for prosperous and massive fishing, fishery-related activities and as major supply of fish products to domestic and offshore markets (Phillips *et al.*, 2012). Two

species of commonly smoked fish [Grey- or silver catfish (*Chrysichthys nigrodigitatus*) and African LadyFish (*Elopslacerta*)] processed individually with three different firewood [*Cola nitida* (Obi), *Funtumia elastica* (Ègbà), and *Alchornea cordifolia* (Ìpà)] commonly used by fish processors were selected during the 3 months study period (October – December, 2018). Sixty samples of smoked fish were randomly collected from fish processors who agreed to use one wood specie per smoking cycle at the processing centers. Thereafter, samples were covered with aluminum foil in order to avoid contamination, stored separately in air-tight nylon, properly labeled and transported to the College of Veterinary Medicine Laboratory, Federal University of Agriculture Abeokuta for PAH analysis.

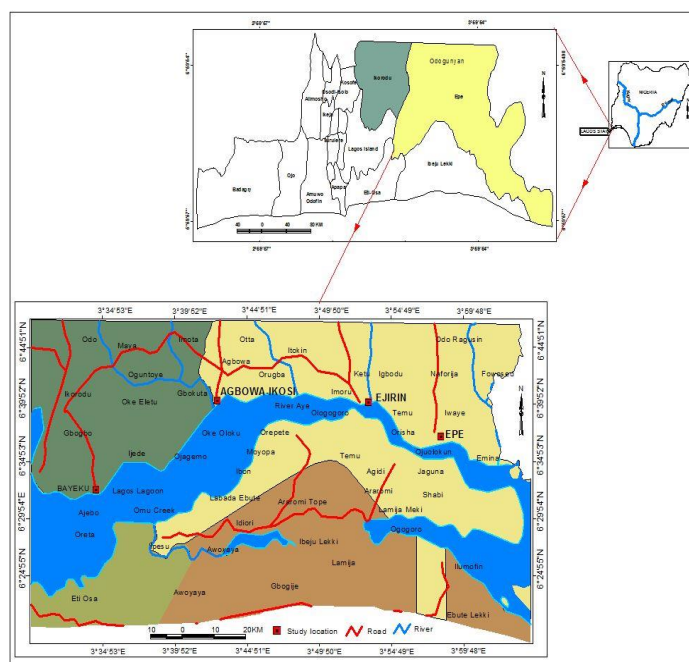


Figure 1. Map of Lagos Lagoon showing sampled fishing communities

Determination of PAHs in smoked fish

Extraction of PAHs

Extraction of PAHs was carried out based on the method described by Tongo *et al.* (2017) with few modifications. Samples were milled separately into fine powder using an electric blender. About 10 g of the fish flour were thoroughly mixed with 40 ml hexane: acetone (1:1), placed into an ultrasonic bath and sonicated for 20 min to separate supernatants of extracts. Mixture was allowed to settle and the solvent layer was decanted. Sample extracts were then concentrated using a rotary evaporator according to the recommended procedures (Buchi® Rotavapor® R-215, Merck KGaA, Darmstadt, Germany) and the contents were collected into 2 ml glass vials.

Chromatographic analysis

The cleaned up extracts were analysed for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo[a]pyrene, chrysene, fluoranthene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene. Samples were analyzed using Agilent 7890B Gas Chromatograph equipped with a flame ionization detector (FID), fitted with a HP-5 capillary column coated with 5 % phenyl methyl siloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness) (Agilent Technologies Inc., Santa Clara, USA). A 1.0 µL of the

samples were injected in split less mode at an injection temperature of 220 °C, at a pressure of 14.861 psi and a total flow of 21.364 mL/min. Purge flow to split vent was set at 15 mL/min at 0.75 min. Oven was initially programmed at 100 °C (2 min) ramped at 10 °C/min to 280 °C (4 min) and then ramped to 300 °C at 10 °C/min. FID temperature was 300 °C with Hydrogen: Air flow at 30 mL/min: 300 mL/min, Nitrogen was used as makeup gas at a flow of 18 mL/min. After calibration, the samples were analysed and corresponding concentrations calculated. Method validation was performed both before and during the sample run to ascertain the functionality and trueness of the whole method by using blank smoked fish fortified with PAH standards at the level of 50.0 µg/kg. The performance values for quality checks were within the acceptable limits as described by the European Union (EU Commission Regulation No. 836/2011). The retention time, correlation, spike recovery and residual standard deviation obtained for each PAHs congener are shown in Table S1.

Table S1. Method precision and relative standard deviation of blank spiked smoked fish (50.0 µgkg⁻¹ PAH)

PAHs	Code	Retention time (min)	Correlation (%)	Spike recovery (%)	Residual Std. Dev.
Naphthalene	NaP	7.115	99.58	102.39	6.29
Acenaphthylene	AcPY	10.454	99.60	101.40	6.88
Acenaphthene	AcP	10.872	99.59	99.00	7.27
Fluorene	Flu	12.016	99.57	99.50	4.90
Phenanthrene	Phe	14.194	99.51	89.98	9.01
Anthracene	Ant	14.296	99.63	92.59	4.79
Fluoranthene	FL	16.947	99.61	100.92	8.23
Pyrene	Pyr	17.450	99.70	115.90	6.30
Benzo(a)anthracene	BaA	20.250	99.80	93.64	6.56
Chrysene	Chr	20.343	99.80	101.14	6.62
Benzo(a)pyrene	BaP	22.705	99.27	99.73	4.11
Benzo(b)fluoranthene	BbFL	23.274	98.84	90.63	11.42
Benzo(k)fluoranthene	BkFL	24.385	98.41	93.46	4.06
Indeno(1,2,3)pyrene	Ind	26.562	95.26	85.95	10.20
Dibenzo(a,h)anthracene	DBA	26.656	95.40	87.63	10.77
Benzo(g,h,i)perylene	BP	27.422	93.89	92.57	10.86

