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Levels of Polycyclic Aromatic Hydrocarbons in European Hake Fish (*Merluccius merluccius*) smoked by Mangrove Wood and Timber Wood

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ABSTRACT: Wood is a classic choice for smoking meat and fish because it has a strong, smoky flavour that can add depth and richness to the meat or fish if smoked sparingly, however, smoking fish could transfer such a strong flavour that can easily overpower the delicate taste of fish and also impact the fish with various volatile organic substances. Hence, the objective of this paper was to evaluate the levels of polycyclic aromatic hydrocarbons in European hake fish (*Merluccius merluccius*) smoked by mangrove wood and timber wood using Gas Chromatography Mass Spectrometry. The results of our study showed that Hake fish smoked with timber wood and Mangrove wood contains naphthalene, acenaphthylene, acenaphthene, fluorine, Phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, benzo(b) fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, dibenzo (a,h)anthracene, indeno (1,2,3-cd) pyrene and benzo(ghi)pyrene While there were variations in the levels of PAHs in hake fish smoked with different types of wood, many of these differences were not statistically significant. However, some specific PAHs showed significant variations, which could have implications for food safety and health considerations. Overall, the PAHs levels in fish smoked with timber wood (4.77±4.18) were significantly higher than those of Mangrove wood (3.515±0.64). The study shows that using both timber and mangrove wood for smoking hake fish may lead to higher levels of Carcinogenic PAHs compared to non-carcinogenic PAHs, raising concerns about the health implications for consumers. The study indicated that the levels of Carcinogenic PAHs exceeded permissible limits, emphasizing the potential health risks associated with consuming smoked hake fish. While more research is needed in this area, these findings provide a basis for making informed decisions that can reduce the health risks associated with the consumption of smoked fish products.

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Polycyclic Aromatic Hydrocarbons (PAHs) represent a class of organic compounds composed of fused benzene rings, characterized by their environmental persistence, lipophilicity, and carcinogenic potential (Ravindra *et al*; 2008). PAHs are widely distributed in various environmental matrices, including air, water, and sediments, and are mainly produced through incomplete combustion processes such as fossil fuel combustion, industrial activities, and biomass burning (Kanchanatip *et al*; 2017). One of the critical pathways for human exposure to PAHs is through the consumption of smoked food products. Smoked fish, a popular delicacy in many cultures, has gained considerable attention as a potential source of PAH contamination (Yazdani *et al*; 2020). PAHs can be formed during the smoking process as the combustion of wood generates heat and smoke, and volatile PAHs may condense onto the fish surface (Kruszewski *et al*;

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2019). Mangrove wood has been traditionally used for smoking fish due to its high calorific value and unique aromatic compounds, enhancing the flavor and taste of the smoked product (Pant *et al*; 2018). However, there is limited research on the potential impact of using mangrove wood for smoking fish in terms of PAH contamination and the associated health risks. Therefore, investigating the levels of PAHs in smoked fish and understanding the potential health implications for consumers is of utmost importance. The study seeks to assess the levels of PAHs in smoked Hake fish, a widely consumed species in many coastal regions, using timber and mangrove wood as the smoking material. Hence, the objective of this paper was to evaluate the levels of polycyclic aromatic hydrocarbons in European hake fish (Merluccius merluccius) smoked by mangrove wood and timber wood.

MATERIALS AND METHODS

Experimental Animals: The fish species used for this experimental study was Hake fish (*Merluccius merluccius*), a species of small pelagic fish that belongs to the *Clupeidae* family. They were bought from Road side vendors, which lies at latitude 4.786687^{o} and longitude 6.988625^{o,} at Mile 2 Diobu, Port Harcourt.

Smoking Process: The weight and length of the hake fish were measured and smoked using traditional method. Three hake fish were smoked using Timber wood and three hake fish were smoked using Mangrove wood. After smoking, they were transported in polythene bag to the Research laboratory of Ignatius Ajuru University, Rivers State for Analysis.

