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Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Smoked Fish (*Hydrocynus Forskahlii*) from Tombia Community-Ekpetiama, Bayelsa State

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Abstract

The assessment of Polycyclic Aromatic Hydrocarbons (PAHs) was done in smoked fish (*Hydrocynus forskahlii*) gotten from Tombia Community-Ekpetiama, Bayelsa State. The smoked fish sample was oven dried and ground thereafter. 1g of the ground fish sample was weighed and digested using n-hexane. The solution was then filtered and the filtrate ran in Gas Chromatograph-flame ionization detector (GC-FID). The following results were obtained, naphthalene 20.36µg/kg, 2-methylnaphthalene 1.91µg/kg, acenaphthylene 1.90µg/kg, acenaphthene 0.34µg/kg, fluorene 0.33µg/kg, flouranthene 7.05µg/kg, pyrene 3.50µg/kg, chrysene 0.07µg/kg, benz (a)anthracene 0.09µg/kg, benzo(b)fluoranthene 2.56µg/kg, benzo(k) fluoranthrene 6.66µg/kg, benzo(a)pyrene 0.087µg/kg, indeno(1,2,3–cd)pyrene 0.27µg/kg, dibenz(a,h)anthracene 0.76µg/kg while two PAHs phenanthrene and anthracene were not detected. The total concentration of the PAH was 45.89µg/kg. The ratios of ([Fla/ (Fla + Py]) is 0.66µg/kg and ([FLa/Py] is 2.02µg/kg]) which indicate the PAHs detected is from the smoking process. The maximum permissible level of the sum of PAH4 (Chrysene, Benz(a)anthracene, Benzo(b) fluoranthene, Benzo(a)pyrene) in smoked fishery products is 0.03 mg/kg (European Union., 2014) and the sum of PAH4 in this research work is 0.0002822mg/kg which is below the Europian Union (EU) recommended standard. The concentration of benzo(a)pyrene is below the E.U recommended limit of 5µg/kg indicating that the fish is healthy for consumption and may not pose cancer and other health risk.

Keywords: Smoked fish; Polycyclic aromatic hydrocarbons (PAHs); *Hydrocynus forskahlii*; River nun; European Union (EU); GC-FID.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds containingtwo or more condense aromatic rings. PAHs are present in water, air, soil and traces in various food products. Food can become contaminated during thermal treatments that occur in the process of food preparation and manufacturing such as drying, smoking, cooking, roasting, baking and fryings [1]. Most PAHs are environmental pollutants that result from the incomplete combustion of organic matter during industrial processing and human activities [2]. Due to their carcinogenic effect, PAHs have been included in the European Union and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Human exposure to PAHs accounts for 58 to 98% of such contamination [3].

PAHs are classified into two broad groups based on their physical and biological properties including High molecular weight (HMW) and low molecular weight (LMW) PAHs. The high molecular weight(HMW) PAHs consist of 4-6 aromatic rings and are less readily bio-degraded by indigenous microorganisms, hence can persist in the aqueous environment, by bio-accumulating in aquatic organisms like fish and mussels and are more carcinogenic [4]. The Low Molecular Weight (LMW) PAHs consists of 2–3 aromatic rings and although less carcinogenic, it also poses toxic effect to many aquatic organisms [5].

Hydrocynus forskahlii is a genus of large characin fish in the family of Alestidae commonly called Tigerfish. Fish are gill-bearing aquatic cremate animals that lacks limbs with digits. Fish is a good source of protein, lysine, phosphorus, calcium and iron. It is high in poly unsaturated fatty acids that are important in lowering blood cholesterol level [6]. One of the greatest problems affecting the fish industry all over the world is fish spoilage. Fish spoilage process starts immediately after the fish death [7]. Fish smoking is one of the techniques for fish preservation to reduce fish spoilage.

Fish smoking belongs to one of the oldest technologies of food preservation, diversified high value added products as an additional marketing option for certain fish species, where fresh fish consumption becomes limited [8]. Traditional smoking techniques involve treating of whole or filleted fish with smoke from wood and burning that comes into direct contact with the product. This process can lead to its contamination with PAHs if the process is not adequately controlled or if very intense smoking procedures are employed [9]. The level of PAHs in smoked food depends on the smoking process including type of smoke generator, combustion temperature and degree of smoking [10].

