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Influence of red mangrove (*Rhizophora mangle*) wood age on polycyclic aromatic hydrocarbons (PAHs) and heavy metal accumulation in smoked atlantic chub mackerel (*Scomber colias*)

Kwadwo Boakye Boadu^{a,*} , Sylvia Bordoh^a, Michael Ayim^a, Caleb Arku^a, Ofori Attah Nkansah^a , Rogerson Anokye^a, Michael Ansong^b , Kwasi Adu Obirikorang^c , Richeal Akuorkor Turkson^a

^a Department of Wood Science and Technology, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

^b Department of Silviculture and Forest Management, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

^c Department of Fisheries and Watershed Management, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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ABSTRACT

Smoking is a widely used method of fish preservation that can enhance flavour but may also introduce harmful contaminants, including polycyclic aromatic hydrocarbons (PAHs) and heavy metals. There is limited research on how the age of red mangrove (*Rhizophora mangle*) wood drives contaminant depositions in smoked fish products. Our study assessed the types and concentrations of polycyclic aromatic hydrocarbons (PAHs), carcinogenic risks related to PAH exposure, and cadmium, lead, and arsenic levels in Atlantic chub mackerel smoked with red mangrove wood aged 8, 11, and 14 years. Eighteen PAH congeners were identified, with four of them classified as probable human carcinogens by the US Environmental Protection Agency. Although Benzo[a]pyrene (B[a]P) concentrations were within the EU limits (1.0 µg/kg), the PAH4 Index exceeded the 12.0 µg/kg limit in fish smoked with 14-year-old wood (12.5 µg/kg), which raises concerns about carcinogenic exposure. The cadmium and lead levels exceeded the EU safety thresholds in all fish samples: the highest contamination was recorded in the fish smoked with older wood. To ensure the safety of smoked fish products, the use of older wood for fish smoking must be restricted, along with the adoption of indirect smoking methods and enforcement of routine food safety monitoring.

Introduction

Fish is an important source of affordable minerals, animal protein and essential fatty acids for many people. Over the past several decades, global fish consumption has exhibited a consistent upward trend, with per capita annual intake increasing from approximately 9.0 kg in the 1960s to around 20 kg in recent years [1]. Fish is an integral part of the diets of many tropical countries like Ghana

* Corresponding author.

E-mail address: kboadu.canr@knust.edu.gh (K.B. Boadu).

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where it plays a vital role in meeting the country's per capita fish consumption of 28 kg year⁻¹ [1,2]. Smoked fish is a staple in many tropical countries, serving as both a vital source of animal protein and a traditional method of fish preservation in regions with limited access to refrigeration. In West Africa, for instance, smoked fish contributes significantly to household food security and income generation, especially among coastal and inland fishing communities [1]. The process enhances shelf life by reducing moisture content and imparts desirable sensory qualities such as flavour and texture, making it a preferred form of fish in local markets [3]. Countries such as Ghana, Nigeria, and Cameroon rely heavily on smoking as a post-harvest processing technique, with red mangrove (*Rhizophora* spp.) and other hardwoods frequently used as fuel due to their availability and high calorific value [4]. Despite its benefits, traditional smoking methods can introduce contaminants such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, raising public health concerns over the safety of smoked fish products [5]. As consumption remains high and demand continues to grow, especially in urban areas, there is an increasing need to assess and improve the safety and quality of smoked fish in tropical regions.

Fish smoking involves incomplete combustion of wood, which can lead to the accumulation of heavy metals and PAHs in fish [5]. PAHs are a class of highly carcinogenic and mutagenic compounds, which are known as eco-toxicants. Heavy metals such as arsenic, cadmium and lead are toxic even at low concentrations and pose significant health risks such as organ damage and carcinogenesis [6]. The European Union (EU) and other regulatory bodies have proposed safety thresholds for heavy metals and PAHs in smoked fish, with a Maximum Allowable Limit (ML) of 2.0 µg/kg for benzo[a]pyrene and 12.0 µg/kg for the PAH4 index [7]. Several studies have documented the accumulation of PAHs and heavy metals in smoked fish products, raising significant concerns regarding food safety and public health. Adeyeye and Ashaolu [8] reported elevated levels of PAHs and heavy metals, including lead and cadmium, in smoked catfish from southwestern Nigeria, with concentrations exceeding international safety limits, thus posing potential carcinogenic and toxicological risks to consumers. Similarly, Aksun Tümerkan [9] identified substantial PAH and elemental contamination in various smoked fish species, emphasizing the influence of smoking techniques on contaminant levels. Zachara et al. [10] found that a significant proportion of smoked meat and fish products in the Polish market contained PAH concentrations above permissible levels. Adeyeye et al. [11] compared traditional drum and kiln smoking methods, revealing that both techniques contributed to the accumulation of PAHs and heavy metals in the bonga shad (*Ethmalosa fimbriata*) and the Senegalese tonguesole (*Cynoglossus senegalensis*). Likewise, Wretling et al. [12] also found high levels of PAHs exceeding recommended limits in commercially smoked fish products in Egypt.

