

Original Article

## Effect of Fish Smoking Methods on Polycyclic Aromatic Hydrocarbons (PAHs) Contamination

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### Abstract

Fish smoking has the tendency to contaminate the smoked fish with Polycyclic Aromatic Hydrocarbons (PAHs) as they are formed as a result of incomplete combustion. Fish smokers in Borno State, Nigeria were known to employ basically 3 unique methods: A. Earth Oven, B. Traditional Open Tray and C. Drum Kiln Smoking methods. These methods differ in the distance between the combustion (heating) chambers and the fish smoking plates and may contaminate the smoked fish with PAHs. The study aimed at assess the effect of the different fish smoking methods on the level of PAHs contamination and its corresponding human health risk. Fish samples of *Carias gariepinus* were brought directly from fishermen at Alau Dam, Maiduguri. The samples were identified and divided into 4 groups. Three of the 4 groups were subjected to a smoking method (A. Earth Oven, B. Traditional Open Tray and C. Drum Kiln Smoking methods), while the 4<sup>th</sup> group labelled D was used as the control. The smoking was done using charcoal from *Khaya senegalensis* wood brought from wood dealers and converted into charcoal by charcoal dealers at Bakassi area, Damboa Road, Maiduguri. The results further reveal the concentration of the 8 out of the 16 priority PAHs compounds detected as follows: Naphthalene (1.02 and 1.03 µg/kg in smoking methods B and C respectively), Methyl Naphthalene (1.01 µg/kg in smoking methods B), Acenaphthylene (2.01 and 1.58 µg/kg in smoking methods B and C respectively), Acenaphthene (1.01 µg/kg in smoking methods B), Pyrene (1.00 and 1.02 µg/kg in smoking methods B and C respectively), Chrysene (2.80 µg/kg in smoking method B), Benz (a) anthracene (1.10 and 1.00 µg/kg in smoking methods B and C respectively), Benzo (b) fluoranthene (1.00 µg/kg in both smoking methods B and C respectively). PAHs were not detected in smoking method A (earth oven) and Group D (unsmoked/control group). In conclusion, the study revealed that fish is most contaminated by PAHs when using the traditional open tray method of fish smoking, whereas the earth oven method is the least contaminated method.

Keyword: Fish smoking methods; Contamination; Charcoal; Ecotoxicology; PAHs

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## INTRODUCTION

Fish smoking is a well-known oldest traditional method of fish processing in Nigeria (Bwala *et al.* 2023a,b). Fish smoking is a lucrative business that helps in meeting the protein needs of hundreds of individuals, provides employment opportunities and serve as a link to the chain involved in the fish business (fishermen – fish smokers – smoked fish traders) (Adah, 2012; Bwala & Imam 2021a). Smoked fish business has high marginal economic returns and this encourages individuals and families who have access to fresh fish to venture into the business of fish smoking and trading (The Nations Newspaper, 2019; Bwala & Imam 2021b).

Fish smoking has recently become a focus in ecotoxicological studies, because the methods used in the smoking involves thermal treatments at high temperature having direct contact with combustion gases which may contaminate the smoked fish with polycyclic aromatic hydrocarbons (PAHs) (Akpambang *et al.* 2009; Hokkanen *et al.* 2018). PAHs may contaminate the ecosystem via anthropogenic activities like effluent discharge into aquatic ecosystems, vehicular and industrial emissions; and the commonest paths of contact with human food is through food processing methods like smoking, frying, roasting etc. (Forsberg, 2011; Topal *et al.* 2014; Lee, *et al.* 2015).

Fish smokers in Borno State, Nigeria were reported to employ three different methods: Earth Oven, Drum Kiln and the Traditional Open Tray Smoking methods (Bwala & Imam, 2021). These methods differ in the distance between the combustion (heating) chambers and the fish smoking plates (Nnaji & Ekwe, 2018). Smoked fish from Borno State is widely consume across the different social strata in Nigeria, Chad, Niger and Cameroun Republic (The Nations Newspaper, 2019).

