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Seasonal variation in contamination of fish flesh of smoked and dried *Chrysichthys nigrodigitatus* (Lacépède, 1803) with polycyclic aromatic hydrocarbons in the locality of Guessabo (Ivory Coast)

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Abstract

Polycyclic aromatic hydrocarbons are organic contaminants, some of which are known to be toxic, carcinogenic and mutagenic for humans. These molecules are ubiquitous pollutants of the environment, and can contaminate foodstuffs such as fish. The aim of this study was to assess the level of polycyclic aromatic hydrocarbons contamination in the flesh of a species of freshwater fish, *Chrysichthys nigrodigitatus*, smoked and dried in the locality of Guessabo. The method used for the determination of these analytes in the

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matrices was high performance liquid chromatography with UV/Visible detector.

Results obtained indicate disproportionate contamination of Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(a)anthracene and Chrysene molecules depending on the mode of transformation and the season. The highest levels of contamination were recorded during the rainy season in the flesh of *Chrysichthys nigrodigitatus* smoked for Benzo(a)pyrene (10.39 ± 1.10 µg/kg), Benzo(b)fluoranthene (46.44 ± 3.77 µg/kg) and the sum of the PAH_4 (59.88 µg/kg). These levels exceed the standard set by Ivorian regulations (5 µg/kg). In dried fish flesh, a low level of toxicity was recorded whatever the season. There were no significant differences between seasons for most of the molecules studied. The flesh of *Chrysichthys nigrodigitatus* dried is less contaminated than smoked *Chrysichthys nigrodigitatus.*

Keywords: Smoked fish, Dried fish, PAHs contamination, Guessabo, Ivory Coast

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are persistent organic pollutants resulting from the incomplete combustion of organic matter (gas heating, road traffic, industry, food, etc.) (Chahin, 2010; Baghdadi *et al*. 2012). These particles can accumulate in the tissues of organisms such as fish, meat and be transferred through the various links in the food chain to reach humans (Baghdadi et *al*.2012). These molecules are toxic, mutagenic and carcinogenic, and can damage human health by causing lung, liver, bladder and skin tumors (Dovonou et *al*. 2023). The Fish is the most widely consumed foodstuff in urban areas, and especially in rural areas, due to its accessibility to all households (Shep et *al*.2016; Boukari. 2017). Guessabo is an area with high fishing potential, accounting for 61,63% of regional production. In this locality, fish is sold fresh, fried, smoked and dried. Most of the fish were sold along the main road during our field surveys. This exposes them to contamination by polycyclic aromatic hydrocarbon molecules from vehicle exhaust fumes. This is likely to have significant harmful effects on people's health, such as pulmonary, integumentary and ocular complications in fish smokers FAO, (2019) and the environment, even at great distances from their source (Amoussou, 2010).

Fresh fish may be contaminated at the base before undergoing the drying process. Similarly, in the smoking process, the incomplete combustion of the wood leads to deposits of various polycyclic aromatic hydrocarbons on the fish. Determining the level of contamination of smoked and dried fish is necessary to better assess the quality of these sales products. The aim of this study was to assess the level of these four molecules (Benzo(a)pyrene;

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Benzo(b)fluoranthene; Benzo(a)anthracene and Chrysene) in both smoked and dried fish sold in the region of Guessabo, using high-performance liquid chromatography with UV detector.

The aim of this contribution is to characterise fish from the Guessabo locality according to processing methods and the level of contamination of the four regulatory hydrocarbon molecules (EC Regulation n°835/2011).

2. Materials and methods

2.1. Study area

This study was carried out in the locality of Guessabo in the Haut-Sassandra region of central-western Ivory Coast Kra, (2016) between latitude 6°30-6°33 North and longitude 6°58-6°46 West (Ahon *et al.*, 2020). This locality is bordered to the north by the Domangbeu and Zoukougbeu , to the south by the Iboguhé , to the east by the Gregbeu and to the west by the Guezon . It is drained to the west by the Sassandra river and benefits from two tributaries, the Lobo and the Davo (Kra, 2016) that make it a key production and sales area for continental fish products **(Figure 1).**