Statistical analysis

Data collected were subjected to descriptive statistics using Statistical Package for Social Sciences (SPSS, version 20.0, 2007; IBM SPSS Statistics, United States). Statistical differences between individual PAH concentrations, low and high molecular weight PAHs, ring types, dietary daily intake (DDI), and carcinogenic potencies of individual PAH concentrations [B(A)Pteq], between the species were performed using Tukey's HSD test in Analysis of Variance (ANOVA) at 0.05 level of significance, while Pearson's correlation analysis was performed for PAHs concentration and health risk exposure indicators.

Human Health Risk Estimations

Potential human risk exposure assessment

To assess human health risks from exposure to PAHs through consumption of smoked fish (dietary intake), human intake models were applied. The Dietary Daily Intake (DDI) concentrations of PAHs from consumption of contaminated smoked fish species were assessed. Carcinogenic risks were also assessed by evaluating the carcinogenic potencies of individual PAH concentrations [B(A)Pteq], the Carcinogenic Toxic Equivalents (TEQs) and the Excess Cancer Risk Index. Values used for parameterization of the human intake models are presented in Table S2.

Table S2. Human Intake Model Parameters

Parameters	Unit	Value	Reference
Concentration of each congener (C_i)	mg kg ⁻¹	Table 1	Table 1
Fish ingestion rate (IFR)	Kg capita ⁻¹ day ⁻¹	0.0548	FAO, 2014
Toxicity equivalence factor (TEF _i)	No Unit	Table S3	Nisbet and LaGoy, 1992
Carcinogenic potency of Benzo[a]Pyrene (Q)	mg kg ⁻¹ day ⁻¹	7.30	Tongo <i>et al.</i> , 2017
Exposure Duration (ED)	Years	70	Tongo <i>et al.</i> , 2017
Adult body weight (BW)	Kg	70	Tongo <i>et al.</i> , 2017
Average life span (ATn)	Days	25,550	FAO, 2014
Concentration of Benzo(a)anthracene (B[a]A)	mg kg ⁻¹	Table 1	Table 1
Concentration of Chrysene (Chr)	mg kg ⁻¹	Table 1	Table 1
Concentration of Benzo(a)pyrene (B[a]P)	mg kg ⁻¹	Table 1	Table 1
Maximum acceptable risk level (RL)	Dimensionless	10 ⁻⁵	USEPA, 2000
Oral Slope Factor (SF)	mg kg ⁻¹ day ⁻¹	7.30	USEPA, 1993
Reference Dose (RfD)	mg kg ⁻¹ day ⁻¹	Table S3	USEPA, 1993

Toxicity equivalence factor (TEF_i) and Reference Dose (RfD) of individual PAHs are shown in Table S2; Concentration of each congener (C_i), Benzo(a)anthracene, Chrysene, and Benzo(a)pyrene used for human intake models are shown in Table 1.

The dietary daily intake (DDI)

The DDI of PAHs in the smoked fish species was assessed for adult population. This was estimated by multiplying the respective PAHs concentration in each fish sample by the fish ingestion rate (IFR) of an average weight adult (70 kg) from Nigeria. The consumption rate for fish in Nigeria for an average adult population was obtained from data of the Food and Agriculture Organization (FAO) on Fishery and Aquaculture Statistics (FAO, 2014). Estimate of DDI was calculated for individual PAH congeners, the sum of the 16 PAHs analysed (TPAHs) and also for the sum of those PAHs considered possible human carcinogens (CPAHs).

$$\text{Dietary Daily Intake (DDI)} = c_i \times \text{IFR} \quad \dots\dots (i)$$

where c_i = concentration of each congener, and IFR = Fish ingestion rate.

Carcinogenic risk indices of PAHs in smoked fish

Cancer risk due to dietary exposure to PAHs in smoked fish was assessed using the PAH4 index, Individual PAH carcinogenic potencies, TEQs and the excess cancer risk index. The PAH4 index was assessed in this study in accordance to the recommendation of the Contaminants in the Food Chain (CONTAM) Panel of the European Food Safety Authority which concluded that PAH4 is a more suitable indicator of PAHs in Food (EFSA, 2008). PAH4 was evaluated as the sum of four different polycyclic aromatic hydrocarbons, which include benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbFL), and benzo(a)pyrene (BaP).

$$PAH4\ Index\ (PAH4) = (BaA) + Chr + BbFL + BaP \quad \dots \quad (ii)$$

The estimated PAH4 index of each fish species was then compared with the maximum permissible level to determine the occurrence and effect of carcinogenic PAHs in the smoked fish samples. The maximum permissible level of 0.03 mg kg⁻¹ for the sum of PAH4 in smoked fishery products as recommended by the European Union (EU) Commission Regulation, No 1327/2014 with respect to maximum levels of PAHs in traditionally smoked fish and fishery products was applied (European Union, 2014).