Recent studies suggest that PAHs contamination on smoked fish can be affected by distance between heating chamber (the smoke source) and the smoking plates (where the fish are placed) (Visciano *et al.* 2006; Nnaji & Ekwe, 2018; Bwala & Imam, 2021b). Traditional open tray method is commonly employed by many households in the study area though it's the least methods used by commercial fish smokers in the study area whereas the earth oven and drum kiln methods were methods commonly used by commercial professional fish processors in the study area (Bwala & Imam, 2021b). All the different smoking methods has the potential of contaminating the fish with PAHs (Akpambang *et al.* 2009; Bwala & Imam, 2021a; Yunker *et al.* 1992), this therefore, necessitate the need to assess the methods and recommend the method that is more hygienic and safer.

Polycyclic aromatic hydrocarbons (PAHs) are formed as a result of incomplete combustion processes, and may originate from petrogenic or pyrogenic sources (Forsberg, 2011; Topal *et al.* 2014; Abdel – Shafy & Mansour, 2016). PAHs are found in smoked fish as combustion by-products and constitute a wide range of toxic, mutagenic and carcinogenic organic compounds, (Forsberg, 2011; Topal *et al.* 2014;

Lee, *et al.* 2015), based on two or more aromatic rings and they belonging to the Food and Environment Contaminants, (Lee, *et al.* 2015; Bwala & Imam, 2021a). Fish can become environmentally contaminated with PAHs mainly processing methods involving high temperature having direct contact with combustion gases, such as smoking, roasting, frying (Ezike & Ohen, 2018; Abdel – Shafy & Mansour, 2016). When fish and their products are being smoked, roasted, barbecued, or grilled, PAHs are then formed as a result of incomplete combustion or thermal decomposition of these organic matter (Amos-Tautua *et al.*, 2013). Pyrolysis of the fats in the smoked fish generates PAH that become deposited on the fish. PAH production by either smoking, roasting, frying or grilling (barbecued) is a function of both the fat content in the food substance and its proximity to the heat source (Amos-Tautua *et al.*, 2013; FSA, 2015).

The study aimed to evaluate the effect of different fish smoking methods on polycyclic aromatic hydrocarbons (PAHs) contamination and its implication on human health risk.

## MATERIALS AND METHODS

### The Study Area

The study was conducted in Borno State, North eastern, Nigeria. The State is located within latitude 10°N and 14°N and longitude 11°31E and 14°41E and has a total area of 61, 435km<sup>2</sup>, the second largest land mass in the Federation. The State occupies the greatest part of the Chad Basin, which is known with its fishing activities, most especially its smoked fish product been exported to the countries it shares borders with, the Republics of Niger to the North, Chad to the North-East and Cameroon to the East (BORMLS, 2008; NMA, 2008; The Nations Newspaper, 2019).

### Fish Sample Collection

#### *Unsmoked fish samples*

Twelve Fresh *Carias gariepinus* fish samples were brought directly from the fishermen at Alau Dam and then divided into 4 groups, 3 fishes in each group. The groups were classified as A-D as follows: A. Earth Oven Smoking Method, B. Traditional Open Tray Smoking Method C. Drum Kiln Smoking Method and D. Control (unsmoked sample).

#### *Smoked fish samples*

Three groups of the fresh unsmoked fish samples were subjected to one of the 3 smoking methods as shown in Table 1.

### Fish Smoking Procedure

A log of *Khaya senegalensis* was purchased from wood dealers and converted into charcoal by charcoal processors/dealers at Bakassi Area, Damboa Road, Maiduguri. Each of

the fish group was subjected to 1 of the 3 smoking methods (earth oven, traditional open tray or drum kiln) as presented in Table 1 by professional fish smokers at Baga Road fish market, Maiduguri.