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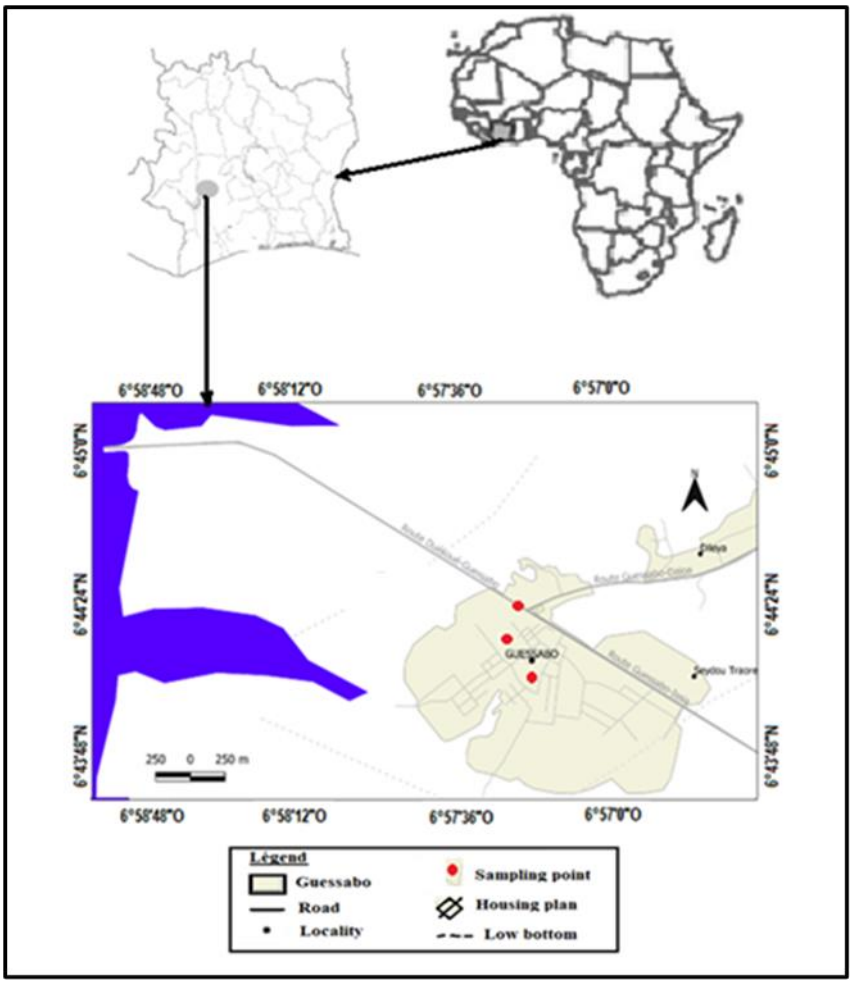

Figure 1. Geographical location of the region of Guessabo and sampling points (Miessan, 2019)

2.2. Sampling

The biological material consisted of 60 samples of smoked *Chrysichthys nigrodigitatus* and 60 samples of dried *Chrysichthys nigrodigitatus*. These samples were purchased from market women, roadside women and storage warehouses. After sampling, the samples were packaged and sent to the official laboratory (LANADA) for physical-chemical analyses. Sampling was carried out throughout the year, twice a season from January to December 2019.

PAH determination

The protocol for the determination of Polycyclic Aromatic Hydrocarbons in the smoked and dried flesh of *Chrysichthys nigrodigitatus*

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was carried out in accordance with ISO 15753-2004 and is based essentially on three analytical steps: extraction, purification and quantification.

Extraction and analysis of PAHs were performed according to ISO 15753/2004. A sample of 2.5 g of fish flesh or reference sample is introduced into a centrifuge tube and then 10 mL of acetonitrile/acetone (V/V, 60/40) are added. The whole is homogenized by vortexing 30 seconds and 5 minutes in ultrasonic bath before being centrifuged for 5 minutes at 4000 rpm. The upper phase was removed and transferred into a conical tube and the solvent is evaporated using a rotary evaporator at 35°C.The extraction was repeated twice with 10 mL of acetonitrile/acetone. The extract is then purified on the cartridges of bonded phase C18 (Waters Sep Pack). To do this, 2 mL of acetonitrile/acetone are introduced into a conical tube containing the sample. The whole was vortexed 15 seconds and centrifuged for 30 seconds. The upper phase was transferred into a tube and the operation was repeated twice. The supernatants were transferred onto a C18 cartridge previously conditioned with 12 mL of methanol and 12 mL of acetonitrile. The elution was performed with 5 mL of acetonitrile/acetone at atmospheric pressure. Then the eluate is concentrated to 50 mg using a rotary evaporator at 35°C.
The purified extract is recovered in 1 mL of hexane. The tube is crimped and stored at -18°C before analysis.