Determination of Polycyclic Aromatic Hydrocarbons: GC-MS analyses of the extracts was conducted using the selected ion monitoring mode (SIM) on a Shimadzu Model 2010 GC-MS (Shimadzu, Japan) installed with an HP-5MS fused silica column (30 m 0.25 mm i.e; 0.25 µm film thickness). Ultra-pure Helium gas at 1 mL/min flow rate was used as the carrier gas. The mass spectrometer was operated in the electron impact mode at 70 eV. Sample extracts were injected with the aid of an auto sampler in the split-less/split mode with a split time of 1 min after each injection, and the injector temperature was programmed from 100 to 280^{o}C at 200^{o}C/minutes. The oven temperature program started at 60^{o}C, ramped to 200^{o}C at 5^{o}C/minutes, then to 250^{o}C at 2^{o}C/minutes, and 280^{o}C at 10^{o}C/minutes (held for 20 minutes), and finally increased to 290^{o}C at 10^{o}C/minutes (held for another 5 minutes). Analyte identification was based on the retention times and fragmented ion profiles of the reference standards matched with the target analytes. Quantification was done with the conventional internal calibration method based on five-point calibration for individual compounds.

Data Analysis: Data for this study were analyzed using SPSS (Statistical Package for Social Science) version 23. Values were expressed as Mean ±SD, with p-values less than or equal to 0.05 being considered statistically significant.

RESULTS AND DISCUSSION

Polycyclic Aromatic Hydrocarbons (PAHs) Content in Hake Fish Smoked with Timber and Mangrove Wood: Three Hake fish were smoked using Timber wood and three Hake fish were smoked using Mangrove wood after which the PAHs levels were analyzed. Table 1 shows PAHs Content of Hake Fish Smoked with Timber and Mangrove Wood.

Table 1: Result Showing the PAHs Content of Hake Fish Smoked with Timber and Mangrove Wood

Parameter	Mangrove Wood(ug/kg)	Timber Wood(ug/kg)	p-value	Inference
Naphthalene	1.114±0.86	1.568±1.514	0.37	NS
Acenaphthylene	14.31±2.24	10.08±4.61	0.20	NS
Acenaphthene	2.22±2.07	2.03±0.5	0.46	NS
Fluorene	2.06±0.74	4.88±5.49	0.88	NS
Phenanthrene	2.98±3.58	4.23±3.53	0.94	NS
Anthracene	1.34±0.25	1.87±0.99	0.05	S
Fluoranthene	1.01±0.89	0.59±0.59	0.4	NS
Pyrene	0.44±0.68	1.29±1.18	0.45	NS
Benzo(a)anthracene	0.35±0.21	0.19±0.16	0.71	NS
Chrysene	0.417±0.25	0.98±1.18	0.05	S
Benzo(b)fluoranthene	1.45±0.65	3.97±4.89	0.05	S
Benzo(k)fluoranthene	1.49±0.7	3.54±5.25	0.28	NS
Benzo(a)pyrene	2.48±0.0.39	2.28±0.44	0.58	NS
Dibenzo(a,h)anthracene	9.98±4.66	8.46±3.59	0.05	S
Indeno(1,2,3-cd) pyrene	10.09±8.34	18.32±21.54	0.05	S
Benzo(ghi)pyrylene	0.96±0.78	2.1±2.67	0.54	NS
Total mean PAHs	3.515±0.64	4.77±4.18	0.01	S

Data are expressed as Mean ± SD of triplicates. Values are statistically significant when p≤0.05.
Key: *NS: Non-significant; S: Significant*

