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Evaluation of Residual Polycyclic Aromatic Hydrocarbon Concentrations of Processed and Unprocessed Fish Body Parts: a Human Health Risk Assessment

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Abstract

Concentrations of polycyclic aromatic hydrocarbons (PAHs) from a dry extract of fresh and smoked body parts of *Clarias gariepinus* were examined to determine associated potential human health risks. Gas chromatography analysis was employed for the PAHs determination. The PAHs levels ranged from 0.001 µg/kg [indeno (1,2,3-cd)pyrene] to 11.7 µg/kg (acenaphthene) in fresh (extract), while the smoked (extract) showed 0.001 µg/kg [indeno (1,2,3-cd)pyrene] to 12.7 µg/kg (pyrene). The liver and head smoked were observed to be more contaminated as compared to fresh parts. Individual PAHs in the fish parts were less than the 12.0 µg/kg limit in food as set by the European Union (EU). Although there was evidence of contamination, the potential health risk associated with the fish consumption revealed no observable potential health risk to consumers.

Keywords: Polycyclic aromatic hydrocarbon, *Clarias gariepinus*, Body parts, Gas chromatography, Health

Introduction

Polycyclic aromatic hydrocarbons (PAHs) formation can be either natural or anthropogenic. They are environmental contaminants formed during the incomplete combustion of carbonaceous materials [1]. Although there are many PAHs, most regulations, analyses, and data reporting focus on only a limited number of PAHs, typically between 14 and 20 individual PAH compounds [2]. US-EPA designated 16 unsubstituted PAHs as priority pollutants. Among the 16 PAHs, seven are considered possible human carcinogens [3]. Humans are exposed to PAHs through dietary and non-dietary sources (e.g., inhalation and skin contact). Among these, dietary sources represent the major exposure route. PAHs are associated with risks to human health, especially carcinogenesis [4-6]. PAHs have been proved to have carcinogenic and mutagenic effects and potent immune suppressants. Effects have been documented on immune system development, humoral immunity, and host resistance [7, 8].

Aquatic biota is an important food source for humans and, therefore, a critical aspect of any toxicological assessments. Fish are among the group of aquatic organisms that represent the largest and most diverse group of vertebrates [9]. Contaminants in general usually do not have a uniform distribution in the environment, and with pesticides, season

matters; unlike other classes of chemicals, they are used particularly at specific times in the growing season for effective control to be achieved because the fish has the ability to bio-accumulate chemicals in the water. Food can become contaminated during thermal treatments in food preparation (drying and smoking) and cooking (roasting, baking, and frying). Smoking is defined as the process of penetration of volatiles resulting from the thermal destruction of wood into the surface of meat or fish products. The levels of PAHs in smoked foods depend on several variables in the smoking process, including the type of smoke generator, combustion temperature, and degree of smoking.

PAHs have been reported in smoked fish [10-14]. Yusuf et al. [15] reported PAHs ranges of 0.19 – 41.3 µg/kg in smoked fish using modern and traditional methods. EU has stressed and recommended that PAHs be measured as wide as possible in food products to obtain data on the occurrence and specific concentrations in various matrices [16, 17].

The study examined the residual levels of PAHs in a dry extract of fresh and smoked body parts of *Clarias gariepinus* to determine associated potential human health risks to the consumers. It should be noted that determination on a dry extract basis brought the original water content in both fresh and smoked body parts at 0.0 g/100g, thereby bringing both samples at par in moisture content.

Materials and Methods

Samples Collection and Preparation

Five fish samples of male *Clarias gariepinus* obtained from a fish vendor based in Basiri quarters of Ado-Ekiti, Nigeria, were used for the experiment. The month of the collection was December 2016. The fish samples were frozen in a container and then taken to the laboratory. Anatomical parts of the fish such as head, trunk, and liver were separately dissected, cleaned, and processed for analysis. Half was analyzed fresh, and a half was analyzed smoke dried. Both were now converted statistically to dry extract samples where both fresh and smoke dried are now at 0.0% moisture content.

Samples were designated as HF, LF, MF, HS, LS, and MS where H = head, L = liver, M = muscle, F = fresh, and S = smoked as the case may be.

Extraction and Clean-up Procedure of the Samples for PAHs Analysis

The extraction and clean-up of the samples were carried out according to the methods of ASTM D3328 [18] and ASTM 3415 [19, 20].