For purification on Bond Elut (C18) cartridges, the Florisil grafted phase was preconditioned with five volumes of 3ml dichloromethane and four volumes of 3ml hexane. The extract was then transferred to the Bond Elut (C18) Florisil-grafted phase cartridge, and the eluate was collected in another tared conical tube (Falcon®). To the eluate, 1ml of hexane/dichloromethane mixture (75/25; v/v) was added and the contents homogenized for 15s be The tared conical tube (Falcon®) was rinsed twice with 2ml of the hexane/dichloromethane mixture. The cartridge was also rinsed with 4ml of hexane/dichloromethane (75/25; v/v) for transferring the eluate to the Bond Elut (C18) Florisil-grafted cartridge.

The solvent was removed and evaporated to 50 µl. Finally, the eluate was weighed to determine the volume of Acetonitrile to be added to obtain 1ml of solution according to the following formula:

V_{ACN} (µl) = 1000- (m/d)

With: V_{ACN} = volume of Acetonitrile to be added
m = weighed sample mass expressed in mg;
d = density of toluene (d = 0.8669)

The eluate is then transferred to the micro-vial. The extract was analyzed using a Shimadzu High-Performance Liquid Chromatograph (HPLC), coupled to an SPD-20A UV/VIS detector.

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Estimation of results

Ivorian legislation (Arrêté 002/MIRAH/CAB/ du 06 JAN 2017) stipulates a level of 5µg/kg for each Polycyclic Aromatic Hydrocarbon molecule detected in smoked fish and a maximum level of 12µg/kg for the sum of the PAH_4. So we calculated the concentration of each molecule (C) in µg/kg, in the samples using the following formula, expressed to the nearest 0.1 µg/ kg.

$C_x = Ai \times Cir \times V / Air \times m$

C_x = Concentration of the molecule sought in the sample in µg/kg, x = molecule

Ai = Peak area (average of the two injections) of the desired molecule in the sample solution

Air = Peak area (average of the two injections) of Benzo(a) pyrene in the standard solution;

Cir = Concentration of Benzo(a) pyrene in the standard solution in µg/kg ;

V = Volume of final extract in ml; m = Sample mass in g.

Statistical analysis

The results of the analyses were processed using XLSTAT.2016 software. Means and standard deviations were processed in the same software. The ANOVA one-factor was used to compare means at the product level of each molecule, and the Student's t-test was used to determine the significant difference between means level at $p< 0,05$.

3. Results

3.1. Concentration of polycyclic aromatic hydrocarbons depending on the method of fish processing.

Figure 2 and Figure 3 show the disproportionate contamination of these four molecules depending on the processing method. Smoked products are heavily contaminated with Benzo(b)fluoranthene and Benzo(a)pyrene, at 81% and 15% respectively. The lowest rates were observed for the Benzo(a)anthracene and Chrysene molecules, at 3% and 1% respectively (Figure 2).

Figure 3 shows contamination of around 20% for most of the molecules tested and slightly high contamination of Benzo(b)fluoranthene, with a level of 33% in dried fish.

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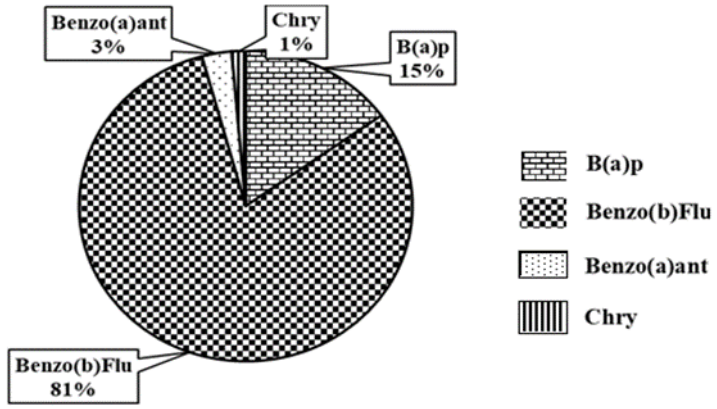

Figure 2. Proportion of PAHs in smoked Chrysichthys nigrodigitatus flesh; B(a)p: Benzo(a)pyrene; Benzo(b)Flu: Benzo(b)fluoranthene; Benzo(a)ant: Benzo(a)anthracene; Chry: Chrysene.