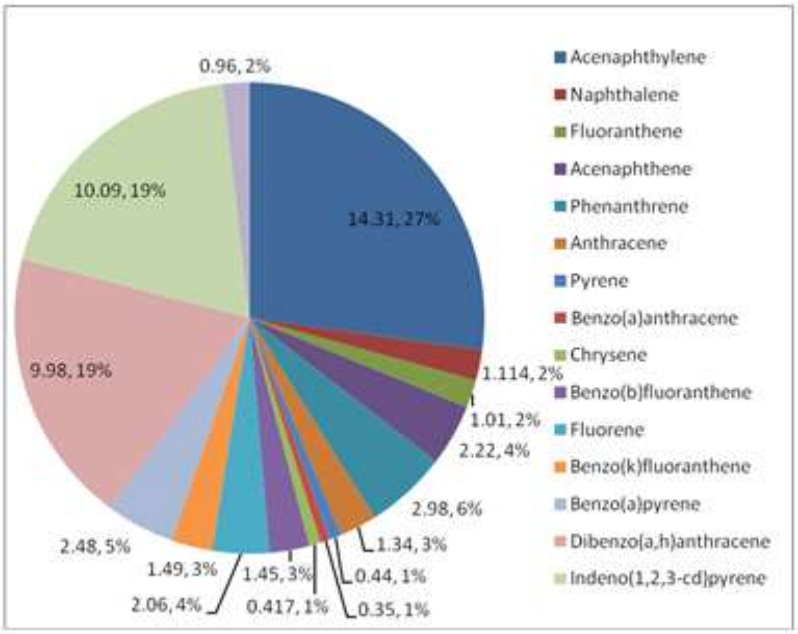

Fig 1: A Pie Chart Showing the Percentage composition of Polycyclic Aromatic Carbons in Hake Fish (*Merluccius merluccius*) Smoked with Mangrove Wood

Among the various types of PAHs in hake fish smoked with Mangrove wood, Acenapthylene (14.31 ±2.24) had the highest concentration while Benzo(a)anthracene has the lowest amount (0.35± 0.21). Among the various types of PAHs in hake fish smoked with Timber wood, Indeno (1, 2, 3-cd) pyrene has the lowest amount while Benzo(a)anthracene has the lowest amount (0.19± 0.164ug/kg). Overall, the PAHs levels in fish smoked with timber wood (4.77±4.18) were higher than those of Mangrove wood (3.515±0.64).

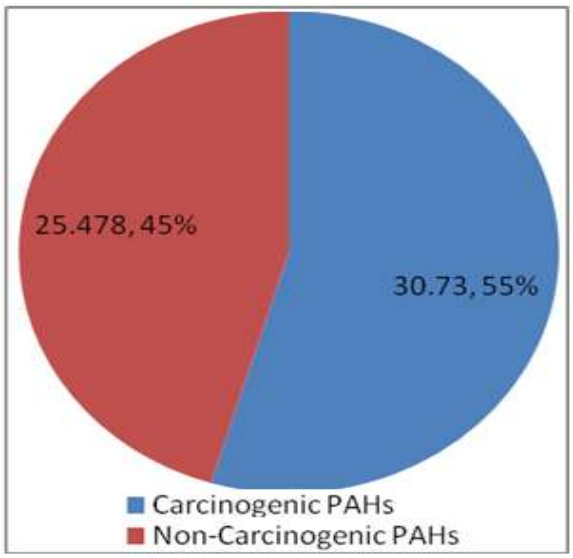

Fig 2: A Pie Chart Showing the Percentage Composition of Carcinogenic and Non-Carcinogenic Polycyclic Aromatic Carbons in Hake Fish (*Merluccius merluccius*) Smoked with Mangrove Wood