Gas Chromatographic Conditions

The gas chromatography (GC) conditions for the analysis of PAHs were as follows: GC model: HP6890 powered with HP ChemStation Rev. A 09.01[1206]; the carrier gas flow rate was 2.0 mL/min; injection type: split injection: 20:1; carrier gas: nitrogen; inlet temperature: 250°C; column type: HP-1; column dimension: (30 m x 0.25 µm x 0.25 mm); oven programme: initial temperature at 60^{o}C for 5 min, first ramping 15°C/min for 14 min, maintained for 3 min, second ramping at 10^{o}C/min for 5 min, maintained for 4 min; detector: flame ionization detector (FID); detector temperature: 320^{o}C; hydrogen pressure: 28 psi; nitrogen: 30 psi; compressed air: 32 psi. The total run time was 31 min.

Benzo(a)pyrene Equivalent Estimation

The carcinogenic risk from exposure to PAHs in fish was carried out according to the USEPA guideline, as described by Cheung

et al. [21]. Overall carcinogenic health risk from the measured PAHs was estimated on toxic equivalent factors (TEFs) derived from the cancer potencies of individual PAH compounds relative to the cancer potency of benzo(a)pyrene [22, 23]. Table 1 shows the TEF and mutagenic equivalent factor (MEF) values [24-27] for each PAH. Toxic equivalent benzo [a] pyrene (TEQ_{Bap}) is the sum of the product of each PAH and its TEF [28]. The sum of each PAH concentration multiplied by the corresponding MEF gives the mutagenic equivalent (MEQ).

$$TEQ_{Bap} = \sum (TEF_i \times C_i \quad (1)$$

$$MEQ_{Bap} = \sum (MEF_i \times C_i \quad (2)$$

where C_i is the measured individual PAH concentration for the (i^{th}) compound with the assigned TEF_i or MEF_i.

Table 1. **Proposed benzo(a) pyrene equivalent factors for carcinogenic (TEF) and mutagenic toxicity (MEF).**

PAHs	TEF	MEF	RfD (mg kg^{-1} day^{-1})	CSF (mg kg^{-1} day^{1})
Naphthalene	0.001		2.00×10^{-2}	
Acenaphthylene	0.001		2.00×10^{-2}	
Acenapthene	0.001		6.00×10^{-2}	
Fluorene	0.001		4.00×10^{-2}	
Phenanthrene	0.001		-	
Anthracene	0.01		3.00×10^{-2}	
Fluoranthene	0.001		4.00×10^{-2}	
Pyrene	0.001		3.00×10^{-2}	
Benzo(a)anthracene	0.1	0.082		7.30×10^{-1}
Chrysene	0.001	0.017		7.30×10^{-3}
Benzo(b)fluoranthene	0.1	0.25		7.30×10^{-1}
Benzo(k)fluoranthene	0.01	0.11		7.30×10^{-2}
Benzo(a)pyrene	1	1		7.3
Indeno(1.2.3-cd)pyrene	1	0.29		7.30×10^{-1}
Dibenzo(a,h)anthracene	0.1	0.31		7.3
Benzo(g,h,i)perylene	0.01		4.00×10^{-2}	

TEF [16], MEF [17, 18], USEPA[21], CSF [22], RfD=reference dose, CSF= cancer slope factor

Dietary exposure to PAHs

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

$$\text{Aveerage daily dose} = \frac{\text{TEQ or MEQ} \times \text{IR} \times \text{CF}}{\text{BW}} \quad (3)$$

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 capital fish consumption of 25 kg for Nigeria [29]. CF is the conversion factor (0.001 mg kg^{-1}), and BW is the bodyweight set at 70 kg.

Non-cancer Hazard, Carcinogenic and Mutagenic Risk Calculations

The risk associated with the dietary exposure to non-carcinogenic PAHs was evaluated using the hazard quotient approach. Hazard quotient represents a ratio of the exposure dose for each PAH divided by reference dose (RfD).

$$\text{Hazard quotient (HQ)} = \frac{\text{Average daily dose (ADD)}}{\text{Re ference Dose (RfD)}\,3333} \quad (4)$$

The summation of individual hazard quotients results gives the hazard index.

$$\text{Hazard Index (HI)} = \Sigma\,(HQ_1 + HQ_2 + \ldots\, HQ_n \quad (5)$$

TEQ_{Bap} and $MEQ_{Bap,}$ according to reference [30] and benzo(a)pyrene slope factor [31] were shown in Table 1.