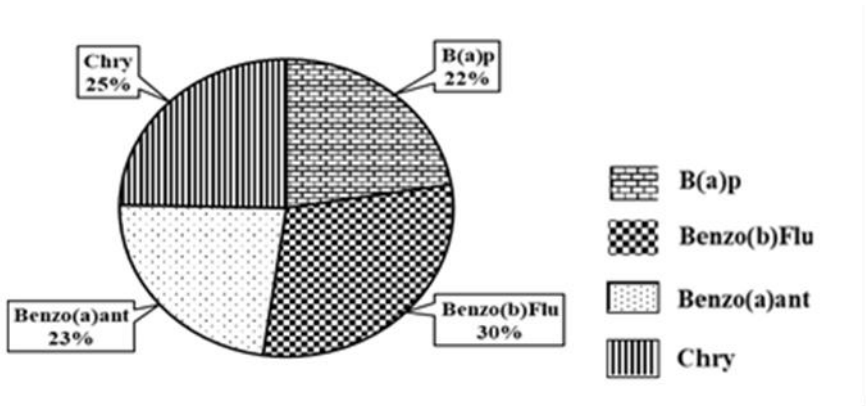

Figure 3: Proportion of PAHs in dried Chrysichthys nigrodigitatus flesh; B(a)p: Benzo(a)pyrene; Benzo(b)Flu: Benzo(b)fluoranthene; Benzo(a)ant: Benzo(a)anthracene; Chry: Chrysene

3.2. Effect of seasonal variation in the PAH_4 in the samples

On a seasonal scale, the highest contamination of PAH molecules in samples was recorded in samples of smoked *Chrysichthys nigrodigitatus*. Average Benzo(a)pyrene levels rose from 0.62 ± 0.48^a µg/kg in the flesh of dried *Chrysichthys nigrodigitatus* to 10.39 ± 1.10^a µg/kg in the flesh of smoked *Chrysichthys nigrodigitatus* during the rainy season. The average levels of Benzo(b)fluoranthene also increased from 0.99 ± 0.45^a µg/kg in the flesh of dried *Chrysichthys nigrodigitatus* to 46.44 ± 3.77^a µg/kg in the flesh of smoked *Chrysichthys nigrodigitatus*. Low levels of Benzo(a)antracene (2.19 ± 1.29^a µg/kg) and Chrysene (0.86 ± 0.49^a µg/kg) were recorded in the

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flesh of smoked *Chrysichthys nigrodigitatus* while these same molecules were also recorded in the flesh of dried *Chrysichthys nigrodigitatus* with low levels going from 0, 60 ± 0.53[a] µg/kg for Benzo(a)antracene to 0.76 ± 0.65[a] µg/kg for Chrysene during the rainy season. During the dry season, average Benzo(a)pyrene levels rose from 0.51 ± 0.43 µg/kg in the flesh of dried *Chrysichthys nigrodigitatus* to 6.38 ± 3.41[b] µg/kg in the flesh of smoked *Chrysichthys nigrodigitatus*. Average Benzo(b)fluoranthene levels ranged from 0.51 ± 0.38 µg/kg in the flesh of dried *Chrysichthys nigrodigitatus* to 44.79 ± 3.70[a] µg/kg in the flesh of smoked *Chrysichthys nigrodigitatus*. On the other hand, both products recorded low levels of Benzo(a)antracene and Chrysene respectively. In the flesh of dried *Chrysichthys nigrodigitatus*, Benzo(a)antracene contamination varied from 0.56 ± 0.46[a] µg/kg to 1.01 ± 0.89[a] µg/kg in the flesh of smoked *Chrysichthys nigrodigitatus*. The Chrysene molecule was recorded at a level of 0.48 ± 0.22[a] µg/kg in the flesh of dried *Chrysichthys nigrodigitatus* and 0.63 ± 0.5[a] µg/kg in the flesh of smoked *Chrysichthys nigrodigitatus*.

The dried *Chrysichthys nigrodigitatus* product contained only a low level of contamination of the PAH_4 regardless of the season. There is no seasonal variability in this product.

