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Market-Specific Polycyclic Aromatic Hydrocarbon Contamination in Smoked African Catfish (*Clarias gariepinus*) from Calabar, Nigeria

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are harmful compounds that can accumulate in smoked fish and pose health risks, especially in developing countries where smoked fish is a common part of the diet. This study measured PAH levels in smoked African catfish (*Clarias gariepinus*) obtained from two markets in Calabar, Nigeria: Marian Market and Watt Market. The aim was to assess the potential health risks associated with their consumption. Gas chromatography with flame ionization detection was used to identify and quantify PAHs. Results showed that total PAH concentrations were significantly higher in Marian Market samples (24.98 ± 1.57 µg/kg) compared to Watt Market samples (8.99 ± 0.35 µg/kg). Marian Market samples also had higher levels of acenaphthene (0.63 ± 0.03 µg/kg) and fluorene (2.87 ± 0.91 µg/kg), while Watt Market samples contained more anthracene (3.47 ± 0.33 µg/kg). Although total PAH concentrations in both markets were below the European Union maximum limit of 30 µg/kg, the level of pyrene in Marian Market samples (15.02 ± 0.48 µg/kg) exceeded the permissible limit of 5 µg/kg. These differences may be due to variations in fish smoking techniques, types of fuel used, and environmental factors such as exposure to vehicle emissions. The study recommends the use of improved smoking methods, regular monitoring of smoked fish in markets, and increased public education to help reduce PAH exposure and protect consumer health.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants made up of two or more fused aromatic rings. They are mainly formed during the incomplete burning or thermal breakdown of organic materials such as coal, wood, and oil **(Mastral & Callén, 2000; Ravindra *et al*., 2008)**. PAHs are known for their chemical stability and unique properties, including high fat solubility, semi-volatility, low water solubility (especially in heavier PAHs), and heat resistance. These features allow PAHs to remain in the environment for a long time and build up in living organisms **(Haritash & Kaushik, 2009; Kim *et al*., 2013; Okon *et al*., 2021)**. Many PAHs are toxic and have been identified as substances that can cause birth defects, gene mutations, cancer, and immune system damage in various organisms **(Bostrom *et al*., 2002; Luch, 2005; Patel *et al*., 2020)**.

The presence of PAHs in the environment is mostly linked to human activities such as industrial operations and urban development, which lead to their release into soil, water, and air **(Wilcke, 2000; Ravindra *et al*., 2008)**. Humans are mainly exposed to PAHs through breathing contaminated air, skin contact, and food consumption. Among these, diet is a major route of exposure, especially through the intake of smoked food items **(Moret & Conte, 2000; EFSA, 2008; Silva *et al*., 2011; Asuquo *et al*., 2018)**. Fish are an important source of nutrition since they contain essential fatty acids, high-quality protein, and valuable micronutrients. They play a key role in food security, especially in developing countries where it forms a regular part of the diet **(Al-Jeddah *et al*., 1999; Eteng & Ifon, 2019)**.

Smoking is a common way of preserving fish in many African communities, including those in Calabar, Nigeria. This method not only helps prevent spoilage but also improves taste and extends shelf life. However, smoking also increases the risk of PAH contamination in fish **(Stołyhwo & Sikorski, 2005; Essumang *et al*., 2012)**. Research has shown that around 70 percent of the PAHs people consume come from food, with smoked fish being a major contributor **(Nnaji & Ekwe, 2018)**. The level of PAHs in smoked fish depends on various factors, including the smoking temperature, humidity, smoke density, ventilation, and the type of material burned to produce the smoke **(Moret *et al*., 1999; Rey-Salgueiro *et al*., 2008)**. Newer technologies such as industrial smoking ovens with filters and controlled smoke generation have been shown to reduce PAH levels more effectively than traditional methods **(Guillen, 1990; Moret *et al*., 1999)**. International organizations, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), recommend reducing PAH formation by avoiding the direct contact of fat with the heat source during smoking **(FAO/WHO, 2005)**.