The carcinogenic potencies of individual PAHs [B(A)Pteq] was estimated by multiplying the PAH concentration in the sample by the individual toxicity equivalency factor (TEF). The TEF is an estimate of the relative toxicity of individual PAH fraction compared to benzo(a)pyrene. The TEFs developed by Nisbet and LaGoy (1992) were applied (Table S3) and these values were used to calculate PAH as benzo[a]pyrene equivalents for a standard adult with 70 kg body weight.

$$[B(A)Pteq] = C_i x TEF_i \quad \dots \quad (iii)$$

where C_i = Concentration of each congener, and TEF_i = Toxicity equivalency factor for individual PAHs.

Table S3. Properties of priority PAHs

Code	No of Aromatic Rings	Molecular weight (g)	Grouping based on molecular weight	Reference dose (RfD)	Toxicity Equivalent Factor (TEF)
NaP	2	128.0626	Low	0.02	0.001
AcPY	3	152.0626	Low	NA	0.001
AcP	3	154.2120	Low	0.06	0.001
Flu	3	166.0783	Low	0.04	0.001
Phe	3	178.0783	Low	NA	0.001
Ant	3	178.0783	Low	0.3	0.01
FL	4	202.0783	Medium	0.03	0.001
Pyr	4	202.0783	Medium	NA	0.001
BaA	4	228.0939	High	NA	0.1
Chr	4	228.0939	High	NA	0.01
BaP	5	252.0939	High	NA	1
BbFL	5	252.0939	High	NA	0.1
BkFL	5	252.0939	High	NA	0.1
Ind	6	276.0939	High	NA	0.1
DBA	6	278.1096	High	NA	1
BP	6	276.0939	High	NA	0.01

Source: USEPA (1993); Nisbet and LaGoy (1992); TEF = Toxicity Equivalent Factor.

Excess Cancer Risk (ECR) induced by dietary exposure to PAHs via smoked fish consumption was assessed using the expression:

$$ECR = \frac{\sum Q \times B(A) \times P \times e \times q \times IFR \times ED}{BW \times ATn} \dots\dots (iv)$$

where Q is the Carcinogenic potency of Benzo(a)pyrene, ED is the exposure duration (70 years), and ATn is the average life span for carcinogens (25550 days).

TEQ is one of the most commonly used indices to facilitate the assessment of toxicity and carcinogenicity of PAHs in smoked fish (Tongo *et al.*, 2017). TEF reports the toxicity-weighted masses of mixtures of individual congeners identified in smoked fish samples assessed. Toxicity equivalency (TEQs) was derived by summing up the values obtained when concentrations of individual PAHs (Ci) was multiplied by its relative toxicity (TEFi).

$$TEQ = \sum (Ci \times TEFi) \dots\dots (v)$$

Screening value (SV) is defined as the concentration of chemicals in edible tissue that are a potential public health concern, and it is used as threshold value against tissue residue level of contamination in similar tissue collected from the environment (US Environmental Protection Agency – USEPA, 2000). In this context, SV is the threshold concentration of total PAHs in fish tissue that is of potential public health concern. SV was calculated using the expression:

$$SV = \frac{[(RL/SF) \times BW]}{IFR} \dots\dots (vi)$$

where SV = screening value (mg kg⁻¹), RL is the maximum acceptable risk level (dimensionless), which was set to 10⁻⁵ (USEPA, 2000) so that the maximum risk would be one additional cancer death per 100,000 persons, if an adult weighing 70 kg consumed 54.8 g of fish daily with the same measured concentrations of PAHs for 70 years. SF is the USEPA oral slope factor for PAHs (mg kg⁻¹day⁻¹), 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 mg kg⁻¹day⁻¹ (USEPA, 1993). BW is the average body weight (g) and was set to 70 kg for the adult population (Tongo *et al.*, 2017). IFR is the fish ingestion rate and was set at 54.8 g day⁻¹ from the annual per capita fish consumption for Nigeria (FAO, 2014).

RESULTS AND DISCUSSION

Concentration of PAHs in smoked fish products

The results of 16 priority PAHs congeners analysed from the smoked fish products are presented in Table 1. Of all the 16 PAHs congener, the smoked fish samples from Lagos Lagoon were contaminated by 9 PAH congener. Pyrene (Pyr) was the most dominant congener in all the fish samples accounting for more than 70 % of total PAHs. This was followed by chrysene (Chr) in *Chrysichthys nigrodigitatus* smoked with *Alchornea cordifolia* (CNA; 76.7991 mg kg⁻¹) and *Chrysichthys nigrodigitatus* smoked with *Funtumia elastica* (CNF; 36.8826 mg kg⁻¹), fluoranthene (FL) in *Chrysichthys nigrodigitatus* smoked with *Cola nitida* (CNC; 15.5608 mg kg⁻¹), *Eloplacerta* smoked with *Alchornea cordifolia* (ELA; 1.142 mg kg⁻¹), and *Eloplacerta* smoked with *Funtumia elastica* (ELF; 10.8137 mg kg⁻¹) as well as anthracene (Ant) in *Eloplacerta* smoked with *Cola nitida* (ELC; 122.9171 mg kg⁻¹). This was dissimilar to the earlier study of Cheung *et al.* (2007) who found naphthalene as the dominant PAH in freshwater and marine fish species from Hong Kong markets. Tongo *et al.* (2017)

reported naphthalene as the most dominant congener in *T. zilli* (0.315 mg kg⁻¹), and *S. scombrus* (1.171 mg kg⁻¹) while Benzo(a)pyrene was the most dominant congener in *C. gariepinus* (0.204 mg kg⁻¹) and *E. fimbriata* (0.288 mg kg⁻¹) from Southern Nigeria. Furthermore, Taiwo *et al.* (2019) revealed that Indole(1,2,3-cd)pyrene and 3-Methylcholanthrene were the major PAHs constituents in smoked fish and other protein food samples from Lagos and Abeokuta, South-western Nigeria. This variation could be connected to the differences in fish species, fuelwood used and/or penetration potential of individual congeners.