Risk (Carcinogenic or mutagenic) = Average daily dose x slope factor (6)

Calculations for the dry extract values followed this formula:

$$\frac{Original\ analysis\ value}{100 - moisture\ content} x100 \quad (7)$$

Statistical methods used are as follows

Standard deviation (s) for the samples were determined using the following formula.

$$S = \sqrt{\frac{\sum \left(X_1 - \overline{X}\right)}{n-1}} \quad (8)$$

Coefficient of variation percent

$$CV\% = \frac{SD}{Mean} \times 100 \quad (9)$$

where SD = standard deviation

Correlation coefficient

$$r_{xy} = \frac{\sum Z \times Zy}{N} \quad (10)$$

$$\text{where } Z_x = \frac{X - \overline{X}}{\sigma_z}, Z_y = \frac{Y - \overline{Y}}{\sigma_y} \quad (11)$$

N= number of pairs of X, Y scores and degree of freedom= n-2.

Degree of association or variance or degree of relationship

$$R_{xy}^2 \quad (11)$$

Lack of relationship or coefficient of alienation

$$C_A = \sqrt{1-(r_{xy})^2} \quad (12)$$

Index of forecasting efficiency (IFE)

$$IFE = 1 - C_A \quad (13)$$

Regression = Y=a +bxyX where a is the point at which the line intersects the y-axis and bxy is the slope. (14)

Equations 8 – 14 are from Oloyo [32] and Chase [33].

Results and Discussion

The concentrations (µg/kg) of PAHs from a dry extract of the body parts (head, liver, and muscle) were presented in Tables 2-4.

The PAHs (µg/kg) levels in the dry extract of the head (fresh) ranged from 0.002 (indeno(1,2,3-cd) pyrene) to 11.7 (acenaphthylene), while the head smoked (extract) had values of 0.001 (indeno(1,2,3-cd)pyrene) - 12.7 (pyrene). The smoked head reported the highest TPAHs (37.5 µg/kg) as compared with the head fresh (33.0 µg/kg) with a build up of 12.0% of total PAHs after smoking. The non-carcinogenic and high molecular weight PAHs also reported an increase of 4.80 and 11.1 µg/kg showing a build up of 14.8% and 78.3%, respectively, while the carcinogenic and low molecular PAHs showed a decrease of 0.219 µg/kg and 6.47 µg/kg after smoking. This showed that some of the carcinogenic PAHs were lost during smoking, while the non-carcinogenic PAHs levels build up more due to smoking. The mean concentration of the individual PAHs in the dry extract from head fresh and smoked ranged from 0.001 ± 0.0003 µg/kg to 10.3 ± 2.71 µg/kg with the highest recorded for non-carcinogenic fluoranthene. Non-carcinogenic and carcinogenic PAHs showed the highest decrease as observed in acenaphthene (99.5%) and the lowest decrease as observed in benzo(a)anthracene (18.9%). Benzo(g,h,i) perylene showed a distinct behaviour, with the fresh and dry reporting the same concentration level. The DHF/DHS coefficient of variation percent (CV%) ranged from 10.1 (benzo(g,h,i)perylene) - 140 (acenaphthene). Non-carcinogenic fluoranthene and pyrene levels in the dry extract from the head smoked were greater than 12.0 µg/kg (EU standard).