Out of more than 100 known PAHs, food safety authorities like the European Union Scientific Committee on Food have listed 15 that are of particular concern because of their strong cancer-causing properties **(EFSA, 2008)**. Benzo[a]pyrene is one of the most toxic PAHs and is often used as a marker to indicate PAH contamination in food products **(European Commission, 2002; IARC, 2010)**. Several studies have found that traditional smoking techniques can significantly increase PAH concentrations in fish muscle tissues, which may raise cancer risks for consumers **(Rey-Salgueiro *et al*., 2008; Kafeelah *et al*., 2015; Nnaji & Ekwe, 2018; Chang *et al*., 2023)**.

The harmful effects of PAHs depend on their structure. Generally, heavier PAHs are more resistant to degradation and more toxic **(Fetzer, 2000; Atagana, 2009)**. Because they do not dissolve well in water, PAHs tend to stick to solid particles and can accumulate in aquatic animals and sediments **(Yu *et al*., 2005; Osuji & Ezeburio, 2006; Ekpo *et al*., 2023)**. Long-term exposure to PAHs has been linked to serious health problems, including cancers of the digestive system, bladder, and lungs, as well as heart, lung, and developmental disorders **(Agency for Toxic Substances and Disease Registry [ATSDR], 1995; Kim *et al*., 2013; Silverman *et al*., 2013)**.

In Calabar, smoked fish continues to be widely consumed despite these risks. Many low-income families rely on smoked fish because it is affordable, nutritious, and readily available. The popularity of roadside vendors selling smoked fish also highlights the need to check and regulate PAH levels in locally sold fish products. This study was designed to measure the levels of PAHs in smoked African catfish (*Clarias gariepinus*) sold in Calabar and to assess the potential health risks associated with their regular consumption. The results are expected to guide public health actions, improve consumer knowledge, and promote safer eating habits in the region.

MATERIALS AND METHODS

Sample collection and study area

Smoked African catfish (*Clarias gariepinus*) samples were collected from two major markets within Calabar Metropolis, Nigeria. These markets were Watt Market, located in Calabar South, and Marian Market, situated in Calabar Municipality. Calabar, the capital of Cross River State in southern Nigeria, includes both Calabar South and Calabar Municipality Local Government Areas. The metropolis covers an area of about 274.6 square kilometers and is positioned between latitudes 4°50′N and 5°10′N and longitudes 8°17′E and 8°20′E. It is bordered to the north by Odukpani Local Government Area and to the east by Akpabuyo Local Government Area **(Ekpo *et al*., 2021a; Ekpo *et al*., 2021b)**. The city is flanked by the Great Kwa River on the eastern side and the Calabar River on the western side. Calabar experiences a tropical equatorial climate marked by consistently high temperatures, high relative humidity, and considerable annual rainfall averaging approximately 2,750 millimeters. The average annual temperature is around 26.1°C. Urban growth and population increase in recent years have led to significant changes in the environment and local climate conditions **(Asuquo & Ifon, 2019; Odum *et al*., 2023)**.