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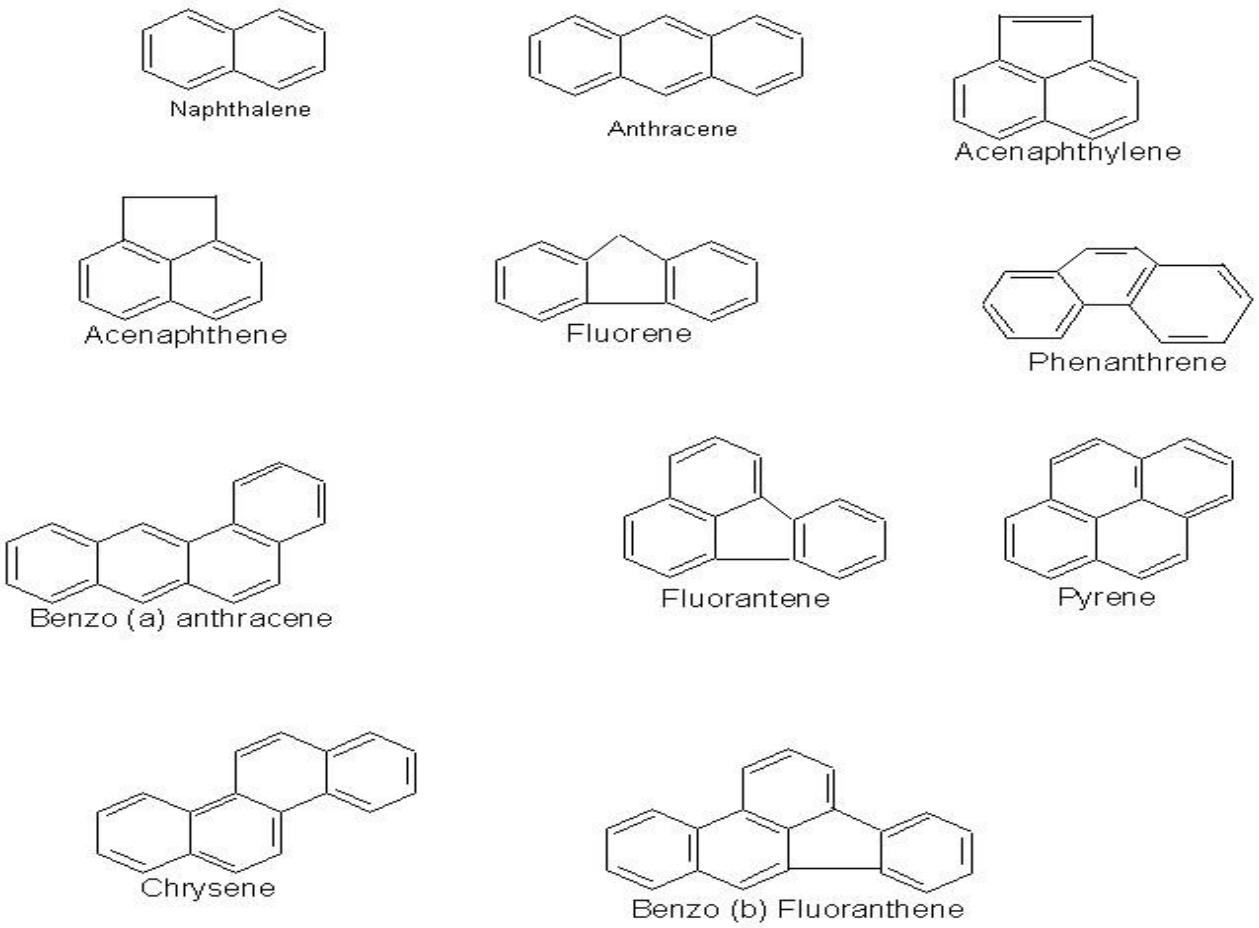
Most indigenes of Tombia Community Ekpetiama depends on fish as one of their source of protein. Human efforts to preserve fishes have affected the quality of it. Many research works have been done on the assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in smoked fish and their possible risk to human health in many parts of the country, but works in Tombia Community Ekpetiama are still scanty.This work therefore seeks to check the quantity of Polycyclic Aromatic Hydrocarbons (PAHs) in smoked fish in Tombia Community Ekpetiama and it likely implications using analytical method.