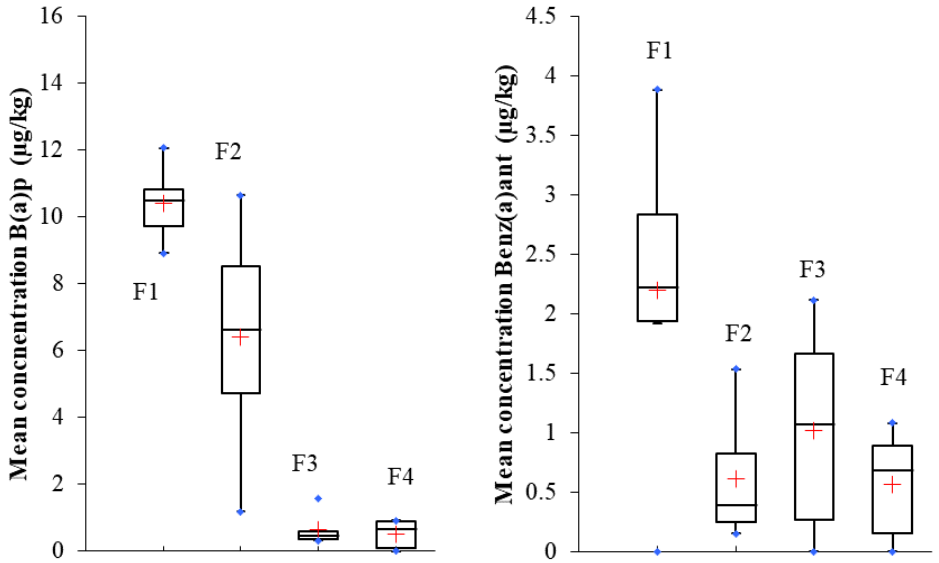

Figure 4. Seasonal variations of the PAH4 in transformed Chrysichthys nigrodigitatus fish; A: B(a)p: Benzo(a)pyrene; B: Benzo(b)Flu: Benzo(b)fluoranthene; C: Benzo(a)ant: Benzo(a)anthracene; D: Chry: Chrysene, F1: Chrysichthys nigrodigitatus smoked in the rainy season, F2: Chrysichthys nigrodigitatus smoked in the dry season, F3: Chrysichthys nigrodigitatus dried in the rainy season, F4: Chrysichthys nigrodigitatus dried in the dry season.

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3.3. Quality gradient for processed fish

Figure 5 shows the quality gradient of the concentration of the four PAHs in smoked and dried flesh *Chrysichthys nigrodigitatus*. According to Ivorian regulations (Arrêté 002/MIRAH/CAB/du 06 JAN 2017), the PAH contamination standard is set at 5 µg/kg for Benzo (a)pyrene and 12 µg/kg for the sum of the PAH_4 in processed fish. Our results showed high average levels of mainly Benzo(a)pyrene (8.38 ± 2.0 µg/kg) and Benzo(b)fluoranthene (45.62 ± 0.82 µg/kg) in the flesh of smoked *Chrysichthys nigrodigitatus* (F1). This makes the smoked *Chrysichthys nigrodigitatus* product unfit for consumption. On the other hand, the average levels of these same molecules, Benzo(a)pyrene (0.56 ± 0.05 µg/kg) and Benzo(b)fluoranthene (0.75 ± 0.23 µg/kg), in the flesh of dried *Chrysichthys nigrodigitatus* (F2) are below the set standard. Contamination levels of Benzo(a)anthracene (1.60 ± 0.59 µg/kg) and Chrysene (0.75 ± 0.11 µg/kg) in the flesh of smoked *Chrysichthys nigrodigitatus* are well below the limit set by Ivorian regulations for processed fish (5 µg/kg). The same is true of dried *Chrysichthys nigrodigitatus* flesh (F2), where the level of Benzo(a)anthracene contamination (0.58 ± 0.02 µg/kg) and Chrysene contamination (0.75 ± 0.11 µg/kg) is well below the limit set by Ivorian regulations. With regard to the sum of the PAH_4, the highest average concentration was recorded in the flesh of smoked *Chrysichthys nigrodigitatus* with an average content of 56.37 ± 15.76 µg/kg above the set standard (12 µg/kg). This makes the product unacceptable for consumption. In the flesh of dried *Chrysichthys nigrodigitatus* (F2), the sum of the PAH_4 with a content of 2.53 ± 0.06 µg/kg is well below the standard set by Ivorian regulations. The dried product is therefore of acceptable quality for consumption.