Table 1. Mean concentration (mg kg⁻¹) of PAHs in smoked fish products from Lagos Lagoon, Lagos State

PAHs	<i>Chrysichthys nigrodigitatus</i>			<i>Eloplacerta</i>		
	<i>Alchorneacordifolia</i> <i>a</i>	<i>Cola</i> <i>nitida</i>	<i>Funtumia</i> <i>elastica</i>	<i>Alchorneacordifolia</i> <i>a</i>	<i>Cola</i> <i>nitida</i>	<i>Funtumia</i> <i>elastica</i>
NaP	ND	0.7914	9.6559	0.1171	1.7650	8.2615
AcPY	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	7.7112
AcP	<0.0001	6.3157	ND	<0.0001	<0.0001	<0.0001
Flu	<0.0001	ND	<0.0001	ND	<0.0001	<0.0001
Phe	53.6785	<0.0001	6.4598	<0.0001	3.1497	<0.0001
Ant	52.3035	10.5423	13.3088	<0.0001	122.9171	1.6149
FL	20.8415	15.5608	8.6057	1.1420	110.0911	10.8137
Pyr	1193.0337	92.1036	278.2586	45.5592	2166.3347	56.9382
BaA	4.4379	ND	2.2043	ND	<0.0001	ND
Chr	76.7991	0.3420	36.8826	<0.0001	27.5938	1.1912
BaP	ND	ND	ND	ND	ND	ND
BbFL	ND	ND	ND	ND	ND	ND
BkFL	ND	ND	ND	ND	ND	ND
Ind	ND	ND	ND	ND	ND	ND
DBA	ND	ND	ND	ND	ND	ND
BP	ND	ND	ND	ND	ND	ND
∑PAHs	1401.0941	125.6568	355.3757	46.8182	2431.8514	86.5306
∑CPAHs	81.2370	0.3420	39.0870	0.0000	27.5938	1.1912
CPAH/TPAH (%)	5.7984	0.2722	10.9988	0.0000	1.1347	1.3761

∑PAHs = Total PAHs (TPAH); ∑CPAHs = Total Carcinogenic PAHs (CPAH);
ND = Not Detected; <0.0001 = Below level of quantification.

The mean concentrations of total and carcinogenic PAHs vary widely between the two fish species processed with different wood species. The observed variation in PAHs concentrations of the different fish species might be attributed to differences in fat and moisture composition of each specie alongside the nature of the skin cover, which influences the rate of smoke penetration and bioaccumulation affinity. Similar findings were reported by Silva *et al.* (2011) who observed variation in PAH contents in *Arius heude loti* (catfish), *Cynoglossus senegalensis* (sole) and *Haake* (fresh stock fish) processed either by smoking or oven drying, using different heat source (sawdust, fire wood and charcoal). The past study of Yusuf *et al.* (2015) found considerably higher PAHs level in catfish compared to sole fish. Tongo *et al.* (2017) observed significant difference in PAHs values among *Clarias gariepinus*, *Tilapia zillii*, *Ethmalosa fimbriata*, and *Scomberscombrus*. The highest total PAHs concentration of 2431.85 mg kg⁻¹ was obtained in ELC, while the lowest value of 46.82 mg kg⁻¹ was observed in ELA. The highest amount of carcinogenic PAHs (81.24 mg kg⁻¹) was found in CNA predominantly

from Chr and BaP comprising 94.54 and 5.46 %, respectively. The discrepancies in data of smoked fish further confirmed that levels of PAHs in smoked fish products may largely depend on the type of smoke generator, combustion temperature, degree of smoking and composition of the smoke. Previous studies (Njai, 2000; Essumang *et al.*, 2014; Ubwa *et al.*, 2015; Babić *et al.*, 2018) have shown that smoking fish using traditional methods (hot smoking with direct exposure of fish to smoke) increased the amount of PAHs formed in smoked fishes. Similarly, Yusuf *et al.* (2015) reported higher PAHs levels in catfish and sole fish smoked using traditional smoking oven compared to their counterparts smoked with modern smoking kiln. A study by Ubwa *et al.* (2015) attributed varying PAH levels observed in five smoked fish samples to the intensities of the smoke and heat generated by the fuel source as well as the contact time with the smoke.

