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Determination Of Polycyclic Aromatic Hydrocarbons (PAHs) In Smoked Clarias Gariepinus And Mugil Cephalus From Bayelsa State, Nigeria.

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Abstract
This study evaluated PAH concentrations in smoked Clarias gariepinus and Mugil cephalus obtained from Nembe River, Bayelsa State. Samples were dehydrated in an oven, pulverized, and precisely weighed before n-hexane extraction. Following filtration, the extracts were analyzed using GC-FID to quantify PAH content. The following results were obtained, (6.0 mg/L, 19.81 mg/L), acenaphthylene (7.0 mg/L, 7.0mg/L), acenaphthene (7.0 mg/L, 7.0mg/L), fluorene (9.00 mg/L, 9.0 mg/L), phenanthrene (7.0 mg/L, 7.0 mg/L), anthracene (7.0 mg/L, 7.0mg/L), fluranthene (7.0 mg/L, 7.0mg/L), pyrene (7.0 mg/L, 7.0mg/L), benzo(c)pyrene (7.0 mg/L, 7.0mg/L), benzo(a)anthrancene, (7.0 mg/L, 7.0mg/L), Chrysene (7.0 mg/L, 7.0mg/L), benzo(k)fluoranthene (7.0 mg/L, 7.0mg/L), benzo(e)pyrene (8.0 mg/L, 1.0mg/L), benzo(a)pyrene (7.0 mg/L, 8.0mg/L), 3-methcarboxy anthracene (3.0 mg/L, 3.0mg/L), benzo(g, h, i)perylene (6.80 mg/L, 7.30mg/L), dibenz(a, l)perylene (60.3 mg/L, 141.63mg/L). While DahA was not detected. Results showed that the detected PAHs originated from the fish smoking process. The total PAH concentrations were substantially lower than the maximum limits established by European Union (EU) regulations. Notably, benzo[a]pyrene levels (below 5 µg/kg) complied with EU safety standards, indicating minimal carcinogenic risk and confirming the smoked fish products' safety for human consumption.
Keywords: *Smoked fish, Clarias gariepinus, Mugil cephalus, GC-FID, GC/MS, Polycyclic aromatic hydrocarbons (PAHs), Bayelsa state.*

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I. Introduction

PAHs represent a class of organic molecules containing multiple condensed aromatic ring structures. These persistent compounds are ubiquitously distributed in environmental matrices (water, air, soil) and occur in trace amounts in various food products. Thermal food processing techniques including smoking, grilling, frying and baking serve as primary contamination pathways. Most PAHs originate as environmental pollutants from incomplete organic matter combustion during industrial and anthropogenic activities (Ishizaki *et al.,* 2021). Recognized for their carcinogenic potential, PAHs are designated as priority contaminants under EU and USEPA regulations. Epidemiological data indicate human exposure accounts for 58-98% of PAH contamination incidents (Farhadian *et al.,* 2021).

Polycyclic aromatic hydrocarbons are systematically classified into two primary categories according to their molecular properties: high molecular weight (HMW) and low molecular weight (LMW) PAHs, with this division reflecting significant differences in their chemical behavior and biological interactions. HMW PAHs contain 4-6 aromatic rings, exhibit slower biodegradation by native microbes, and tend to persist in aquatic systems. Due to their bioaccumulation potential in marine life (e.g., fish and mussels) and heightened carcinogenicity, they pose significant environmental risks *(*Rocher *et al.,* 2024*)*. PAHs with low molecular weight (LMW), comprising 2-3 aromatic ring structures, demonstrate significantly reduced carcinogenic potential when compared to high molecular weight (HMW) PAHs. However, they still demonstrate significant toxicity to numerous aquatic species, making them environmentally concerning. *(*Brown *et al.,* 2018).

Smoking represents one of the most traditional techniques for fish preservation and processing. This method involves exposing fish to smoke generated from burning wood or plant matter. The process typically incorporates simultaneous salting, drying, heating and smoking within a specialized chamber. Its preservative effects primarily stem from moderate dehydration and the deposition of both aliphatic and aromatic compounds on the fish's surface (Simpko, 2017). High-temperature food preparation techniques including smoking, drying, roasting, baking and frying are well-established as significant contributors to PAH contamination in food products (Yurchenko and Mölder, 2019).