***Table 2*. Concentration (µg/kg) of PAHs from a dry extract of the head.**

PAHs	DHF	DHS	DIFF	% Diff	Mean	SD	CV %
Naphthalene^{+}	0.968	0.010	+0.958	+99.0	0.489	0.678	139
Acenaphthylene^{+}	0.019	0.008	+0.011	+57.9	0.013	0.008	60.0
Acenaphthene^{+}	11.70	0.063	+11.70	+99.5	5.90	8.26	140
Fluorene^{+}	0.010	0.092	-0.082	-820	0.051	0.059	115
Phenanthrene^{+}	0.016	1.73	-1.71	-10713	0.872	1.21	139
Anthracene^{+}	6.03	10.40	-4.37	-72.5	8.24	3.12	37.8
Fluoranthene*	8.38	12.20	-3.82	-45.6	10.3	2.71	26.3
Pyrene*	6.76	12.70	-5.94	-87.9	9.71	4.16	42.9
Benzo(a) anthracene**	0.159	0.129	+0.030	+18.9	0.144	0.021	14.8
Chrysene**	0.149	0.080	+0.069	+46.3	0.115	0.049	42.5
Benzo(b)fluoranthene**	0.089	0.011	+0.078	+87.6	0.050	0.055	110
Benzo(k)fuoranthene**	0.108	0.008	+0.100	+92.5	0.058	0.071	123
Benzo(a)pyrene**	0.073	0.129	-0.056	-76.7	0.101	0.039	39.0
Indeno(1,2,3-cd)pyrene**	0.002	0.001	+0.001	+50.0	0.001	0.0003	25.6
Dibenzo(a,h)anthracene**	0.006	0.008	-0.002	-33.3	0.007	0.001	13.6
Benzo(g,h,i)perylene*	0.006	0.006	0	0	0.006	0	-
TPAHs	33.0	37.50	-4.50	-13.6	35.3	3.18	9.02
∑7C-PAHS	0.584	0.365	+0.219	+37.5	0.475	0.155	32.6
∑NC-PAHS	32.40	37.20	-4.80	-14.80	34.80	3.40	9.76
∑LMW	18.8	12.3	+6.50	+34.6	15.6	4.58	29.4
∑HMW	14.1	25.2	-11.1	-78.7	19.7	7.82	39.8

DHF= dry extract from head fresh; DHS= dry extract from head smoked; DIFF = difference; %DIFF = percentage difference; SD= standard deviation; CV=coefficient of variation; $^{+}$indicates PAHs classified as low molecular weight PAHs; * = high molecular weight and non-carcinogenic PAHs; ** = high molecular weight and carcinogenic PAHs; ∑7c-PAHs= sum of seven carcinogenic PAHs, ∑nc-PAHs= sum of non-carcinogenic PAHs; ∑LMW-PAHs= sum of low molecular weight PAHs; ∑HMW-PAHs= sum of high molecular weight PAHs

***Table 3*. Concentration (µg/kg) of PAHs from a dry extract of the liver.**

PAHs	DLF	DLS	DIFF	%Diff	Mean	SD	CV%
Naphthalene^{+}	0.010	0.010	0	0	0.010	0	-
Acenaphthylene^{+}	0.016	0.008	+0.008	+50.0	0.012	0.006	49.0
Acenaphthene^{+}	0.013	0.063	-0.050	-384.6	0.038	0.035	93.8
Fluorene^{+}	0.010	0.094	-0.084	-840	0.052	0.059	115
Phenanthrene^{+}	0.019	1.73	-1.71	-9005	0.873	1.21	138
Anthracene^{+}	6.89	10.60	-3.71	-53.8	8.72	2.60	29.8
Fluoranthene*	8.03	12.00	-3.97	-49.4	10.00	2.80	28.0
Pyrene*	6.86	12.70	-5.84	-85.1	9.75	4.10	42.0
Benzo(a) anthracene**	0.165	0.129	+0.036	+21.8	0.147	0.026	17.5
Chrysene**	1.75	0.804	+0.946	+54.1	1.28	0.666	52.2
Benzo(b)fluoranthene**	0.079	0.011	+0.068	+86.1	0.045	0.048	107
Benzo(k)fuoranthene**	0.095	0.009	+0.086	+90.5	0.052	0.061	117
Benzo(a)pyrene**	0.067	0.129	-0.541	-92.5	0.098	0.044	44.9
Indeno(1,2,3-cd)pyrene**	0.001	0.001	0	0	0.001	0	-
Dibenzo(a,h)anthracene**	0.006	0.008	-0.002	-33.3	0.007	0.001	13.6
Benzo(g,h,i)perylene*	0.006	0.004	+0.002	+33.3	0.005	0.001	25.6
TPAHs	21.3	38.2	-16.9	-79.3	29.7	12.0	40.2
∑7C-PAHS	0.587	1.09	-0.503	-85.7	0.839	0.356	42.4
∑NC-PAHS	20.7	37.1	-16.4	-79.2	28.9	11.6	40.2
∑LMW	6.95	12.4	-5.45	-78.4	9.69	3.87	40.0
∑HMW	14.3	25.7	-11.4	-79.7	20.0	8.08	40.3