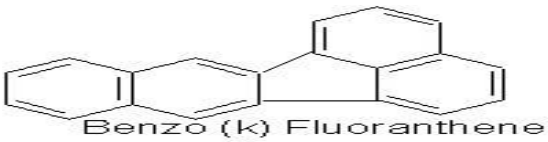
Processing of food at high temperatures from grilling, smoking, roasting and frying are major sources of PAHs in food. PAHs are found in coal, coal tar, and in the creosote oils, oil mists, and pitches formed from the distillation of coal tars [11]. The sources of PAHs in the costal environment are described as either petrogenic (if the source is derived from petroleum, e.g. natural oil seepage and oil spills) or pyrogenic (if the source is derived from the incomplete combustion of organic matter and fossil fuel [12, 13].

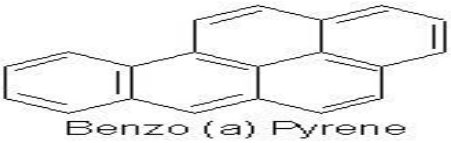
The quantity of PAHs in raw food depend primary on its source of the food, for example, fish, foods, fruit or vegetables obtained from polluted area generally contain higher concentration of PAHs than those from less polluted area. In cooked foods, the method of food preparation is generally the primary determinant of the PAHs and this content varies considerably, depending on the cooking habits. The presence of PAHs in non-processed (raw) foods is associated with environmental pollution from human, industrial activities and contamination of soil. Among sample of foods analysized for the presence of PAHs benzo(a)pyrene was the most common PAHs determined in food [14].

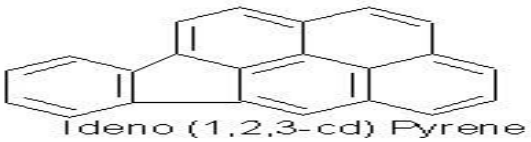
The health effects caused by exposure to PAHs depend on how much has entered the body, how long one have been exposed to PAHs, and how the body responds to PAHs. Increased incidences of lung, skin, and bladder cancers are associated with occupational exposure to PAHs. Epidemiologic reports of PAH-exposed workers have noted increased incidences of skin, lung, bladder, and gastrointestinal cancers. These reports, however, provide only qualitative evidence of the carcinogenic potential of PAHs in humans because of the presence of multiple PAH compounds and other suspected carcinogens. Some of these reports also indicate the lack of quantitative monitoring data [15-17]. Health effects of exposure to PAHs may include cataracts, kidney and liver damage, and jaundice. Repeated skin contact to the PAH naphthalene can result in redness and inflammation of the skin. Breathing or swallowing large amounts of naphthalene can cause the breakdown of red blood cells. Benzo(a)pyrene is the most common PAH to cause cancer in animals and human. The most significant endpoint of PAHs toxicity is cancer. Other non-cancer effects of PAHs involve primarily the pulmonary, gastrointestinal, renal, and dermatologic systems. It is difficult to fixed observed health effects to specific PAHs because most exposures are to PAHs mixtures. The earliest human PAH-related epidemiologic study was reported in 1936 by investigators in Japan and England [18].

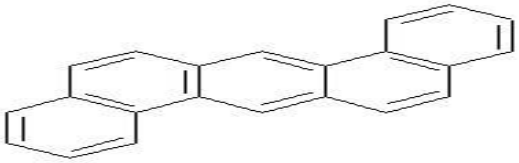
Fig-1. Structures of PAHs











2. Materials and Methods

This section provides the study area, apparatus, reagents, principles and methods used in the research work.

2.1. Study Area

Tombia Community Ekpetiama is a Community in Yenagoa Local Government Area of Bayelsa State, it lies on latitudes 05° 0'0" N, longitudes 06° 16'0" E and has an altitude of54m. It is situated in the southern part of the Niger Delta of Nigeria. The indigenous people are the Ijaws, the major occupations of the people are farming and fishing and hasa population of 5,612. The community also harbor a large number of non-indigenes who arebusiness men, students and staff of the Bayelsa State School of Nursing.

Fig-2. Map of Tombia Community Showing the Sampling Location

2.2. Sample Collection and Preservation

Tigerfish *(Hydrocynus forskahlii)* was caught in river Nun by Tombia Community Ekpetiama Yenagoa Local Government Area of Bayelsa and was smoked alongside other fishes using Mahogany tree (Mellicae). The smoked fish was transported to the Chemical Science Department Laboratory, Niger Delta University, Bayelsa state.