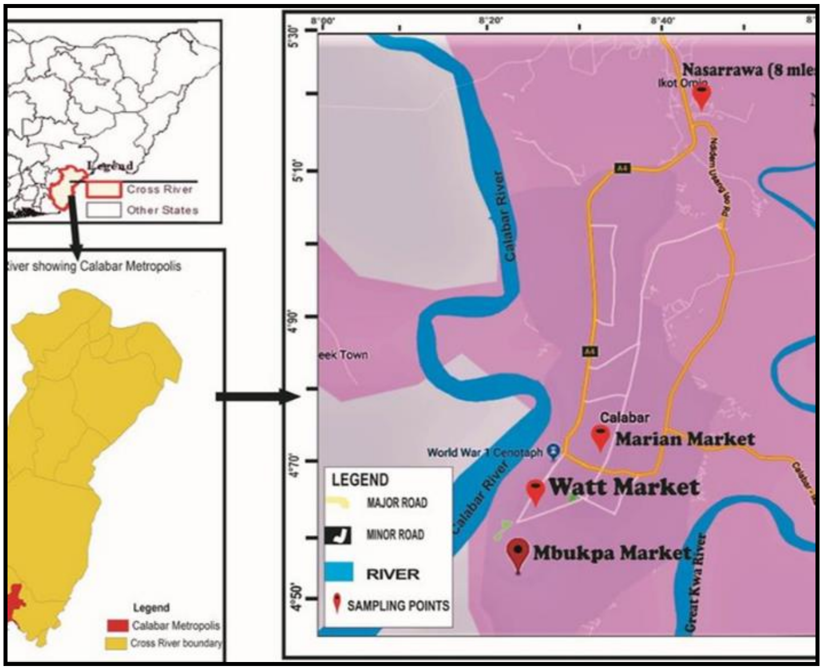

Fig. 1. Map of the study area showing sampling stations. Source **(Google map, 2024)**

Laboratory analysis

The analysis of polycyclic aromatic hydrocarbons (PAHs) in the smoked fish samples was carried out at Mifor Consult Laboratory in Calabar using a Hewlett Packard 5890 Series II gas chromatograph fitted with a flame ionization detector (GC-FID). The procedure was adapted to ensure precision and reliability for detecting PAHs in smoked fish, following standardized protocols suitable for such food matrices **(Moret *et al*., 1999)**.

For each sample, 50 milligrams of homogenized fish tissue were weighed and extracted with 10 milliliters of a 1:1 volume-to-volume mixture of hexane and dichloromethane. The resulting extract was then purified using a silica gel column, prepared with 3 grams of anhydrous sodium sulfate and preconditioned with hexane. This step helped remove lipids and other substances that could interfere with the analysis **(Moret *et al*., 1999)**. After purification, a 1-microliter portion of the cleaned extract was injected into the gas chromatograph.

Chromatographic separation was performed using a 30-meter long and 0.25-millimeter internal diameter DB-5MS fused silica capillary column with a film thickness of 0.15 micrometers. Helium was used as the carrier gas, while hydrogen and air served as ignition gases. The column head pressure was maintained at 20 psi, and the flow rate was set to 1 milliliter per minute. The oven temperature was programmed to start at 55°C with a hold time of 0.4 minutes, followed by a ramp to 200°C at a rate of 25°C per minute. It was then increased to 280°C at 8°C per minute and finally to 300°C at 25°C per minute with a final hold for 2 minutes. Identification and quantification of the PAHs were based on retention times, with lighter compounds eluting before heavier ones **(Yurchenko, 2011)**.

Statistical analysis

The data were evaluated using both descriptive and inferential statistical methods. Descriptive statistics included the calculation of mean values and standard deviations for the concentration of PAHs. Inferential analysis was conducted using independent t-tests to determine if there were statistically significant differences in individual and total PAH concentrations between fish samples obtained from Marian Market and those from Watt Market. Statistical testing was done with SPSS version 25, and results were considered significant at a probability level of less than 0.05 **(Asuquo *et al*., 2025)**. This approach follows established practices for assessing PAH contamination in smoked fish **(Ubwa *et al*., 2015; Borodi *et al*., 2022)**.

RESULTS

Levels of PAHs in smoked catfish from Calabar markets

The analysis of smoked African catfish (*Clarias gariepinus*) collected from Marian Market (MM) and Watt Market (WM) in Calabar confirmed the presence of nine polycyclic aromatic hydrocarbons (PAHs). These included naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, and indeno(1,2,3-cd)pyrene. Other PAH compounds were not detected, as their concentrations were below the instrument's detection limits.