Wood smoke comprises numerous PAHs and their derivatives, including carcinogenic compounds like Benzo[a]Pyrene (BaP). BaP serves as the primary indicator for carcinogenic PAHs in smoked fish, with a

regulatory limit of 2µg/kg. Following metabolic conversion to diol-epoxides in mammalian cells, PAHs form covalent bonds with DNA and other macromolecules. This interaction induces DNA replication errors and mutations, initiating carcinogenesis a activation pathway shared, with minor variations, by all carcinogenic PAHs. (Falco *et al.*, 2016).

This study primarily aimed to quantify the levels and assess the potential health risks associated with polycyclic aromatic hydrocarbons (PAHs) in smoked mullet and river catfish commonly consumed in Bayelsa State, Nigeria.

II. Materials

The study utilized smoked African catfish (*Clarias gariepinus*) and flathead mullet (*Mugil cephalus*) samples. Laboratory equipment included a drying oven, spatula, homogenization tools (mortar and pestle) and analytical balance. Extraction procedures utilized n-hexane and dichloromethane with extraction bottles and filter paper. Quantitative analysis was conducted using an American-manufactured HP 5890 Series II gas chromatograph with flame ionization detection (GC-FID).

III. Methods

Extraction of Fish Sample

Smoked ***Clarias gariepinus and Mugil cephalus*** was purchased from a road side vendor at Basambiri, Nembe, while smoked ones was purchased from Swali market in Yenagoa, Bayelsa State. The purchased smoked fishes were chopped finely and homogenized, after which bone fragments were removed using tweezers. The fish sample were subjected to oven drying at 60°C for 48 hours. Following dehydration, the samples were removed and pulverized. A precisely weighed 1g aliquot of homogenized fish material was measured into an extraction vessel, to which 10 ml of n-hexane was added. After a 72-hour standing period, the solution was vacuum-filtered through Whatman filter paper, the clarified extract was then subjected to GC-FID analysis for PAH quantification.

Gas Chromatography-Flame Ionization Detector (GC-FID) Analysis

Chromatographic separation was conducted using an HP 5890N Series II GC system configured with split/splitless injection capability and FID detection. An Agilent HP-608 capillary column (30 m length × 0.53 mm internal diameter, 0.5 µm stationary phase thickness) achieved compound resolution. The thermal profile began at 70°C (2 min isothermal), increased at 6°C/min to 260°C (15 min final hold), with injector and detector maintained at 280°C and 300°C respectively. Nitrogen served as carrier gas at 4.0 mL/min constant flow rate. ChemStation software controlled instrument operation and data processing. PAH identification involved retention time matching with pure standards, with GC/MS confirmation for samples showing elevated PAH levels.

IV. Results And Data Presentation

Table 1: Level of PAHs in *Clarias gariepinus* and *Mugil cephalus*
Permissible limits of PAHs in *Clarias gariepinus and Mugil cephalus by international bodies.*

PAHs	*Clarias gariepinus (mg/Kg)*	*Mugil cephalus (mg/Kg)*	Permissive Limit(mg/kg)	Source
Nap	6. 0E-02	19.81		
Ace	7. 0E-02	7. 0E-02		
Acep	7. 0E-02	7.0E-02	0.06	USEPA
Flru	9. 0E-02	9. 0E-02	0.06	SON
Phe	7. 0E-02	7. 0E-02	0.1	USEPA
Anth	7. 0E-02	7. 0E-02	0.3	USEPA
Flura	7. 0E-02	7.0E-02		
Pyr	7. 0E-02	7. 0E-02	0.06	SON
B(c)P	7. 0E-02	7. 0E-02	3mg/kg	WHO
B(a)A	7. 0E-02	7. 0E-02	10mg/kg	WHO
Chr	7.0E-02	7. 0E-02	10mg/kg	WHO
BkF	7. 0E-02	7. 0E-02	0.10	USEPA
B(e)P	8. 0E-02	1. 0E-01	5.10	USEPA
B(a)P	7. 0E-02	8. 0E-02		
3MCA	3. 0E-02	3. 0E-02		
BghiP	6. 8E-01	7. 30E-01	0.05	SON
DahA	0.001	0	0.03	SON
D(a,l)P	60.03	120.09		

Table 1 shows the quantitative analysis results for PAH presemt in both *Clarias gariepinus* and *Mugil cephalus* with recommended values by standard organizations. The predominant PAHs found in the smoked fishes are Nap, D(a,i)P. Although, the two PAHs are not among biomarkers of PAHs that are dangerous to human health

but continual consumption might result in bioaccumulation in the body of the consumers which may cause ill health.