The higher carcinogenic to total PAHs ratio was obtained in CNF compared with other smoked fish samples (Table 1). This probably explains the lipophilic nature of PAHs and relative solubility in organic solvents which cause higher affinity for fats or lipids stored in fish flesh (Essumang *et al.*, 2014; Ezike and Ohen, 2018). Ake Assi *et al.* (2012) opined that PAHs are liposoluble substances which have higher chance of being absorbed in an oily matrix. Moreso, it was observed that concentrations of total PAHs in smoked fish species assessed in this study was higher than those previously reported in Nigeria (Akpambang *et al.*, 2009; Yusuf *et al.*, 2015; Tongo *et al.*, 2017; 2018), Egypt (Hafez *et al.*, 2017), China (Wen-Jing *et al.*, 2012), and Republic of Serbia (Babić *et al.*, 2018). This could probably imply that the higher quantities of smoke with concomitant concentration of PAHs were emitted by the fuelwood used. Previous work by Essumang *et al.* (2014) reported that the use of modified traditional kiln containing charcoal filters or modified kiln reduced total PAH in smoked fish by 69 % while B(a)P concentration was below the permissible limit of 5.0 mg kg⁻¹ set by the European Commission (2005). In agreement, when different filters (Zeolite, activated carbon and gravel) were incorporated into traditional smoking kiln, Babić *et al.* (2018) observed more than 64 % reduction in total PAHs in smoked carp meat compared to the control group whereas BaA and Chr were below detection level in group with activated carbon filter because they were able to remove substantial amounts of congeners from the smoke before getting into contact with the fish being smoked thereby producing safer and healthier smoked fish products.

Classification of PAHs based on aromatic rings, molecular weight and carcinogenicity

Results showing the classification of PAHs based on aromatic rings, molecular weight and carcinogenicity are presented in Figures 2 to 4. The PAHs composition pattern by ring type showed a considerable predominance of the four-ring PAHs followed by three and two-ring PAHs, whereas five and six-ring PAHs was not detected because they are below the limit of quantification (Figure 2). The concentration of two-ring PAH (Nap) averaged 3.4318 mg kg⁻¹, of which Nap content in ELF was almost 10 times higher than that of CNA. The three-ring PAH (AcPY, AcPh, Flu, Phe, Ant), averaged 46.3336 mg kg⁻¹ with the Anthracene as the major contributor. The mean value of four-ring PAH (FL, Pyr, BaA, Chr) was 691.46 mg kg⁻¹ of which Pyr alone contributed 92.3 percent.

This study showed higher proportion of medium molecular weight (MMW) PAHs was observed in all the smoked fish products investigated accounting for more than 78 % of the total PAHs while high molecular weight (HMW) PAHs was the least (Figure 3). The mean concentration of lower (LMW), medium (MMW), and high (HMW) molecular weight PAHs were 49.7654, 666.5471, and 24.9085 mg kg⁻¹, respectively. HMW PAHs containing 5 or more condensed aromatic rings are considered to be more dangerous to human health than 2 or 3-rings PAHs (EFSA, 2008). The lipophilic nature of the PAHs released by the firewood used as well as fish skin could have provided better protection from the HMW PAHs than others.