DLF= dry extract from liver fresh; DLS= dry extract from liver smoked; DIFF = difference; %DIFF = percentage difference; SD= standard deviation; CV=coefficient of variation; $^{+}$indicates PAHs classified as low molecular weight PAHs; * = high molecular weight and non carcinogenic PAHs; ** = high molecular weight and carcinogenic PAHs; ∑7c-PAHs= sum of seven carcinogenic PAHs, ∑nc-PAHs= sum of non carcinogenic PAHs; ∑LMW-PAHs= sum of low molecular weight PAHs; ∑HMW-PAHs= sum of high molecular weight PAHs

Table 4. Concentration (µg/kg) of PAHs from a dry extract of the muscle.

PAHs	DMF	DMS	DIFF	%Diff	Mean	SD	CV%
Naphthalene^{+}	0.010	0.013	-0.003	-30.0	0.011	0.003	22.9
Acenaphthylene^{+}	0.025	0.034	-0.009	-36.0	0.030	0.006	20.7
Acenaphthene^{+}	0.016	0.055	-0.039	-243.8	0.035	0.028	78.1
Fluorene^{+}	0.016	0.062	-0.046	-288	0.039	0.032	83.5
Phenanthrene^{+}	0.019	1.77	-1.75	-9216	0.895	1.24	138
Anthracene^{+}	6.10	8.54	-2.44	-40.0	7.32	1.73	23.6
Fluoranthene*	8.10	8.93	-0.830	-10.2	8.51	0.592	6.96
Pyrene*	7.68	8.45	-0.766	-10.0	8.07	0.542	6.72
Benzo(a) anthracene**	0.156	0.132	0.024	15.4	0.144	0.017	11.6
Chrysene**	0.171	0.077	0.094	55.0	0.124	0.067	53.7
Benzo(b)fluoranthene**	0.117	0.015	0.102	87.2	0.066	0.072	109
Benzo(k)fuoranthene**	0.156	0.008	0.148	95.0	0.082	0.105	128
Benzo(a)pyrene**	0.073	0.157	-0.084	-115.1	0.115	0.060	51.8
Indeno(1,2,3-cd)pyrene**	0.002	0.001	0.001	50.0	0.001	0.0003	32.8
Dibenzo(a,h)anthracene**	0.006	0.008	-0.002	-33.3	0.007	0.001	13.6
Benzo(g,h,i)perylene*	0.006	0.006	0	0	0.006	0	-
TPAHs	22.2	28.3	-6.10	-27.6	25.2	4.28	16.9
∑7C-PAHS	0.679	0.398	0.281	41.4	0.539	0.199	36.9
∑NC-PAHS	21.6	27.8	-6.20	-28.7	24.7	4.44	18.0
∑LMW	6.19	10.5	-4.31	-69.6	8.33	3.03	36.3
∑HMW	16.1	17.8	-1.70	-10.6	16.9	1.24	7.3

DMF= dry extract from muscle fresh; DLS= dry extract from muscle smoked; DIFF = difference; %DIFF = percentage difference; SD= standard deviation; CV=coefficient of variation; $^{+}$indicates PAHs classified as low molecular weight PAHs; * = high molecular weight and non carcinogenic PAHs; ** = high molecular weight and carcinogenic PAHs; ∑7c-PAHs= sum of seven carcinogenic PAHs, ∑nc-PAHs= sum of non carcinogenic PAHs; ∑LMW-PAHs= sum of low molecular weight PAHs; ∑HMW-PAHs= sum of high molecular weight PAHs