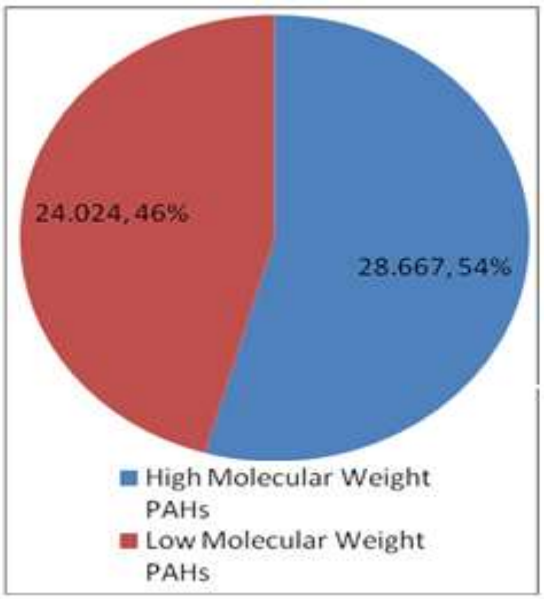

Fig 3: A Pie Chart Showing the Percentage Composition of High Molecular and Low Molecular Polycyclic Aromatic Carbons in Hake Fish (*Merluccius merluccius*) Smoked with Mangrove Wood.

The levels of naphthalene were found to be higher in hake fish smoked with timber wood (1.568 µg/kg) compared to mangrove wood (1.114 µg/kg), but the difference was not statistically significant (p=0.37). These results were higher than those of Emmanuel *et al*. (2021), whose results showed naphthalene levels to be 0.04 µg/kg. Acenaphthylene content was slightly higher in fish smoked with mangrove wood (14.31 µg/kg) compared to timber wood (10.08 µg/kg), with no significant difference observed (p=0.20). This is

not consistent with the research results of Emmanuel *et al*. (2021), whose results showed Acenaphthylene levels in smoked hake fish to be 0.01 µg/kg. Both wood types resulted in similar Acenaphthene levels, with no statistically significant difference (p=0.46).

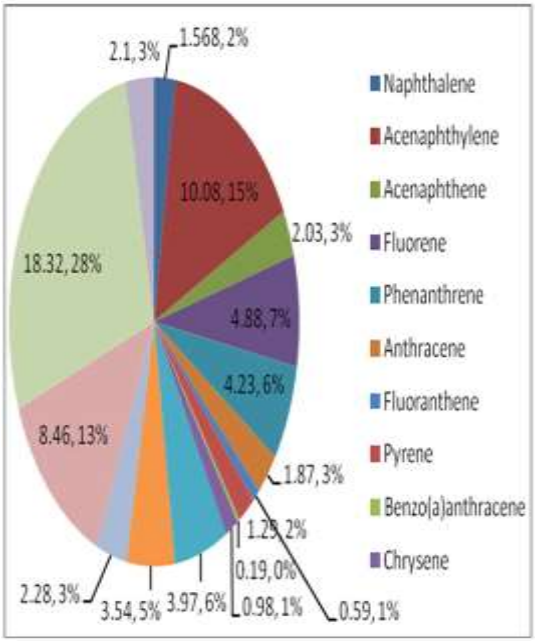

Fig 4: A Pie Chart Showing the Percentage composition of Polycyclic Aromatic Carbons in Hake Fish (*Merluccius merluccius*) Smoked with Timber Wood

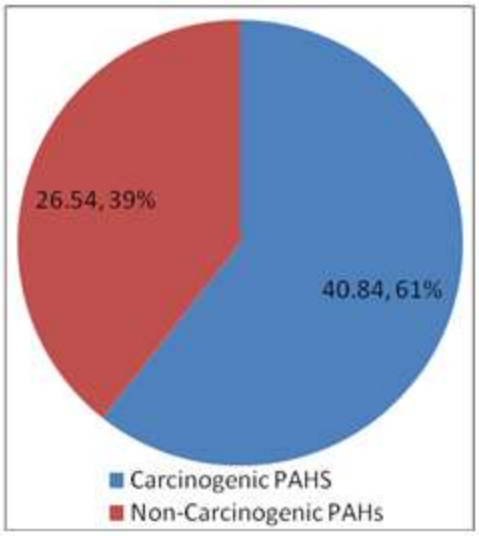

Fig 5: A Pie Chart Showing the Percentage Composition of Carcinogenic and Non-Carcinogenic Polycyclic Aromatic Carbons in Hake Fish (*Merluccius merluccius*) Smoked with Timber Wood