**Table 1:** Fish Sample Grouping

Group	Smoking Method	Distance Between the Heating Chamber and the Smoking Plates (m)
A	Earth Oven	1.63
B	Traditional Open Tray	0.26
C	Drum Kiln	0.67
D	Unsmoked Fish (Control)	-

### Sample Processing

The smoked fish samples obtained from each smoked group were oven dried at 105°C temperature for 10h, while the fresh fish samples were oven dried for 144h (6 day). The samples will then be ground and kept in air tight containers prior to extraction process (Olabemiwo *et al.* 2011). The grounded samples were marked according to the grouping.

activated silica gel (100 – 200 Mesh) onto a chromatographic column (1cm internal diameter). These were topped with anhydrous Na<sub>2</sub>SO<sub>4</sub> and then conditioned with dichloromethane. The concentrated extracts were then loaded and eluted with dichloromethane, aimed at removing the polar lipids from the exact. The extracts were kept in amber bottle to avoid oxidation. The procedures were repeated for all the samples (Nnaji & Ekwe, 2018).

### Polycyclic Aromatic Hydrocarbons (PAHs) Determination

#### Soxhlet Extraction

The processed sample from each group were subjected individually to soxhlet extraction technique where 5g was measured and transferred into the extraction thimble and placed in the extraction chamber of the soxhlet extraction unit. The soxhlet extraction unit consisted of a 250ml round bottom flask, condenser and extraction tube, placed on a heating mantle with wide temperature range. Soxhlet extraction of the samples with hexane and dichloromethane (3:1 v/v) at 50°C for 6h was adopted according to the EPA 3540 Method (USEPA, 1994) with slight modification. The extract was concentrated in a rotary evaporator at 60°C to 2ml.

#### Clean-up of extracts

Clean-up was done for the extracted samples by passing them through a silica gel column prepared by loading 10g of

#### GC - MS Analysis

The purified sample was injected three times into the GC – MS (Agilent Technologies 7890B GC System coupled together with Agilent Technologies 5977A MSD) for analysis at Chemistry Department, Yobe State University.

#### Statistical Analysis

Data obtained was tabulated using Microsoft Excel 2010 and simple descriptive statistical tool was used.

#### The Carcinogenic Risk Assessment Using TEF (TEQ)

The Carcinogenic Risk Assessment of the smoked fish samples were estimated adopting the formula cited in Nyarko *et al.* (2011); Essumang *et al.* (2012); Aheto *et al.* (2014) and SFMP, (2017). PAHs and their TEFs values are shown in the Table 2.

**Table 2:** TEFs Values of Carcinogenic PAHs Compounds

S/N	PAH	TEF Value (USEPA, 1993)
1	Chrysene	0.001
2.	Benz(a)anthracene	0.100
3.	Benzo(b)fluoranthene	0.100
4.	Benzo(k)fluoranthene	0.010
5.	Benzo(a)pyrene	1.000
6.	Indeno(1, 2, 3-cd)pyrene	0.100
7.	Dibenz(a, h)anthracene	1.000
8.	Naphthalene	0.001

Source: Aheto *et al.*, (2014)

Benzo[a]pyrene equivalent concentrations was calculated using the formula below:

$$TEQ\ BaP = \sum (TEFi \times Ci)$$

Where **Ci** stands for the level of individual PAHs concentrations in the ‘**ith**’ compound with the assigned **TEFi** (Essumang *et al.* (2012) cited in Aheto *et al.* (2014); SFMP, (2017).

$$BaPEQ\ Daily\ Dose\ (mgkg^{-1}day^{-1}) = \frac{Ci \times IngR}{BW}$$

Where **Ci** stands for the level of individual PAHs concentrations in the ‘**ith**’ compound.

**IngR** - Ingestion Rate (0.5kg<sup>-1</sup>day<sup>-1</sup>)

**BW** – Body weight (adults 60kg)

$$Life\ Time\ Excess\ Carcinogenic\ risk\ (LECR) = \frac{Ci \times IngR \times EF \times ED \times CF \times SFO}{BW \times AT}$$

Where **Ci** stands for the level of individual PAHs concentrations in the ‘**ith**’ compound.