Table 3 depicts the concentration (µg/kg) of PAHs from a dry extract of the liver fresh and smoked. The concentrations of the dry extract from the liver fresh (µg/kg): 0.001 (indeno(1,2,3-cd)pyrene - 8.03 (fluoranthene), whereas the liver smoked had values from 0.001 (indeno(1,23-cd)pyrene) - 12.7 (pyrene). The liver showed a distinct behaviour as compared to other parts. The TPAHs, ∑7C-PAHS, ∑NC-PAHS, ∑LMW, and ∑HMW all showed a concentration increase in the liver after smoking. A concentration build up (µg/kg): 0.503-16.9 was observed to have a high percentage difference of 78.8% (low molecular weight) to 85.6% (carcinogenic PAHs). Decrease in DLF/DLS percentage concentrations were observed in acenapthylene (51.5), benzo(a) anthracene (22.0), chrysene (53.9), benzo(b) fluoranthene (86.1), benzo(k) fluoranthene (90.8) and benzo (g,h,i) perylene (30.7), respectively. The average concentration of the DLF/DLS varied from 0.001 to 10.0 ±2.80 µg/kg (fluoranthene) with CV % of 13.6 (dibenzo (a,h)anthracene) and 138 (phenanthrene). The PAHs concentration in the liver except pyrene (12.7 µg/kg) in the smoked were lower than the EU limit of 12.0 µg/kg. The sum of non-carcinogenic types was comparatively lower than the sum of the seven carcinogenic PAHs.

The levels of PAHs from a dry extract of the muscles part are shown in Table 4. The PAHs concentration (µg/kg) were observed to range between 0.002 (indeno(1,2,3-cd)pyrene) to 8.10 fluoranthene for fresh muscle (extract)

and 0.001 to 8.93 muscle smoked (extract), respectively. The TPAHs (27.2), ∑NC-PAHs (29.1), ∑LMW (69.2), and ∑HMW (10.9) were percent values that were found to have increased after smoking, respectively, whereas the carcinogenic PAHs showed a high decrease of up to the level of 41.4%. This observation was noted for carcinogenic PAHs in the head and muscle, where the total carcinogenic PAHs concentration also increased after smoking. All the seven carcinogenic PAHs concentrations except benzo(a)pyrene and dibenzo(a,h)anthracene were seen to decrease after smoking, while all the non-carcinogenic PAHs showed a build up with an increase of 10.0 - 9199%. Benzo(g,h,i)perylene showed similar levels in smoked and fresh muscle parts.

In Table 5, we have the statistical analysis results of the data from Tables 2 (DHF/DHS), 3 (DLF/DLS) and 4 (DMF/DMS). Considered in Table 5, the values of correlation coefficient (r_{xy}), coefficient of determination or variance (r_{xy}^2), regression coefficient (R_{xy}); the grand mean, the standard deviation (SD), and the coefficient of variation (CV%) of DHF, DHS, DLF, DLS, DMF, and DMS; also calculated for the coefficient of alienation (C_A) and index of forecasting efficiency (IFE). The r_{xy} values were subjected to values from the critical Table at $r_{=0.01}$ (df of n -2 = 16 - 2 = 14) to see if significant differences existed between the values of DHF/DHS, DLF/DLS, and DMF/DMS. The r_{xy} values were all positively high and significant between DHF/DHS, DLF/DLS and DMF/DMS with the following trend (regression calculation value, r_c and star meaning significant difference): DHF/DHS, $r_{c = 0.6364*}$ < DLF/DLS, $r_{c = 0.9835*}$ < DMF/DMS, $r_{c = 0.9845*}$. The (variance) r_{xy}^2 was low to high in values as the corresponding r_{xy}^2 range from above was 0.4050 < 0.9673 < 0.9693. There was a somersault in the R_{xy} values as shown: DHF/DHS (R_{xy} = 0.7904) < DLF/DLS (R_{xy} = 1.59) > DMF/DMS (R_{xy} = 1.15). The value of R_{xy} in each pair group was a reflection of the concentration of the values in each pair group sample. In DHF/DHS, the R_{xy} value of 0.7904 meant that when the total values of DHF increased by 1.0 µg/kg, those of DHS would increase by a value of 0.7904 µg/kg. A similar argument could be applied to the R_{xy} of DLF/DLS and DMF/DMS. The mean values were generally low for all the samples with values in the six samples ranging from 1.50 ± 2.90 µg/kg – 2.35± 4.71 µg/kg showing the SD values to be higher than mean values in all cases denoting that the statistical values were highly heterogeneously spread. Since the mean values were low with corresponding high SD values, definitely the CV% would be high since CV% was derived from the values of mean and the SD. All CV% values were higher than 100%; actually, the values ranged from 176 - 208%.

Table 5. Statistical analysis of the data values obtained from Tables 2, 3 and 4.