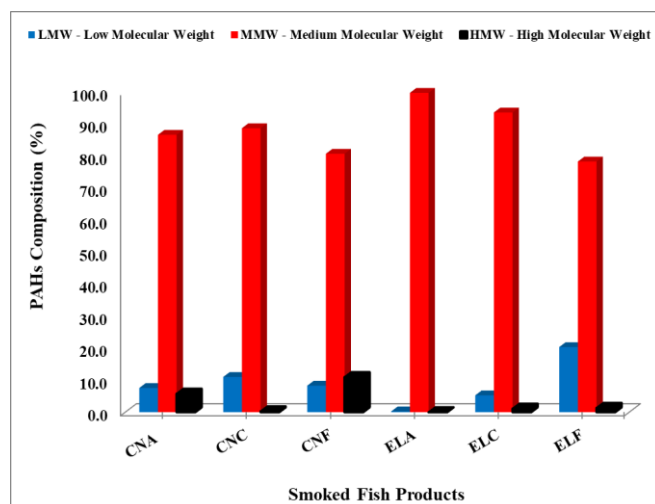


Figure 2. PAHs classification based on molecular weight

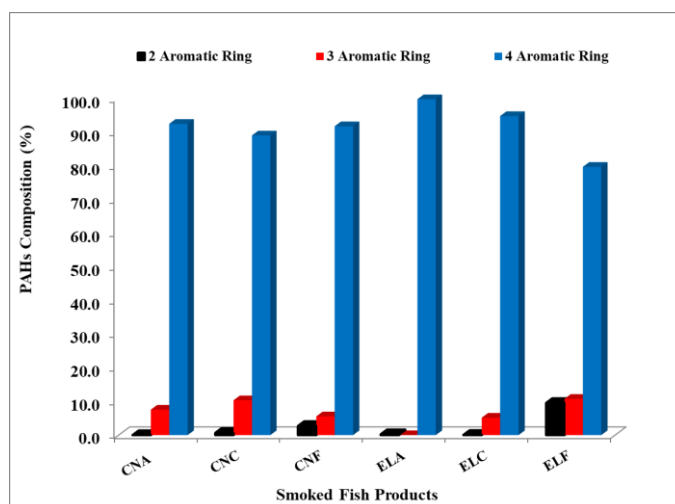


Figure 3. PAHs classification based on aromatic rings

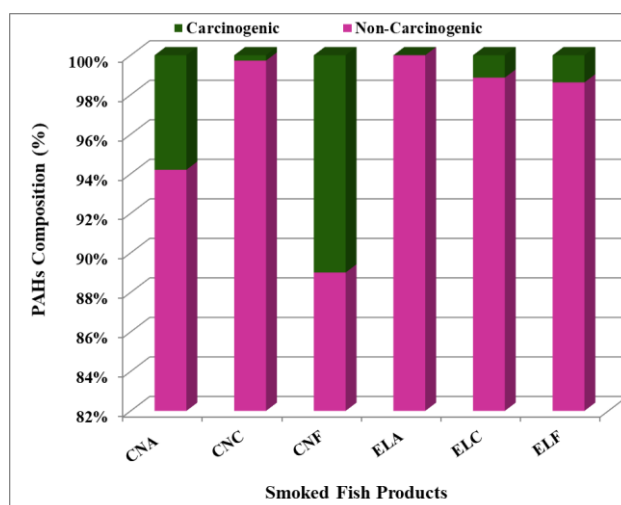


Figure 4. PAHs classification based on carcinogenicity

In addition, the mobility of PAHs is also determined by molecular weight in that PAHs with higher molecular weight usually exhibit reduced mobility when compared with those having low molecular weight (EFSA, 2008). The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) have reported that higher absorption of lower molecular mass PAHs were generally observed in processed foods compared to higher molecular mass PAHs, which are poorly absorbed (EFSA, 2008). This result is in agreement with previous findings such as Cheung *et al.* (2007), Hafez *et al.* (2017) and Yusuf *et al.* (2015) which found the dominance of ≤ 4 -ring PAHs in fish samples. Akpambang *et al.* (2009) and Tongo *et al.* (2017), however, detected six-ring PAHs as well as HMW PAHs in smoked fish which is in contrary with what was found in this study. Taiwo *et al.* (2019) also reported dominance of high molecular weight PAHs in smoked fish, meat and crayfish samples from Lagos and Abeokuta, Nigeria.

Based on proportion of carcinogenicity presented in Figure 4, majority ($\geq 89\%$) of PAHs detected in smoked fish products were non-carcinogenic. Meanwhile, highest proportion (11%) of carcinogenic congeners was detected in CNF, followed by CNA (5.8%), ELF (1.4%) and ELC (1.1%), whereas such PAHs were below level of quantification in ELA. This is because, majority of the high molecular weight PAHs (which are known to have higher carcinogenic and mutagenic potential) were below detectable limit. It should be noted that most dreadful carcinogenic congeners such as B(a)P, D(ah)A and BbFL were not detected in this study. Previous studies by Ubwaet *et al.* (2015) also reported that B(a)P was not detected in smoked Mud minnow, *Cynoglossus senegalensis*, and Blunt hwake. In the same vein, Yusuf *et al.* (2015) also revealed B(a)P and D(ah)A measured below detection limit in catfish and solefish smoked with traditional oven.

Human Risk Exposure Assessment

Dietary Daily Intake

For risk assessment, dietary exposure to PAHs, the non-carcinogenic and carcinogenic risks were estimated. The dietary daily intake (DDI, $\text{mg kg}^{-1} \text{ body weight day}^{-1}$) of PAHs in the analysed smoked fish products for an adult (70 kg) population is presented in Table 2.

Table 2. Estimated Dietary Daily Intake (DDI; $\text{mg kg}^{-1} \text{ day}^{-1}$) of PAHs in smoked fish from Lagos Lagoon, Lagos State

PAHs	CNA	CNC	CNF	ELA	ELC	ELF
NaP	0.00E+00	9.20E-05	6.20E-04	1.38E-03	7.56E-03	6.47E-03
AcPY	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	6.04E-03
AcP	0.00E+00	0.00E+00	4.94E-03	0.00E+00	0.00E+00	0.00E+00
Flu	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Phe	4.10E-02	0.00E+00	8.25E-03	9.62E-02	1.04E-02	1.26E-03
Ant	4.20E-02	0.00E+00	0.00E+00	2.47E-03	5.06E-03	0.00E+00
FL	1.63E-02	8.94E-04	1.22E-02	8.62E-02	6.74E-03	8.47E-03
Pyr	9.34E-01	3.57E-02	7.21E-02	1.70E+00	2.18E-01	4.46E-02
BaA	3.47E-03	0.00E+00	0.00E+00	0.00E+00	1.73E-03	0.00E+00
Chr	6.01E-02	0.00E+00	2.68E-04	2.16E-02	2.89E-02	9.33E-04
BaP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BbFL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BkFL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Ind	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

DBA	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

CNA = *Chrysichthys nigrodigitatus* smoked with *Alchorneacordifolia*, CNC = *C. nigrodigitatus* smoked with *Cola nitida*, CNF = *C. nigrodigitatus* smoked with *Funtumia elastica*, ELA = *Eloplacerta* smoked with *A. cordifolia*, ELC = *E. lacerta* smoked with *C. nitida*, and ELF = *E. lacerta* smoked with *F. elastica*

The results showed that DDI values ($\text{mg kg}^{-1}\text{day}^{-1}$) estimated from individual PAH concentration in smoked fish ranged from 0 to 0.9340 (CNA), 0-0.0357 (CNC), 0-0.0721 (CNF), 0-1.70 (ELA), 0-0.2180 (ELC), and 0-0.0446 (ELF). From this study, the highest DDI was observed in Pyr when compared with other PAHs congener among all fish samples. Also, the order of DDI for Pyr in smoked fish products follows the trend of ELA > CNA > ELC > CNF > ELF > CNC. Furthermore, the estimated DDI of Pyr and FL were found to be higher than the reference dose ($0.03 \text{ mg kg}^{-1}\text{day}^{-1}$) set by the European Commission (EC 2006; Miculis *et al.*, 2011) which suggested higher risk to human health when consumed. Higher DDI values for total and carcinogenic PAH were obtained in ELC and CNA. Implying that consumption of ELC in preference to the other fish species may result in higher risk of exposure to PAHs, while the consumption of CNA may pose significant health threats to consumers due to carcinogenic and possibly mutagenic effects. On the contrary, Tongo *et al.* (2017) reported that the estimated daily intake of PAHs was generally lower the reference dose for all the smoked fish species assessed.