Differences in the PAH profiles between samples from the two markets were clearly observed. Naphthalene and indeno(1,2,3-cd)pyrene were found only in samples from Watt Market, with concentrations of 0.28 ± 0.04 µg/kg and 1.19 ± 0.04 µg/kg, respectively. On the other hand, pyrene was detected exclusively in samples from Marian Market, where it appeared at a relatively high concentration of 15.02 ± 0.48 µg/kg.

In general, Marian Market samples contained higher average levels of acenaphthylene (1.44 ± 0.95 µg/kg), acenaphthene (0.63 ± 0.03 µg/kg), fluorene (2.87 ± 0.91 µg/kg), phenanthrene (0.76 ± 0.23 µg/kg), and fluoranthene (2.87 ± 0.09 µg/kg) compared to those from Watt Market. In contrast, anthracene was more abundant in Watt Market samples, showing a concentration of 3.47 ± 0.33 µg/kg, which was higher than the 1.39 ± 0.29 µg/kg observed in Marian Market samples.

The total average PAH concentration was significantly greater in fish obtained from Marian Market, measured at 24.98 ± 1.57 µg/kg, compared to 8.99 ± 0.35 µg/kg recorded for Watt Market samples. Among all PAHs quantified in the study, pyrene in Marian

Market samples had the highest concentration, while naphthalene in Watt Market samples had the lowest (Table 1 & Fig. 2).

Table 1. Average concentration of PAHs in smoked catfish from two markets in Calabar

PAH (μg/kg)	Marian Market	Watt Market
Naphthalene	-	0.28±0.04
Acenaphthylene	1.44±0.95	1.21±0.03
Acenaphthene	0.63±0.03	0.33±0.05
Fluorene	2.87±0.91	0.98±0.03
Anthracene	1.39±0.29	3.47±0.33
Phenanthrene	0.76±0.23	0.18±0.06
Fluoranthene	2.87±0.09	1.35±0.09
Pyrene	15.02±0.48	-
Indeno(1,2,3-cd)pyrene	-	1.19±0.04
Total	**24.98±1.57**	**8.99±0.35**