Parameter	DHF	DHF/DHS	DHS	DLF	DLF/DLS	DLS	DMF	DMF/DMS	DMS
r_{xy}		0.6364*			0.9835*			0.9845*	
r_{xy}^2		0.4050			0.9673			0.9693	
Rxy		0.7904			1.59			1.15	
Mean	2.15		2.35	1.50		2.39	1.42		1.77
SD	3.79		4.71	2.90		4.69	2.94		3.44
CV%	176		201	193		196	208		195
C_A		0.7714			0.1809			0.1751	
IFE		0.2286			0.8191			0.8249	

For DHF, DHS (see Table 2), DLF, DLS (see Table 3), DMF, DMS (see Table 4); r_{xy} = correlation coefficient; r_{xy}^2 = coefficient of determination (variance); R_{xy} = regression coefficient; SD = standard deviation; CV% = coefficient of variation; C_A = coefficient of alienation; IFE = index of forecasting efficiency, * = result is significantly different at r_{xy} or $r_{=0.01}$ [Note that degree of freedom (df) = n -2 =16 - 2 = 14; $r_{=0.01}$ critical level = 0.684.]

The C_A values ranged from high to low with 0.7714 – 0.1751 with corresponding low to high values of IFE: 0.2286 – 0.8249. From this statement, it could be noted that C_A is the opposite of IFE. Whilst C_A measures alienation or non-relationship, the IFE measures the prediction of relationship. Whilst C_A measures the error of prediction, and the IFE measures the reduction in the error of prediction. Hence, when C_A is high, prediction of relationship is difficult, but when C_A is low, the error of prediction is low, and prediction of relationship is easy. That is C_A > IFE (prediction of relationship is difficult and low), C_A < IFE (the above relationship is reversed). Going to the C_A and IFE values in Table 6, it should be noted that DHF/DHS C_A + IFE (= 1.00) and this was observed for DLF/DLS and DMF/DMS. Since C_A > IFE in DHF/DHS, the probability is high that sample DHF would be difficult to be able to predict its PAHs activities vis-a-vis those of DHS. However, in DLF/DLS and DMF/DMS where C_A < IFE in each case, sample DLF PAHs activities could be used to predict the PAHs activities of DLS; also the PAHs activities of DMF could be used to predict the PAHs activities of DMS. It could be concluded that whilst the DHF/DHS samples might not be highly biochemically or physiologically related, those of DLF/DLS and DMF/DMS pairs were likely biochemically or physiologically related or both.

The level of PAHs (104 and 76.5 µg/kg) reported for total PAHs in the fresh and smoked fish extracts from the present study were comparatively lower than those reported for *Periophthalamus koeleuteri* (172 µg/kg), *Crassostrea virginica* (105 µg/kg) [34]; higher than *M. undulatus* (9.4 – 17.7 µg/kg), *O. niloticus* (12.6 – 18.7 µg/kg) and *S. lalandi* (16.1 – 20.2 µg/kg) [35], *Liza abu* (2.30 – 16.7 µg/kg), *Carassius auratus* (1.09 – 8.67 µg/kg) [36], while *Crassostrea virginica* (97.2 – 105 µg/kg) [23] from Kpoghor and Iko showed a similar trend. In the comparison of the dry extract concentration from the smoked and fresh samples in the groups of the head, liver, and muscles (Tables 2 - 4), the head, liver, and muscle all showed build up values in the TPAH, total non-carcinogenic PAHs, and total high molecular PAHs after smoking. The total carcinogenic PAHs showed an increase in the liver, while the head and muscle decreased after smoking. For the individual PAHs in all the body parts, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)pyrene, and dibenzo(a,h) anthracene showed an increase or build up in all the body parts while benzo (a) anthracene, chrysene, benzo(b)fluoranthene and benzo (k) fluoranthene showed a decrease.

Human health risk assessment

Table 6 shows the risk assessment associated with dry extract of the fish samples. Non-carcinogenic equivalent concentration ranged from 0.000012 (fluorene) to 0.0634 (anthracene) and 0.000017 (acenaphthylene) to 0.0985 (anthracene). The smoked (0.123) showed the highest sum of benzo(a)pyrene as compared to the fresh (0.083). The daily intake (mg kg^{-1} day^{-1}) of NC-PAHs for the fresh extract was 8.12×10^{-8}, while the smoked extract had 1.20×10^{-7}. The hazard index of less than 1.0 from the present study showed no potential human health risk. The study, therefore, showed that the concentration level of the non-carcinogenic PAHs showed no potential health risk or hazard to people feeding on them.