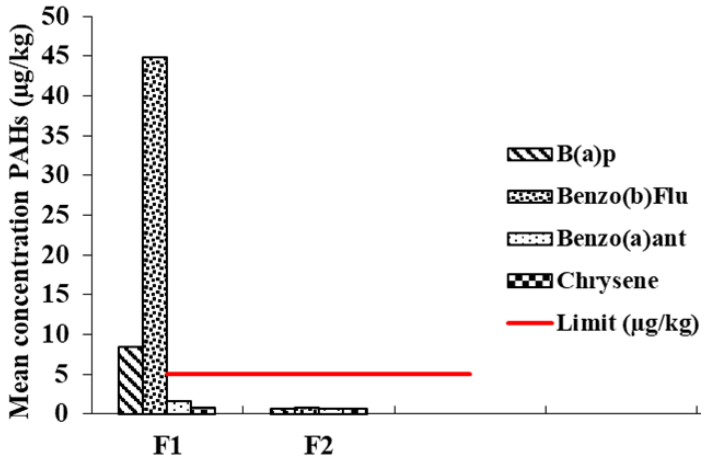

Figure 5. Quality gradient for fish processed from Chrysichthys nigrodigitatus; F1: Chrysichthys nigrodigitatus smoked , F2: Chrysichthys nigrodigitatus dried ; B(a)p: Benzo(a)pyrene, Benzo(b)Flu: Benzo(b)fluoranthene, Benzo(a)ant: Benzo(a)anthracene, Chrysene.

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Discussion

4. Discussion

The analyses revealed a high level of contamination by Benzo(b)fluoranthene and Benzo(a)pyrene, with proportions of 81% and 15% respectively in the flesh of smoked *Chrysichthys nigrodigitatus*. However, in dried *Chrysichthys nigrodigitatus* flesh, the majority of PAH molecules were around 20%, with Benzo(b)fluoranthene predominating at 33%. In addition, PAH contamination in the samples analysed was more pronounced during the rainy season, particularly in the flesh of smoked *Chrysichthys nigrodigitatus*, with an average Benzo(a)pyrene content of 10.39 ± 1.10^{a} µg/kg and 46.44 ± 3.77^{a} µg/kg for the Benzo(b)fluoranthene molecule. This high level of contamination in the wet season could be explained by the closed enclosures used, which concentrate more smoke on the products during the smoking process. This high contamination of smoked *Chrysichthys nigrodigitatus* flesh with Benzo(a)pyrene and Benzo(b)fluoranthene could be explained by the use of wood during the smoking process, exposure to the open air of the fish during smoking and especially at the side of the main road where many vehicles circulate. Also, the high Polycyclic Aromatic Hydrocarbons contamination of the products analysed during the rainy season is thought to be due to the increase in relative humidity during this season Botta et *al*. (2014 ; Ndrianaivo, 2016). During this period, the smoking time could therefore be longer, which could lead to an increase in PAH contamination during smoking and also reduce the amount of sunlight penetrating the dried fish during drying (Abdoullahi et *al*. 2018).

Smoking is the most widely used technique for preserving fish after capture in rural areas of Côte d'Ivoire. This technique is used by processors to extend the shelf life of finished products (Sene et *al*. 2010; Ndrianaivo, 2016). Unfortunately, this technique is thought to be responsible for the high Polycyclic Aromatic Hydrocarbons contamination of smoked fish through the use of fuels (Djessouho, 2015). Indeed, diesel-powered transport vehicles could deposit their exhaust fumes on these products at the time of sale. On this subject, Baghdadi et *al.* (2012) affirmed that road traffic and heating smoke are proven sources of Polycyclic Aromatic Hydrocarbons. In addition, the work of Aké Assi, (2018) revealed that foodstuffs, in particular smoked fish, could be contaminated by Polycyclic Aromatic Hydrocarbons through the environment (air, water, soil). Other sources of contamination include the processing of raw materials into smoked fish, industrial effluents, tidal currents and boat engine oils. These results are in line with those of several authors who have stated that the presence of in foods Polycyclic Aromatic Hydrocarbons depends on their source of emission. So the area of Polycyclic Aromatic Hydrocarbons pollution could influence the state of the fish (Almulsi, 2011; N'diaye, 2012; Dina, 2012).