The concentrations were 2.22 µg/kg for mangrove wood and 2.03 µg/kg for timber wood. However, Emmanuel *et al*. (2021) recorded Acenaphthene levels of 0.01 µg/kg. Fish smoked with timber wood showed higher levels of Fluorene (4.88 µg/kg) compared to mangrove wood (2.06 µg/kg), but the difference was not statistically significant (p=0.88). However, the results of Emmanuel *et al*. (2021) recorded Fluorene levels to be 0.05 µg/kg. In the results, Phenanthrene concentrations were similar for both wood types, and the difference was not statistically significant (p=0.94). Mangrove wood had 2.98 µg/kg, while timber wood had 4.23 µg/kg. This was quite higher than the results obtained by Emmanuel *et al*. (2021).

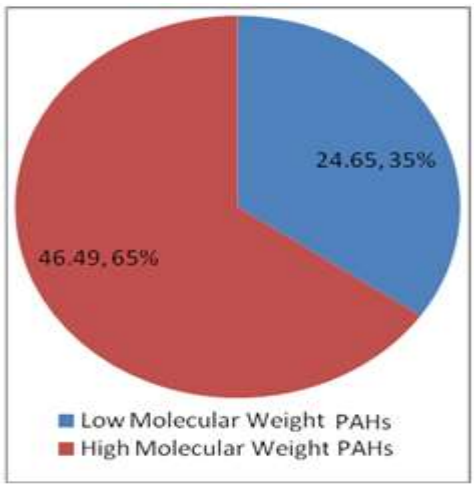

Fig 6: A Pie Chart Showing the Percentage Composition of High Molecular and Low Molecular Polycyclic Aromatic Carbons in Hake Fish (*Merluccius merluccius*) Smoked with Timber Wood

In their research, they obtained Phenanthrene concentration of 0.088 µg/kg. The Anthracene levels were significantly higher in Hake fish smoked with timber wood (1.87 µg/kg) compared to mangrove wood (1.34 µg/kg) (p=0.05). There was no significant difference in Fluoranthene levels between the two wood types (p=0.4). The values were 1.01 µg/kg for mangrove wood and 0.59 µg/kg for timber wood. However, in the research by Emmanuel *et al*. (2021), the levels of Anthracene in smoked Hake fish were 0.012 µg/kg.

Both wood types yielded similar Pyrene concentrations, with no significant difference (p=0.45). Mangrove wood had 0.44 µg/kg, while timber wood had 1.29 µg/kg. In the results by Emmanuel *et al*. (2021), Pyrene levels were 0.966 µg/kg, which was higher than the results obtained in our study for Hake fish smoked with Mangrove wood and lower than that of timber wood. There was no significant difference in Benzo(a)Anthracene levels between the two wood types (p=0.71). The values were 0.35 µg/kg for mangrove wood and 0.19 µg/kg for timber wood. However, these results were higher than that of Emmanuel *et al*. (2021), who obtained levels of 0.05 µg/kg in smoked Hake fish. Chrysene levels were significantly higher in fish smoked with

timber wood (0.98 µg/kg) compared to mangrove wood (0.417 µg/kg) (p=0.05). These were higher than the results obtained by Emmanuel *et al*. (2021). In their results, the value of Chrysene in smoked Hake fish was 0.22 µg/kg. Similarly, Benzo(b)fluoranthene concentrations were significantly higher in fish smoked with timber wood (3.97 µg/kg) compared to mangrove wood (1.45 µg/kg) (p=0.05). However, these results were higher than the results obtained by Emmanuel *et al*. (2021), whose results showed fluoranthene levels to be 0.179 µg/kg. No significant difference was observed in Benzo(k)fluoranthene levels between the two wood types (p=0.28). The concentrations were 1.49 µg/kg for mangrove wood and 3.54 µg/kg for timber wood. The levels of Benzo(a)pyrene did not significantly differ between mangrove wood (2.48 µg/kg) and timber wood (2.28 µg/kg) (p=0.58). However, the results obtained by Emmanuel *et al*. (2021) were 0.096 µg/kg. The levels of Dibenzo (a, h) anthracene were similar between the two wood types, with significant difference (p=0.05). Mangrove wood had 9.98 µg/kg, while timber wood had 8.46 µg/kg. However, these were quite higher than the results obtained by Emmanuel *et al*. (2021). In their results, they obtained levels of 0.162 µg/kg.