Recently, many studies reporting data on PAHs occurrence and health risk assessment have been published investigating whether a potential risk exists when consuming certain foods. Bogdanovic et al. [37] investigated 180 samples of fish and meat products obtained in Croatia. Although they observed high levels of PAHs, they concluded that these products do not present health risks to consumers based on the margin-of-exposure (MOE) results.

Table 6. **Risk assessment based on, non-carcinogenic equivalent, carcinogenic equivalent, mutagenic equivalent, average daily dose and risk associated with the dry extract fish samples.**

PAHs	Non-carcinogenic equivalent		PAHs	Carcinogenic equivalent		Mutagenic equivalent	
	Fresh	*Smoked*	*Carcinogenic*	*Fresh*	*Smoked*	*Fresh*	*Smoked*
Naphthalene	0.00033	0.000011	Benzo(a)anthracene	0.016	0.013	0.01312	0.0107
Acenaphthylene	0.00002	0.000017	Benzo(b)fluoranthene	0.0095	0.0012	0.02375	0.003
Acenapthene	0.00393	0.00006	Benzo(k)fluoranthene	0.0012	0.00008	0.0132	0.0009
Fluorene	0.000012	0.000083	Benzo(a)pyrene	0.071	0.138	0.071	0.138
Phenanthrene	0.000018	0.00174	Dibenzo(a,h)anthracene	0.0006	0.0008	0.00186	0.0025
Anthracene	0.0634	0.0985	Chrysene	0.00069	0.00032	0.01171	0.0054
Fluoranthene	0.00817	0.0111	Indo(1,2,3-cd)pyrene	0.001	0.0081	0.0003	0.0235
Pyrene	0.0071	0.0113	∑BaP TEQ	0.0999	0.234	0.1349	0.2066
Benzo(g,h,i)perylene	0.00006	0.00005	BaP TEQ daily dose mg kg^{-1} day^{-1}	9.8×10^{-8}	2.3×10^{-7}	1.3×10^{-7}	2.0×10^{-7}
∑BaP TEQ	0.083	0.123	LECR	8.0×10^{-8}	1.8×10^{-7}	5.0×10^{-8}	1.6×10^{-7}
BaP TEQ daily dose mg kg^{-1} day^{-1}	8.12×10^{-8}	1.20×10^{-7}					

LECR= life time excess carcinogenic risk

The toxic risk assessments of the fish samples from the study area were also presented in Table 6. The (TEQ_{Bap}) and (MEQ_{Bap}) ranged from 0.0006 (dibenzo (a,h)anthracene) to 0.071 (benzo(a)pyrene) and 0.00008 (benzo(k) fluoranthene) to 0.138 (benzo(a)pyrene), respectively. The sum of carcinogenic equivalent relative to benzo(a)pyrene for the dry extract fresh was 0.999, whereas the smoked gave 0.234. The toxic risk values were lower than the USEPA [38, 39] unit risk of 1×10^{-5}. The observed values thus indicated no risk to the human beings since the values were lower than the said standard values [40]. The study, therefore, revealed that the dry extracts of the fish samples pose no likely toxic risk to consumers.

Conclusion

The study revealed evidence of residual levels of polycyclic aromatic hydrocarbons in the dry extract of fresh and smoked body parts of *Clarias gariepinus*. The non-carcinogenic and high molecular weight PAHs revealed an increase with a build-up of 14.8% and 78.3%, respectively, after smoking. Some of the carcinogenic PAHs were lost during smoking, while the non-carcinogenic PAHs levels build up more. The smoked head reported the highest TPAHs as compared with the head fresh with a build-up of 13.6% of total PAHs after smoking. The total carcinogenic PAHs also revealed an increase in the liver, while head and muscle decreased after smoking. The concentration level of the non-carcinogenic PAHs in the dry extract fresh and smoked fish pose no potential non-carcinogenic and carcinogenic health risks or hazard to people feeding on them. Based on the findings, there is a need for continuous survey and monitoring programmes for PAHs in all smoked fish products in order to protect the end users from unexpected exposure to PAHs.

Conflict of Interest

The authors declare no conflict of interest.

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