Fig-3. Fish sample (Tigerfish; *Hydrocynusforskahlii*)Mahogny tree (Mellicae)

3. Materials

3.1. Apparatus/Reagents

Smoked fish (*Hydrocynus forskahlii),* Electric oven, Mortar and pestle, Weighing balance, Spatula, Extraction bottle, n-hexane, Filter paper, GC-FID; HP 5890 series II gas chromatograph (GC) coupled with flame ionization detector (FID). Equipment manufactured in USA.

3.2. Equipment: Gas Chromatograph-Flame Ionization Detector (GC-FID)

Gas Chromatography-Flame Ionization Detector (GC-FID) is an instrumental analytical technique comprised of a gas chromatograph with a flame ionization detector. It is a very common analytical technique that is widely used in the petrochemical, pharmaceutical and natural gas markets. An FID typically uses a Hydrogen/Air flame into which the sample is passed to oxidize organic molecules and produces electrically charged particles (ions). The ions are collected and produce an electrical signal which is then measured. As common with other GC techniques, a carrier gas is required with low Water and Oxygen impurities since Water and Oxygen can interact with the stationary phase and cause significant problems such as high baseline noise and column bleed in the output gas chromatogram which both reduces the analyzer sensitivity and decreases column lifetime. The FID is also extremely sensitive to Hydrocarbon impurities in the Hydrogen and Air supply for the flame. Hydrocarbon impurities can cause increased baseline noise and reduce the detector sensitivity.

3.3. Sample Extraction

The fish sample was oven dried at a temperature of 60^{o}C for fourty-eight hours. The dried fish sample was removed from the oven and ground. 1g of the ground fish sample was weighed into an extraction bottle. 10ml of n-hexane was then added into the extraction bottle and allowed to stand for seventy-two hours. The solution was then filtered with whatman-Filter paper and the filtrate taken for GC-FID analysis.

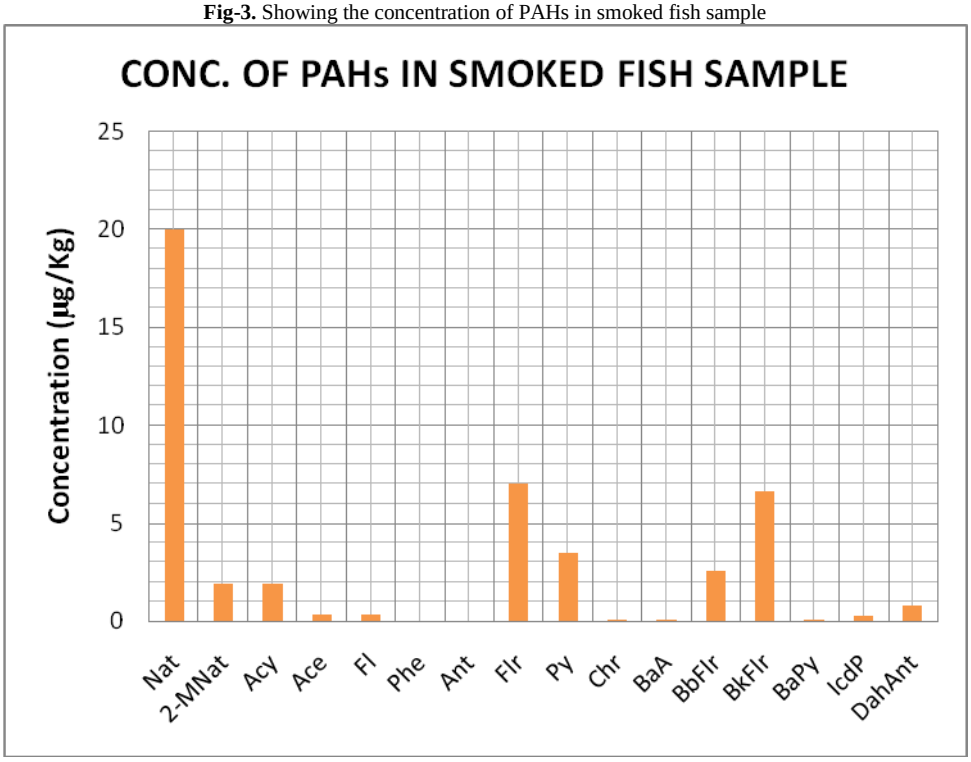
4. Results and Discussion

4.1. Results

Table-1. The concentration of Polycyclic Aromatic Hydrocarbons (PAHs) present in smoked fish sample