Fish smoked with timber wood had significantly higher indeno (1,2,3-cd) pyrene levels (18.32 µg/kg) compared to mangrove wood (10.09 µg/kg) (p=0.05). There was no significant difference in benzo(ghi)pyrene levels between the two wood types (p=0.54). The values were 0.96 µg/kg for mangrove wood and 2.1 µg/kg for timber wood. However, these results were higher than that of Emmanuel *et al*. (2021). In their results, they obtained levels of 0.001 µg/kg. These variations between the levels of PAHs obtained in our study and those of Emmanuel *et al*. (2021) may be due to the duration and type of wood used for smoking. Overall, the mean PAHs levels in fish smoked with timber wood (4.77±4.18) were significantly higher than those of Mangrove wood (3.515±0.64), this is comparatively lower than that of Nworu *et al;* (2019) who recorded 313.43±0.67mg/kg in the muscle of Tilapia fish. According to Ingenbleek *et al*. (2019), the maximum permissible limit for PAH4 set by the European Union is 12 µg/kg. From our study, the levels of PAH4 in timber wood and Mangrove wood were 4.7 µg/kg and 7.42 µg/kg, respectively, which were much lower than the maximum permissible limit. Also, Tumerkan & Elif (2022) stated that the maximum permissible limit of benzo (a) pyrene is 2 µg/kg. However, our results showed that the levels of benzo(a)pyrene in both hake fish smoked with Timber wood and Mangrove wood (2.48±0.39 µg/kg) and (2.28±0.44 µg/kg) were higher than the maximum permissible limit. Tongo *et al*. (2018) stated that benzo(a)pyrene is carcinogenic to humans. Thus, using Mangrove and Timber wood for smoking hake fish is unsafe for humans based on the results of our study. The levels of Carcinogenic PAHs in hake fish smoked with Mangrove wood were 30.734 µg/kg, while those of non-carcinogenic PAHs were 25.477 µg/kg. The percentage of Carcinogenic PAHs was 55%, while that of non-carcinogenic PAHs was 45%. The levels of Carcinogenic PAHs in hake fish smoked with Timber wood were 40.84 µg/kg, while those of non-carcinogenic PAHs were 26.54 µg/kg. Also, the percentage of Carcinogenic PAHs was 61%, while that of non-carcinogenic PAHs was 39%. According to Tongo *et al.* (2018), Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene are non-carcinogenic, while Benzo (a)anthracene, Chrysene, Benzo (k) fluoranthene, Benzo (a)pyrene, Benzo (b) fluoranthene, Indeno (1,2,3) pyrene, Dibenzo (a,h) anthracene, and Benzo(g,h,i,) perylene.

These results show that the levels of Carcinogenic PAHs in Hake fish smoked with both timber and Mangrove wood are higher than those of non-carcinogenic PAHs, which raises a serious health concern for the consumers of smoked hake fish. The levels of High molecular weight PAHs in hake fish smoked with Mangrove wood were 28.667 µg/kg, while those of Low molecular weight were 24.02 µg/kg. The percentage of High molecular weight PAHs in hake fish smoked with Mangrove wood was 54%, while that of Low molecular weight PAHs was 46%. Also, the percentage of High molecular weight PAHs in hake fish smoked with Timber wood was 65%, while that of Low molecular weight PAHs was 35%.

Conclusion: The research study on the polycyclic aromatic hydrocarbons (PAHs) content in hake fish smoked with mangrove and timber wood provides valuable insights into the safety and quality of smoked fish products. This finding underscores the importance of wood selection in food preparation The light of these findings can serve as a foundation for further investigations into the health implications of consuming smoked fish products and the development of guidelines for safe smoking practices, processes, as well as its potential health implications for consumers.

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