The type of wood species used in fish smoking plays a crucial role in determining the levels of polycyclic aromatic hydrocarbons (PAHs) and heavy metals that accumulate in the final product. Different wood species vary in their chemical composition, density, moisture content, and combustion properties, all of which influence the quantity and type of smoke generated during the smoking process. Hardwoods, particularly those rich in lignin and low in resin content, tend to produce higher levels of PAHs when burned at incomplete combustion temperatures [13]. The red mangrove for instance has widespread use in many countries for fish smoking as a result of its slow burning characteristics and perceived unique flavouring effects on the fish [3], but studies have shown smoking fish with the red mangrove can lead to significant PAH and metal contaminations depending on the age and condition of the wood [14,3]. Additionally, some wood types naturally contain trace amounts of heavy metals, which can be transferred to smoked fish through deposition of ash or particulate matter during smoking [15]. Wood age affects its chemical composition such as the percentage of lignin, cellulose and hemicellulose [16], which could influence the release of PAHs and heavy metals during combustion. Although red mangrove is extensively used in fish smoking, research on how the age of red mangrove wood affects the levels of PAHs and heavy metals in smoked fish is scanty. This creates a gap in both scientific understanding and regulatory practices which warrants investigations.

This study, thus, sought to evaluate the types and concentrations of PAHs and heavy metals (arsenic, cadmium and lead) in Atlantic chub mackerel smoked with red mangrove wood aged 8, 11, and 14 years. The study analyzes contaminant levels and compared them to EU regulatory limits to assess the health risks associated with the consumption of smoked fish. The findings will provide rich insights for policymakers and stakeholders in the fish smoking industry about safer wood choices for reducing contamination.

Materials and methods

Collection and chemical characterization of red mangrove wood

The stems of red mangrove wood of varying ages (8-, 11-, and 14-year-olds) were obtained from Salo market in Keta in the Volta Region of Ghana. The area experiences a warm and tropical climate with temperatures ranging from a minimum of 24.7 °C to a maximum of 32.7 °C [17]. The wood samples were air-dried to 16 % MC, and their chemical characteristics, including the cellulose, hemicellulose, lignin, and extractive contents, were analyzed using the ASTM methods (ASTM D 1103–60, ASTM D 1104–96, ASTM D 1105–96, and ASTM D 1106–96) and methods described by Boadu and Sackey [18], Boadu et al. [19], and Boadu et al. [20]. The selection of 8-, 11-, and 14-year-old red mangrove wood for the current study was based on the fact that traditional fish smokers prefer wood within this age range due to its perceived optimal burning characteristics. Also, as a common practice in mangrove plantation management in Ghana, harvesting of the wood typically begins after 8 years of growth, which makes these age classes both culturally relevant and practically representative of what prevails in the fish smoking industry.

Collection, processing and smoking of Atlantic Mackerel

Twenty-four (24) samples of fresh Atlantic Mackerel (*Scomber colias*) with an average mass of 300 g were purchased from a fish sales outlet in Kumasi, Ghana (latitude: 6°40'27.92" N; longitude: 1°33'24.14" W). Eighteen (18) of them were prepared for smoking