S/N	PAHs	Rings	Conc (PPM)	Conc. (PPB)
1	Naphthalene	2	2.03623×10^{-3}	20.3623
2	Methyl Naphthalene	2	1.91167×10^{-4}	1.91167
3	Acenaphthylene	3	1.90898×10^{-4}	1.90898
4	Acenaphthene	3	3.42455×10^{-5}	0.34245
5	Fluorene	3	3.32430×10^{-5}	0.33243
6	Phenanthrene	3	-	-
7	Anthracene	3	-	-
8	Flouranthene	4	7.04467×10^{-4}	7.04467
9	Pyrene	4	3.48593×10^{-4}	3.48593
10	Chrysene	4	7.93619×10^{-6}	0.07936
11	Benz(a)anthracene	5	9.53944×10^{-6}	0.09539
12	Benzo(b)fluoranthene	5	2.55998×10^{-4}	2.55998
13	Benzo(k)fluoranthrene	5	6.65610×10^{-4}	6.6561
14	Benzo(a)pyrene	5	8.77003×10^{-6}	0.0877
15	Indeno(1, 2, 3-cd)pyrene	6	2.70254×10^{-5}	0.27025
16	Dibenz(a, h)anthracene	5	7.62692×10^{-5}	0.76269
Total			4.58999×10^{-3}	45.89999

Fig-3. Showing the concentration of PAHs in smoked fish sample



4.2. Discussion

Table-2. Table of Comparism of results with standards

S/N	PAHs	Present study	European Commission
1	Benzo(a)pyrene	0.08770 µg/kg	5.00 µg/kg
2	PAH4	0.000822 mg/kg	0.03 mg/kg

PAH4 = Chrysene, Benz (a)anthracene, Benzo(b)fluoranthene, Benzo(a)pyrene

The results of the analysis of PAHs in smoked fish shows the presence of four-teen PAHs which include naphthalene 20.36µgkg, 2-methylnaphthalene 1.91µg/kg, acenaphthylene 1.90µg/kg, acenaphthene 0.34µg/kg, fluorene 0.33µg/kg, flouranthene 7.05µg/kg, pyrene 3.50µg/kg, chrysene 0.07µg/kg, benz(a)anthracene 0.09µg/kg, benzo(b)fluoranthene 2.56µg/kg, benzo(k)fluoranthrene 6.66µg/kg, benzo(a)pyrene 0.087µg/kg, indeno(1,2,3–cd)pyrene 0.27µg/kg, dibenz(a,h)anthracene 0.76µg/kg. This agrees with inferences made by other researchers that raw foods do not normally contain high levels of PAHs but they are formed during processing, roasting, baking, smoking or frying [19-21].

Among this priority PAHs, eight PAHs (PAH8) are carcinogenic:benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthrene, chrysene, dibenz(a,h)anthrancene, indeno(1,2,3-cd)pyrene and benzo[*g,h,i*]perylene while naphthalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene,anthracene, flouranthene, pyrene are not carcinogenic [22].

4.3. Source of PAHs

[Fla/(Fla + Py)] = [7.04467/ (7.04467+3.48593)]µg/kg
= 0.66897µg/kg
≈ 0.67µg/kg
FLa/Py = [7.04467/3.48593]µg/kg
= 2.02089µg/kg
≈ 2.02µg/kg

Table-3. Calculation of PAHs

PAHs Ratio	Petroleum	Wood	Present study	Reference
[Fla/(Fla + Py)]	<0.40	>0.50	0.67	Yunker, *et al.* [23]
Fla/Py	<1.00	>1.00	2.02	Baumard, *et al.* [12]

PAH4 Index (PAH4) = ∑(B[a]A + Chr + B[b]Fl + B[a]P)
$=9.53944 \times 10^{-6} + 7.93619 \times 10^{-6} + 2.55998 \times 10^{-4} + 8.77003 \times 10^{-6}$
$=2.822 \times 10^{-4}$ mg/kg = 0.2822µg/kg

In table 2above the ratio of fluoranthene to (fluoranthene + pyrene) in this study is 0.67µg/kg which is greater than 0.50µg/kg indicating the smoking process as thesourceof PAH.The ratio of fluoranthene (Fla) to pyrene (Pyr) is often used to verify the sources of PAHs detected in fish. Ratio of fluoranthrene to pyrene greater than one

(Fla/Pyr>1) is attributed to pyrolytic source while Fla/Pyr<1 is attributed to petrogenic source(petroleum hydrocarbon source) [12]. In this study the ratio of Fla/Pyr obtained from the smoked fish sample is 2.02µg/kg. Which indicate the PAHs detected in the fish originated from the smoking process.