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As with the sources of Polycyclic Aromatic Hydrocarbons contamination mentioned by some of the above authors, the studies by Dagnogo et *al*. (2022) added the origin of the fish, the fat content of the processed fish and the fuel used. The average levels of Benzo(a)pyrene contamination (8.38 ± 2.0 µg/kg) obtained in the flesh of smoked *Chrysichthys nigrodigitatus* and of this same molecule in the flesh of dried *Chrysichthys nigrodigitatus* (0.56 ± 0.05 µg/kg) are lower than those obtained by (Aké Assi, 2018). This author's work revealed a high level of Benzo(a)pyrene contamination in smoked fish, mainly in the communes of Yopougon (70.60 ± 61.29 µg/kg) and Port-Bouët, with a level of 101.64 ± 79.55 µg/kg. The studies by Traoré, (2016) and Boukari, (2017) also reported high PAH levels compared to our results in smoked fish. The average contamination level was recorded mainly for Benzo(a)pyrene (21, 29 µg/kg) and Chrysene in the fish analysed during the work by (Traoré, 2016). Similarly, the sum of the PAH_4 had a very high value of 46961.63 µg/kg dominated by Chrysene and Benzo(a)anthracene. The work of Boukari, (2017) also revealed a preponderance of average contamination of smoked and smoked/dried fish *Ethmalosa fimbriata* and *Cypselerus cyanopterus* in Benzo(a)antracene and Chrysene, which were 86.94 µg/kg and 151.39 µg/kg respectively.

Samples of barbecue fish analysed by Dagnogo et *al*. (2022) showed PAH contamination levels below our results. This author's studies showed levels ranging from 0.167 ± 0.275 µg/kg for Benzo(a)pyrene and 0.858 ± 1.681 µg/kg for Benzo(b)fluoranthene in braised mackerel, considered to be the most contaminated product. The low level of Polycyclic Aromatic Hydrocarbons contamination in dried *Chrysichthys nigrodigitatus* could be due to the low contamination of the raw material used. Our results confirm those of Boukari, (2017) who would have claimed that the source of Polycyclic Aromatic Hydrocarbons contamination in processed fish could also be due to the initial contamination of the raw material. This low level of Polycyclic Aromatic Hydrocarbons contamination in the flesh of dried *Chrysichthys nigrodigitatus*, where the average levels varied from 0.56 ± 0.05 µg/kg for Benzo(a)pyrene to 0.75 ± 0.23 µg/kg for Benzo(b)fluoranthene for can be explained by the ability of fish, during their lifetime, to reduce the level of contamination Polycyclic Aromatic Hydrocarbons in their bodies through metabolic reactions, and these Polycyclic Aromatic Hydrocarbons are then excreted through the bile and urine, according to the work of (Ndadani et *al*. 2016). The smoking process would therefore be the main source of the high Polycyclic Aromatic Hydrocarbons contamination in the flesh of smoked *Chrysichthys nigrodigitatus*. Our results are in agreement with some authors who have made the same observations.

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Conclusion

This study has shown that both smoked and dried fish are contaminated with PAHs. Smoked *Chrysichthys nigrodigitatus* are the most contaminated through the smoking process. Benzo(a)pyrene and Benzo(b)fluoranthene molecules predominate in the flesh of smoked *Chrysichthys nigrodigitatus*, making it non-compliant with European regulations (EC N°1881/2006) and Ivory Coast (Arrêté 002/MIRAH/CAB/du 06 JAN 2017) stipulating for 5 µg/kg for B(a)P and 12 µg/kg for the sum of the PAH_4).

In view of the results of the study and with a view to reducing the level of polycyclic aromatic hydrocarbons in fish flesh. It is imperative that women smokers are made aware of the rules of hygiene and manufacturing practices. This would mean using the red wood *Chlorophora excelsa*, coconut flakes and chips or charcoal during the smoking process. Similarly, a policy of increasing the number of improved traditional ovens (FTT-thiaroye ovens) should be considered, making them accessible to all processors. Smoked and dried fish should be protected from microbial and chemical contamination when sold.

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Data Availability: All data are included in the content of the paper.

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Animal Studies: This research was approved by the Chemistry Department Review Board and the principles of the Helsinki Declaration were followed.

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