Graphical Presentation Of The PAHs Concentration In *Clarias Gariepinus And Mugil Cephalus.*

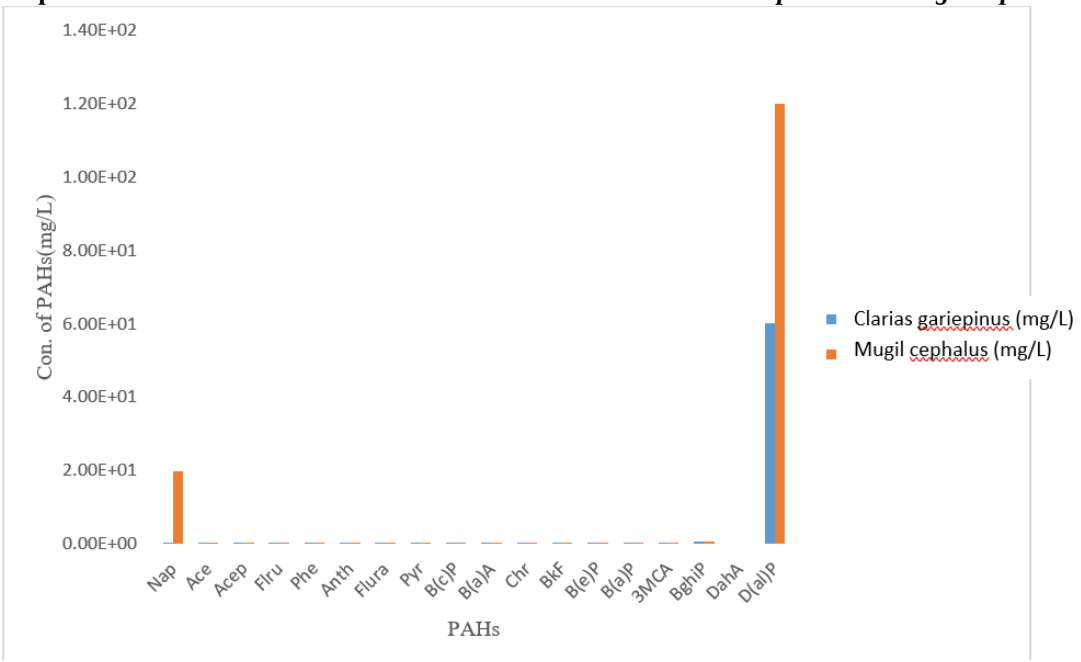

Table 1: Level of PAHs in *CLARIAS GARIEPINUS* AND *MUGIL CEPHALUS*

PAHs	*Clarias gariepinus (mg/L)*	*Mugil cephalus(mg/L)*
Nap	6.00E-02	19.81
Ace	7.00E-02	7.00E-02
Acep	7.00E-02	7.00E-02
Flru	9.00E-02	9.00E-02
Phe	7.00E-02	7.00E-02
Anth	7.00E-02	7.00E-02
Flura	7.00E-02	7.00E-02
Pyr	7.00E-02	7.00E-02
B(c)P	7.00E-02	7.00E-02
B(a)A	7.00E-02	7.00E-02
Chr	7.00E-02	7.00E-02
BkF	7.00E-02	7.00E-02
B(e)P	8.00E-02	1.00E-01
B(a)P	7.00E-02	8.00E-02
3MCA	3.00E-02	3.00E-02
BghiP	6.80E-01	7.30E-01
DahA	0	0
D(a,l)P	60.03	120.09
Sum	6.17E+01	141.63
Mean	3.43	7.868333
Standard deviation	±0.33	±0.669

From **Table 1**: It shows the level of PAHs in my sample with recommended values by standard organization. The predominant PAHs found in the smoked fishes are Nap, D (a,i)P. Although, the two PAHs are not among biomarkers of PAHs that are dangerous to human health but continual consumption might result in bioaccumulation in the body of the consumers which may cause ill health.

Table 2 Pearson's Correlation interpretation.