**IngR** - Ingestion Rate (0.5kg)

**BW** – Body weight (adults 60kg)

**EF** – Exposure Frequency (365 days)

**ED** – Exposure Duration (70 years)

**CF** - Conversion factor (1 x 10<sup>-6</sup>)

**SFO** – Oral Slope Factor

**AT** – Average Time for Carcinogens

## RESULTS AND DISCUSSION

### Polycyclic Aromatic Hydrocarbons (PAHs) Contamination on Smoked Fish Subjected to Different Smoking Methods

Table 3 reveals the different level of concentration of the 16 priority PAHs compounds obtained on the smoked fish using different smoking methods. The results indicate 8 out of the 16 priority PAHs compounds were detected on the smoked fish. The results revealed that only two smoking methods were contaminated by PAH (Smoking method B i.e. traditional open tray and C i.e. drum kiln methods).

**Table 3:** The Concentration of PAHs ( $\mu\text{g}/\text{kg}$ ) on Smoked Fish Subjected to different Smoking Methods

Smoking methods/ PAHs	No. of rings																
	2	2	3	3	3	3	3	4	4	5	5						
	Naphthalene	Methyl Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Chrysene	Benz (a) anthracene	Benzo (b) fluoranthene	Benzo (k) fluoranthene	Benzo (a) pyrene	Indeno (1, 2, 3 - cd) pyrene	Dibenz (a, h) anthracene	PAH4
<b>A</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0
<b>B</b>	1.02	1.01	2.01	1.01	BDL	BDL	BDL	BDL	1.00	2.80	1.10	1.00	BDL	BDL	BDL	BDL	5.90
<b>C</b>	1.03	BDL	1.58	BDL	BDL	BDL	BDL	BDL	1.02	BDL	1.00	1.00	BDL	BDL	BDL	BDL	3.02
<b>D</b>	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0

**BDL:** Below Detectable Limit, **PAH4:** Chrysene, Benz (a) anthracene, Benzo (b) fluoranthene, Benzo (a) pyrene

In smoking method B (the traditional open tray fish smoking method), all the 8 detected PAHs compounds were present in the different concentrations (Naphthalene 1.02 µg/kg, Methyl Naphthalene 1.01 µg/kg, Acenaphthylene 2.01 µg/kg, Acenaphthene 1.01 µg/kg, Pyrene 1.00 µg/kg, Chrysene 2.80 µg/kg, Benz (a) anthracene 1.10 µg/kg and Benzo (b) fluoranthene 1.00 µg/kg). This smoking method further recorded the sum total of the 4 PAHs (Chrysene, Benz (a) anthracene, Benzo (b) fluoranthene, Benzo (a) pyrene) (5.90 µg/kg) which is below the maximum permissible limits (12 µg/kg) as adopted by the EU Scientific Committee on Food (SCF) and the Food Safety Authority of Ireland (FSA, 2015). The smoking method B (traditional open tray fish smoking method) is the most contaminated fish smoking method and has the highest recorded total sum of the 4 PAHs (5.90 µg/kg). This implies that even though it's within the EU – SCF and the FSA set permissible limits (12 µg/kg), this method still needs strict monitoring as was reported to possess major health risk (SFMP, 2017; Fronthea *et al.* 2013).