Carcinogenic Potential [B(a)Peq]

Individual PAH carcinogenic potencies [B(a)Peq] varied significantly ($P < 0.001$) among the smoked fish species studied (Table 3).

Table 3. Carcinogenic Potential [B(a)Peq] of PAHS from consumption of smoked fish

PAHs	CNA	CNC	CNF	ELA	ELC	ELF
NaP	0.00E+00	7.91E-04	9.66E-03	1.17E-04	1.77E-03	8.26E-03
AcPY	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	7.71E-03
AcP	0.00E+00	6.32E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Flu	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Phe	5.23E-01	1.05E-01	1.33E-01	0.00E+00	1.23E+00	1.62E-02
Ant	5.37E-02	0.00E+00	6.46E-03	0.00E+00	3.15E-03	0.00E+00
FL	2.08E-02	1.56E-02	8.61E-03	1.14E-03	1.10E-01	1.08E-02
Pyr	1.19E+00	9.21E-02	0.28E-01	4.56E-02	2.17E+00	5.69E-01
BaA	4.44E-01	0.00E+00	2.20E-01	0.00E+00	0.00E+00	0.00E+00
Chr	7.70E-01	3.42E-03	3.69E-01	0.00E+00	2.76E-01	1.19E-02
BaP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BbFL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BkFL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Ind	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
DBA	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Footnote – same as in Table 2

Pyr had the highest carcinogenic potency in CNA (1.190 mg kg^{-1}), ELA (0.046 mg kg^{-1}), ELC (2.170 mg kg^{-1}) and ELF (0.569 mg kg^{-1}), while Phe (0.105 mg kg^{-1}) and Chr (0.369 mg kg^{-1}) were observed highest in CNC and CNF, respectively. The individual PAH carcinogenic

potency [B(a)Peq] in smoked fish samples assessed in this current study was significantly higher than the recommended maximum acceptable level of 0.005 mg kg⁻¹ for benzo(a)pyrene in smoked fish (European Commission, 2005). Highest values for FL, Phe and Pyr were observed in *Eloplacerta* smoked with *C. nitida*, while values for Benzo(a)anthracene and Chrysene were observed to be above the maximum acceptable level in *Chrysichthys nigrodigitatus* smoked with *A. cordifolia*. More so, the values were comparably higher than levels reported for smoked fish species by Yusuf *et al.* (2015) and Tongo *et al.* (2007). This indicated that people consuming these smoked fish are at potential risk of exposure to the carcinogenic and other health-related effects of various types of such PAHs.

Excess Cancer Risk (ECR)

Results of excess cancer risk (ECR) shown in Table 4 followed a similar trend as that of [B(a)Peq]. The highest ECR values were measured in Pyr for CNA (1.90×10^{-5}), ELA (1.60×10^{-6}), ELC (3.40×10^{-5}) and ELF (1.01×10^{-6}), while Phe was highest in CNC (2.00×10^{-6}) and Chr (6.02×10^{-6}) in CNF. Of all the six smoked fish products investigated, only *Eloplacerta* smoked with *A. cordifolia* had ECR value that was lower than the acceptable guideline value of 1.0×10^{-6} set by the United States Environmental Protection Agency (USEPA, 1993). The implication, therefore, is that daily consumption of 54.8 g of smoked fish, over a 70-year lifetime period could increase the probability of a lifetime cancer risk of more than one person in a million. In a study by Tongo *et al.* (2017), cumulative ECR for *E. fimbriata* and *C. gariepinus* were also found to exceed the USEPA's acceptable cancer risk level of 10^{-6} thus indicating the likelihood of Nigerians being exposed to cancer risk due to regular consumption of traditionally-smoked fish products.

Table 4. Excess Cancer Risk (ECR) of PAHS from consumption of smoked fish

PAHs	CNA	CNC	CNF	ELA	ELC	ELF
NaP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
AcPY	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
AcP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Flu	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Phe	8.00E-06	2.00E-06	2.00E-06	0.00E+00	1.90E-05	0.00E+00
Ant	1.00E-06	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
FL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.00E-06	0.00E+00
Pyr	1.90E-05	1.00E-06	4.00E-06	1.00E-06	3.40E-05	1.01E-06
BaA	7.10E-06	0.00E+00	3.00E-06	0.00E+00	0.00E+00	0.00E+00
Chr	1.20E-05	0.00E+00	6.02E-06	0.00E+00	4.00E-06	0.00E+00
BaP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BbFL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BkFL	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Ind	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
DBA	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
BP	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Carcinogenic Risk Indices

The estimated carcinogenic risk indices such as DDI, TEQ, PAH4 and SV are presented in Table 5.