S/No	Degree of Correlation	Types of Correlation
1	±0.00 to ±0.20	Negligible
2	±0.20 to ±0.40	Low
3	±0.40 to± 0.70	Moderate
4	±0.70 to± 0.90	High
5	±0.90 ±1.00	Very High
6	±1.00	Perfect

Table 3: Interrelationship between the analyzed PAHs in the selected Fishes samples from Bayelsa State

	Clarias gariepinus	*Mugil cephalus*
Clarias gariepinus	1	
Mugil cephalus	0.986448	1

Interrelationship between the analyzed PAHs in the selected Fishes samples from Bayelsa State

During this study, high correlations between *Clarias gariepinus* and *Mugil cephalus* (r = 0.986448), was observed in samples of selected smoked fishes. Hence, correlations among the Fishes show that the analyzed sample (*Clarias gariepinus and Mugil cephalus*) had sources of PAHs from the same point/ origin. The consumers of these smoked fishes will be obtaining PAHs in their diet, which will bioaccumulated in their body and had adverse effect in their well-being and general health conditions.

t-Test: Two-Sample Assuming Equal Variances		
	Clarias gariepinus	*Mugil cephalus*
Mean	3.43	7.868333
Variance	199.5511	805.8976
Observations	18	18
Pooled Variance	502.7243	0.986448
Hypothesized Mean Difference	0	0
df	34	17
t Stat	-0.59385	1.286381
P(T<=t) one-tail	0.278272	0.107777
t Critical one-tail	1.690924	1.739607
P(T<=t) two-tail	0.556544	0.215554
t Critical two-tail	2.032245	2.109816

V. Discussion

The data in table 1 shows that the analyzed samples contain 17 varieties of polycyclic aromatic hydrocarbons including naphthalene (6.0 mg/L, 19.81 mg/L), acenaphthylene (7.0 mg/L, 7.0mg/L), acenaphthene (7.0 mg/L, 7.0mg/L), fluorene (9.00 mg/L, 9.0 mg/L), phenanthrene (7.0 mg/L, 7.0 mg/L), anthracene (7.0 mg/L, 7.0mg/L), fluranthene (7.0 mg/L, 7.0mg/L), pyrene (7.0 mg/L, 7.0mg/L), benzo(c)pyrene (7.0 mg/L, 7.0mg/L), benzo(a)anthracene, (7.0 mg/L, 7.0mg/L), Chrysene (7.0 mg/L, 7.0mg/L), benzo(k)fluoranthene (7.0 mg/L, 7.0mg/L), benzo(e)pyrene (8.0 mg/L, 1.0mg/L), benzo(a)pyrene (7.0 mg/L, 8.0mg/L), 3-methcarboxy anthracene (3.0 mg/L, 3.0mg/L), benzo(g, h, i)perylene (6.80 mg/L, 7.30mg/L), dibenz(a, l)perylene (60.3 mg/L, 141.63mg/L). These findings corroborate previous studies demonstrating that while raw food products typically contain minimal PAH concentrations, these hazardous compounds primarily form during thermal processing techniques including smoking, roasting, baking and frying (Kayali *et al.,* 2019). Within this group of priority PAHs, five compounds exhibit carcinogenic properties: chrysene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[g,h,i]perylene and benz[a]anthracene. In contrast, fluorene, acenaphthylene, pyrene, acenaphthene, phenanthrene, naphthalene, anthracene and fluoranthene are classified as non-carcinogenic (Kafeelah *et al.*, 2019).

High significant concentration of different polycyclic aromatic hydrocarbons were observed in samples of selected smoked fishes. Hence, correlations among the fishes show that the analyzed samples smoked **(*Clarias gariepinus and Mugil cephalus*)** had sources of PAHs from the same point/ origin. For the *Clarias gariepinus*, the concentrations ranged from 6.0 mg/L for Nap, to 60.03 mg/L for (a,i)P. On the other hand, the concentrations of PAHs in the *Mugil cephalus* ranged from 1.0 mg/L for B(e)P to 120.09 mg/L D(a,l)P. The consumers of these smoked fishes will be obtaining PAHs in their diet, which may accumulate in their body and cause adverse effects that will affect their well-being and general health conditions.

VI. Conclusion

Analysis revealed that PAH concentrations in smoked *Clarias gariepinus* and *Mugil cephalus* samples fell below the safety thresholds established by the European Commission, FAO, and WHO. These low levels suggest the smoked fish products are safe for human consumption and unlikely to present carcinogenic or other health risks to Bayelsa State residents or other consumers. The detected PAHs were predominantly pyrogenic in origin, resulting from the smoking process itself.

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