Smoking method C (drum kiln fish method) revealed 5 out of the 8 detected PAHs compounds (Naphthalene 1.03 µg/kg, Acenaphthylene 1.58 µg/kg, Pyrene 1.02 µg/kg, Benz (a) anthracene 1.00 µg/kg and Benzo (b) fluoranthene 1.00 µg/kg). This smoking method also recorded the sum total of the 4 PAHs (3.02 µg/kg) and is below the maximum permissible limits (12 µg/kg) as adopted by the EU Scientific Committee on Food (SCF) and the Food Safety Authority of Ireland (FSA), (2015). This method is the second most contaminated fish smoking method after the traditional open tray smoking method. This method was reported by Fronthea *et al.* (2013) to be safer than the traditional open tray method and also has the tendency of providing qualitative smoked fish. The time taken for the fish samples to be smoked using this method is reported to be longer than when using the traditional open tray method (Fronthea *et al.* 2013), this means that time taken to smoke fish samples may not be a determining factor in assessing level of PAHs contamination.

The result further suggest that fish smoking methods may have impact on the level of PAHs contamination (Olabemiwo

*et al.* 2011; Nnaji & Ekwe, 2018). This study presented a much lower level of PAHs contamination which negates the findings of Akpambang *et al.* (2009); Olabemiwo *et al.* (2011); Aheto *et al.* (2017); SFMP (2017); Orudu & Peri, (2018), who reported higher concentrations of PAHs on smoked fish but a similar trend was reported by Amos-Tautua *et al.* (2013); Fronthea *et al.* (2013); Kafeelah *et al.* (2015); Bwala & Imam (2021).

PAHs were not detected in smoking method A (earth oven) and Group D (unsmoked/control group). The results indicated that smoking method B (traditional open tray method) was the most PAHs contaminated fish smoking method followed by the drum kiln. This suggest that the distance between the heating chamber and the smoking plates may have contributed to the contamination, and is with conformity with the studies of Visciano *et al.* (2006); Akpambang *et al.* (2009); Orodu & Peri, (2018); Bwala & Imam, (2021).

#### Benzo (a) pyrene Equivalence Dose/Human Risk Exposure

The Benzo (a) pyrene Equivalence Dose/Human Risk Exposure presented in **Table IV** revealed that the B(a)P eq dose were only obtained at smoking group B and C (1.8E-6 and 1.7E- mg/kg/day<sup>-1</sup> in smoking method group B and C respectively). This corresponds to carcinogenic risk value of 5.475E-12 and 5.170E-12 in smoking method group B and C respectively. The calculated value estimate that in both smoking method group B and C about 5 out of 100,000,000,000 adults are likely to experience cancer in their life. The further confirmed that though both smoking methods (traditional open tray and drum kiln methods) may contaminate the fish with PAHs but yet it's safe for human consumption as PAHs contaminations as the carcinogenic risk values were very minimal. This study is consistent with the findings of Nyarko *et al.* (2011); Aheto, (2017); Orodu & Peri, (2018); Bwala & Imam, (2021b).

**Table 4:** Benzo (a) pyrene Equivalence Dose/Human Risk Exposure

Carcinogenic Equivalency/Smoking Methods	A	B	C	D
Benz (a) anthracene	ND	0.00011	0.00010	ND
Benzo (b) fluoranthene	ND	0.00010	0.00010	ND
Benzo (k) fluoranthene	ND	ND	ND	ND
Chrysene	ND	0.0000028	ND	ND
Indeno (1, 2, 3 – cd) pyrene	ND	ND	ND	ND
Dibenz (a, h) anthracene	ND	ND	ND	ND
$\sum$ TEQ	-	0.0002128	0.00020	-
BaPEQ Daily Dose (mgkg <sup>-1</sup> day <sup>-1</sup> )	-	0.0000018	0.0000017	-
Life Time Excess Carcinogenic risk (LECR)	-	5.475E-12	5.170E-12	-

ND: Not Detected

## CONCLUSION

In conclusion, the study reveals that smoking methods affect the level of PAHs contamination on smoke fish. Traditional open tray method of fish smoking is the most affected method whereas the earth oven method is the least affected methods. The study was only limited to the effect of smoking methods but other variables like the type of fuel/material use in smoking fish, fat content in the fish, sprinkling of cooking oil during smoking, type of fish being smoked etc may have effect on the level of PAHs contamination, and therefore calls for further study on other variables.

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