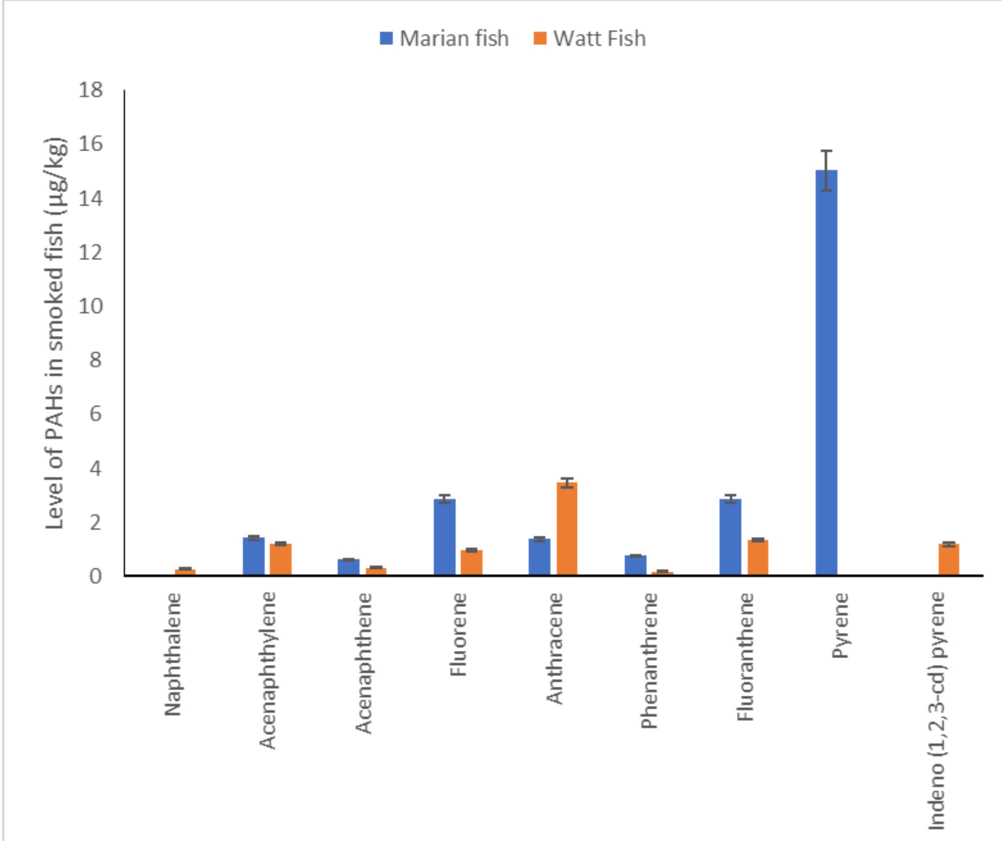

Fig. 2. Variation in levels of PAHs in smoked fish from Calabar markets

Statistical differences in PAH concentrations

Results from statistical analysis revealed that there were significant differences ($P < 0.05$) in the total PAH content and in the concentrations of most individual PAHs between the two markets. The only exception was acenaphthylene, for which the difference was not statistically significant ($P > 0.05$). Because naphthalene, pyrene, and indeno(1,2,3-cd)pyrene were not detected in both markets, statistical comparisons could not be made for these compounds (Table 2).

When compared with the European Union (EU) standards specified in Regulation EC No 1881/2006, the combined concentration of four priority PAHs in samples from both Marian Market (24.98 ± 1.57 µg/kg) and Watt Market (8.99 ± 0.35 µg/kg) remained below the EU maximum allowable limit of 30.0 µg/kg for smoked fish products. However, the concentration of pyrene in Marian Market samples (15.02 ± 0.48 µg/kg) exceeded the EU regulatory limit of 5.0 µg/kg for individual PAHs. This finding suggests a potential health concern for consumers who regularly consume smoked catfish from that market.

Table 2. Differences in the individual and total PAHs found in locally consumed smoked fish in Calabar markets

PAH	**T-test**	***P*-value**	**Significance**
Acenaphthylene	2.23	1.04	Not significant
Acenaphthene	6.39	0.042	Significant
Fluorene	8.46	0.028	Significant
Anthracene	11.24	0.011	Significant
Phenanthrene	5.28	0.046	Significant
Fluoranthene	5.89	0.038	Significant
Total PAHs	16.22	0.00	Significant

DISCUSSION

This study presents a detailed evaluation of polycyclic aromatic hydrocarbon (PAH) contamination in smoked African catfish (*Clarias gariepinus*) sold in Calabar, Nigeria. It reveals differences in PAH levels between two major markets, pointing to the need for market-specific interventions. The presence of PAHs, although generally at low levels, was consistent across all samples. This indicates that consumers may be exposed to PAHs on a regular basis. The total mean concentration of PAHs was significantly higher in samples from Marian Market (24.98 ± 1.57 µg/kg) compared to those from Watt Market (8.99 ± 0.35 µg/kg). This difference may be linked to environmental factors and smoking practices that vary from one market to another. According to earlier studies, locations with high traffic and industrial activity often have higher PAH levels in food and environmental samples **(Rengarajan *et al*., 2015; Sánchez-Arévalo *et al*., 2020)**. The very high concentration of pyrene (15.02 ± 0.48 µg/kg) in Marian Market is particularly concerning, as it exceeds the European Union (EU) limit of 5 µg/kg for individual PAHs **(European Commission, 2011)**.