On a study by Amos-Tautua, *et al.* [24] on theevaluation of polycyclic aromatic hydrocarbons and some heavy metals in roasted food snacks in Amassoma, Niger Delta, Nigeria. PAH was not detected in the raw (not smoked) fish sample (Scomber scombrus) which is in conformity with the statement made by Stolyhwo and Sikorski [25] that fish and marine invertebrates may naturally contain smallor undetectable amount of different PAHs absorbed from the environment. The concentration of PAHs in this research work ranges from (0.07936 – 20.3623)µg/kg.Naphthalene is the highest PAH with a concentration of 20.3623µg/kg. The maximum concentration of naphthalene in human body is not established by WHO [26]. On a study by Kefeelah, *et al.* [22] on the influence of fish smoking method on polycyclic aromatic hydrocarbons contents and it possible risks to human health, the concentration of naphthalene is 0.02 mg/kg which is above the concentration of naphthalene (0.00203 mg/kg) in this research work. On another research work by Hafez, *et al.* [27] on the Safety assessment of polycyclic aromatic hydrocarbons (PAHs) in cold smoked fish (*Mugil Cephalus*) using GC-MS, benzo(a)pyrene was not detected in farm A and B, concentration of fluorene was 6.6 µg/kg in farm A and was not detected in farm B, the concentration of benzo(a)pyrene in this work was 0.08770 µg/kg and the concentration of fluorene is 0.33243µg/kg.

European Union (EU) suggested that taking into your body each day the following amounts of individual PAHs: 0.3 mg of anthracene, 0.06 mg of acenaphthene, 0.04 mg of fluoranthene, 0.04 mg of fluorene, and 0.03 mg of pyrene per kilogram (kg) of your body weight (one kilogram is equal to 2.2 pounds) is not likely to cause any harmful health effects [11]. The concentration of acenaphthene, fluoranthene, fluorene and pyrene detected in the smoked fish sample are0.00003425 mg/kg, 0.0007045 mg/kg, 0.00003324 mg/kg and0.0003324mg/kg respectively, which implies that one must eat more than 1000kg of the smokedfish sample to reach theEU suggested concentration of acenaphthene and fluorene intake per day,and 100kg of the smoked fish sample to reach the EUsuggested concentration offluoranthene and pyrene intake per day which is impossible.The maximum permissible level of the sum of PAH4 (Chrysene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(a)pyrene) in smoked fishery products is 0.03 mg/kg [28], the concentration of PAH4 in this research work is 0.0002822 mg/kg (0.2822µg/kg) which is below the EU recommended standard.

In Brazil maximum levels for benzo[a]pyrene in smoke flavorings is 30 µg/kg [29]. The concentration of benzo(a)pyreneis 0.08770µg/kgin the smoked fish samples analyzed which is below the acceptable limit of (5 µg/kg) for smoked meats and smoked meat products specified by European Commission (EU) and Food and Agriculture Organizationof the United Nations (FAO) and World Health Organization (WHO) limit of (10 µg/kg) for smokedand barbecuedfish. This indicates that the fish is healthy for consumption and may not pose cancer and other health risk.

5. Conclusion

The concentration of some Polycyclic Aromatic Hydrocarbons (PAHs) detected in the smoked fish (*Hydrocynus forskahlii)*is low andbelow the Europian Commission andFood and Agriculture Organisation of the United Nations (FAO) and World Health Organization (WHO) accepted limit, which indicate that the smoked fish is healthy for consumption and may not pose cancer and other health risk to the people of Tombia Community Epketiama and other consumers.The PAHs detected in the smoked fish sample is pyrogenic (from smoking process).

Recommendation

1. The standard of this fish smoking process should be maintained because it imputes little concentration of PAHs.
2. Oven drying method of fish preservation is advised because it is void of pyrogenic PAHs.

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