Table 5. Estimated Carcinogenic Risk Indices of PAHS in smoked fish from Lagos Lagoon, Lagos

Carcinogenic Risk Index (mg kg ⁻¹)	<i>Chrysichthys nigrodigitatus</i>			<i>Eloplacerta</i>		
	<i>Alchorneacordifolia</i>	<i>Cola nitida</i>	<i>Funtumia elastica</i>	<i>Alchorneacordifolia</i>	<i>Cola nitida</i>	<i>Funtumia elastica</i>
∑DDI	1.0969 ^b	0.0984 ^d	0.2782 ^c	0.0367 ^f	1.9038 ^a	0.0677 ^e
∑DDI for carcinogenic PAH	0.0636 ^a	0.0003 ^d	0.0306 ^b	0.0000 ^e	0.0216 ^c	0.0009 ^d
Excess Cancer Risk (ECR)	4.70E-05 ^b	3.50E-06 ^d	1.61E-05 ^c	7.33E-07 ^f	5.93E-05 ^a	1.75E-06 ^e
TEQ	3.0024 ^b	0.2236 ^d	1.0253 ^c	0.0468 ^f	3.7865 ^a	0.1118 ^d
PAH4	81.2370 ^a	0.3420 ^e	39.0870 ^b	0.0000 ^f	27.5938 ^c	1.1912 ^d
Screening Value (SV)	12.8315	12.8315	12.8315	12.8315	12.8315	12.8315

^{a-f} Mean values across the row with different superscript differ significantly at p<0.01

The DDI value for TPAH was highest for ELC, while that for CPAH was highest in CNA. The estimated ECR values ranged from 7.33×10^{-7} to 5.93×10^{-5} mg kg⁻¹ while TEQ ranged from 0.0468 to 3.7865 mg kg⁻¹, and the highest value was found in ELC. PAH4 index showed significant ($p < 0.01$) variation with highest value recorded in CNA. The increasing order of PAH4 is as follows; CNA > CNF > ELC > ELF > CNC > ELA. The calculated Screening Value (SV) was 12.83 for all the fish samples. TEQ is a popular index commonly used to facilitate the assessment of toxicity and carcinogenicity of PAHs in smoked fish (Tongo *et al.* 2017). TEQ values obtained were lower than the calculated SV of 12.83 which is an indication of low risk of developing cancer through consumption of assessed smoked fish in this study area. The screening value (SV) is defined as the threshold concentration of chemicals (PAHs) in edible tissues that could pose serious public health concern (Cheung *et al.* 2007). This result is similar to those previous studies (Cheung *et al.*, 2007; Yusuf *et al.*, 2015; Tongo *et al.* 2017) where lower TEQ than the SV were reported.

Correlation between PAHs concentration and health risk exposure indicators

The correlation coefficient between PAHS concentration and human health risks are presented in Table 6. There was statistically significant positive correlation between TEQ and TPAHs ($r = 0.977$; $p < 0.01$), Non-carcinogenic PAHs ($r = 0.970$; $p < 0.01$), PAH4 ($r = 0.705$; $p < 0.01$) and DDI ($r = 0.977$; $p < 0.01$). Significantly positive correlations between TEQ and PAHs concentrations suggested potential exposure to cancer and other health-related issues through dietary consumption of sampled smoked fish products. Unexpectedly, positive correlations ($p < 0.05$) also existed between Non-carcinogenic PAHs and PAH4 as well as DDI for carcinogenic PAHs. This probably might be an indication of other non-cancer effects of PAHs. Damon (1997) had reported that some non-carcinogenic PAHs could interact in many steps of the carcinogenic process, promote the initiation phase of carcinogenesis by generating free radicals as well as mutation genes resulting from DNA damage, and impair the body's immune defence mechanism due to their immunosuppressive effects. In view of this, fish processors should be enlightened on safer processing and preservation techniques in order to deliver healthier smoked fish products to Nigerians.

Table 6. Correlation coefficient between PAHs concentration and health risk exposure indicators

Variables	Total DDI	DDI for carcinogenic	TEQ
Total PAHs	1.000**	0.538*	0.977**
Non-Carcinogenic PAHs	1.000**	0.514*	0.970**
PAH4 Index	0.538*	1.000**	0.705**

Correlation level of significance (* = $P < 0.05$; ** = $P < 0.01$) at 2-tailed.

CONCLUSION

This present study examined PAHs levels and potential health risk of smoked fish processed with different wood species. The highest measured PAH congener in all the smoked fish samples was Pyrene. The highest and lowest PAH concentrations were recorded in *Eloplacerta* smoked with *Cola nitida* and *Alchornea cordifolia*, respectively. The order of PAH4 abundance follows the trend of CNA > CNF > ELC > ELF > CNC > ELA. The smoked fish products were generally dominated by medium molecular weight, four-ring PAHs. Non-carcinogenic PAHs accounted for $\geq 89\%$ of the total PAHs in smoked fish assessed. The estimated DDI of Pyr and FL were found to be higher than the reference dose ($0.03 \text{ mg kg}^{-1} \text{ day}^{-1}$) set by the European Commission. Both *Eloplacerta* smoked with *Cola nitida* (ELC) and *Chrysichthys nigrodigitatus* smoked with *Alchornea cordifolia* (CNA) had higher potential carcinogenic risk above the safe tolerable limits for consumers. Hence, *Cola nitida* is recommended as a suitable fuelwood for *Chrysichthys nigrodigitatus* while *Eloplacerta* could be smoked with *Alchornea cordifolia* and *Funtumia elastica*. Furthermore, the possible linkage between smoked fish consumption and consumers' health risk necessitate the advocacy for adoption of improved fish processing techniques that guarantee minimal PAHs level in smoked fish meant for local and global markets.

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