The differences in PAH concentrations between the two markets may be explained by a few factors. Traditional smoking methods vary, and the use of open-fire techniques common in the area allows for more direct contact between the smoke and the fish. This can lead to higher PAH concentrations compared to more controlled methods such as smoking kilns **(Stołyhwo & Sikorski, 2005; Essumang *et al*., 2012)**. The type of fuel used also plays a major role. Sawdust and charcoal, which are commonly used for smoking, release a mixture of PAHs when they are not fully burned **(Sojinu *et al*., 2019)**. Although not directly observed in this study, the possible use of plastics or waste materials in smoking could further increase PAH contamination **(Bandowe *et al*., 2014)**.

In addition, environmental pollution from traffic and industrial emissions may contribute to the overall PAH burden in areas like Marian Market, which experiences heavy commercial activity **(Kim *et al*., 2013)**.

The levels of PAHs in fish are influenced not only by smoking methods but also by their habitats. Fish from natural habitats are often exposed to higher environmental pollution, leading to greater PAH accumulation due to factors like organic matter and sediment composition. In contrast, artificial habitats such as managed fish ponds typically have better water quality and lower pollutant exposure, resulting in reduced PAH levels. A study in Calabar South, Nigeria, demonstrated significant differences in physico-chemical parameters between natural and artificial habitats, which affect contaminant uptake in fish. Thus, the habitat origin plays a key role in baseline PAH contamination before smoking and should be considered in risk assessments **(Inyang-Etoh *et al*., 2025)**.

The specific types of PAHs found also differed between the markets. Marian Market samples had higher concentrations of compounds such as acenaphthene and fluorene, possibly reflecting pollution from nearby sources. On the other hand, Watt Market had more anthracene, which may be due to different smoking methods or sources of fish. Acenaphthylene showed similar levels in both markets, which might suggest that some contamination sources are common to both areas. These differences support the idea that PAH contamination is influenced by a complex mix of factors. The concentrations observed in this study are higher than those reported by **Kwaghvihi *et al*. (2018)** and **Wangboje (2024)**, but they are consistent with findings by **Asamoah *et al*. (2021)**, who noted that improved smoking kilns reduce PAH levels. The dominance of high molecular weight PAHs, as noted by **Palm *et al*. (2011)** and **Amos-Tautua *et al*. (2013)**, further supports the possibility of bioaccumulation in smoked fish products.

Although previous studies such as that by **Yusuf *et al*. (2015)** suggest that the health risks associated with smoked catfish are generally below acceptable levels, long-term exposure, especially among frequent consumers, should not be ignored. Certain PAHs, like naphthalene, are considered possible carcinogens, while others such as acenaphthene are known to affect the liver **(ATSDR, 1999; U.S. EPA, 2001)**. Reference values for safe daily intake, like 0.30 mg/kg for anthracene and 0.06 mg/kg for acenaphthene provided by the ATSDR, offer useful benchmarks for assessing health risks. Therefore, it is important to implement measures to reduce PAH exposure. Improving smoking techniques by using better combustion methods can help reduce PAH formation **(Hamad *et al*., 2023)**. Regular inspection and enforcement of food safety regulations are also necessary to ensure compliance **(European Commission, 2011)**. Public education campaigns could raise awareness about the risks of PAHs and encourage safer smoking and consumption practices. These steps are in line with current efforts to promote sustainable food safety practices that take both human health and the environment into account **(Guiné *et al*., 2024)**.

CONCLUSION

This study has shown that PAH contamination in smoked catfish varies by market in Calabar, Nigeria. Differences in smoking methods and environmental exposure seem to influence the levels of PAHs detected. The particularly high level of pyrene in Marian Market samples is above the EU safety limit, which poses a potential health concern. While other PAHs remain within acceptable levels, their presence suggests the need for ongoing monitoring. To protect public health, it is necessary to promote safer fish smoking practices, enforce existing food safety regulations, and educate consumers about the risks of PAH exposure. Further studies should examine the long-term health effects of PAH intake and assess how effective current PAH reduction efforts are.

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