while six (6) were not smoked and served as the control. The fish was thoroughly rinsed to ensure total removal of dirt and to aid preservation. The fish samples were smoked using a pre-heated traditional oven built with a fitted mesh rack (mesh size: 4" x 4") at the demonstration farm of the Department of Fisheries and Watershed Management at the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi-Ghana. The air-dried red mangrove woods of different ages were used in the smoking process. Six fish samples were smoked using the 8-year-old red mangrove wood, another six were smoked using 11-year-old red mangrove wood, and the last batch of six fish samples were smoked using the 14-year-old red mangrove wood. The fish samples were arranged on the mesh rack in single layers with spaces between them to allow the smoke to circulate. The smoking process took about 40 min for each set. After the initial 15 min of equilibration, a digital thermometer was used to record the temperatures of the flames and the oven at four separate sites for every five minutes of smoking [13]. In line with the suggestions by Nti et al. [21], the oven temperature recorded ranged between 36.2 °C and 58.6 °C. The average temperatures recorded across the three wood age treatments were comparable and not statistically different ($p > 0.05$) throughout the 40-minute smoking period. Once the smoking process was over, the fish samples were removed from the mesh and allowed to cool. Samples were individually wrapped in sheets of labeled aluminum foil to avoid cross-contamination. Whole smoked fish samples of each wood type were collected after the smoking period, composited, descaled, and homogenized according to wood type for further preparation and extraction prior to analysis using the gas chromatography/mass spectrometry (GC/MS) method. The average sizes of the smoke-cured fish were measured. The homogenized fish samples were kept in amber bottles and refrigerated at temperatures below 4 °C before analysis. The smoked and unsmoked fish samples were analyzed at the pesticide laboratory at the Ghana Standards Authority (GSA) for Polycyclic Aromatic Hydrocarbons (PAHs) and at the laboratory of the Faculty of Agriculture (KNUST) for heavy metal accumulation.

PAHs extraction and extract purification

The analysis of the PAHs was done following the standard methods described in Essumang et al. [5]. All PAH analyses were done at the laboratory of the Ghana Standards Authority. A Soxhlet extraction apparatus, which consisted of a 500 mL round-bottom flask, an extraction chamber, a condenser, and water circulators, was placed on heating mantles with a temperature control mechanism. For each of the extractions, 5 g of the smoked fish powder was homogenized together with 10 g of Na₂SO₄ until a completely dry mixture was obtained. The homogenized sample was then poured into an extraction thimble, and placed in the Soxhlet extractor. A methanol-KOH solution obtained by dissolving 5 g of KOH in 10 mL distilled water and diluting to 100 mL with methanol was added to the extraction chamber. The Soxhlet extraction was done using 300 mL dichloromethane (DCM) with an average solvent circulation rate of four cycles hour⁻¹ over a 24 h period. The extract was then cooled to room temperature. To separate the aqueous and organic layers, 100 mL of methanol-water mixture (2:8) was added using a separatory funnel. The organic layer was washed twice with 50 mL distilled water to remove residual stearates. The extract was concentrated to approximately 5 mL using a Rotavapor R-114 at 45 °C, then further reduced to about 1 mL under a nitrogen gas stream following USEPA Method 3540C. The concentrated extract was subjected to purification in a silica gel column composed of 10 g of activated silica gel with 1 g of anhydrous sodium sulfate on top. Both ends were sealed with glass wool. The column was pre-conditioned with 5 mL of a 1:3 dichloromethane/hexane mixture. The 1 mL extract was loaded onto the column and eluted first with 20 mL hexane to remove n-hydrocarbons. This was followed by two washes with about 20 mL of dichloromethane/hexane (1:3). Polar lipid residues were retained at the top of the column. In order to ensure analytical precision, the samples were analyzed in triplicate and the procedural blanks were included in the analysis of each batch. Calibration curves were prepared using the certified PAH standards (Supelco® EPA 610 PAH Mix). The linearity ($R^2 > 0.995$) was confirmed across the expected concentration ranges. Before the GC/MS analysis was performed, about 5 mL of a 0.5 mg/mL internal standard solution in dichloromethane was added to each extract and its triplicate, which was then reduced to 1 mL under nitrogen gas. The GC/MS system (Varian 3800 GC, Varian, Inc., Palo Alto, CA), which had an 8400 auto-sampler (mass data type: centroid), was employed for the PAH analysis. The mass spectrometer was operated in ionization mode with a scan range of 45–450 *m/z* and automatic gain control. The chromatographic conditions included the use of a DB-5MS capillary column (30 *m* × 0.25 mm, 0.25 μm film thickness), helium as the carrier gas (flow rate: 1 mL/min), injector temperature: 280 °C, initial oven temperature: 60 °C (held for 2 min), ramped at 10 °C/min to 290 °C (held for 10 min). Selected ion monitoring (SIM) was performed by comparing the base peaks of target PAHs. The NIST standard reference material (1941), which is used in evaluating analytical methods for the determination of specific PAHs, PCB congeners, and chlorinated pesticides in marine sediments and similar matrices like smoked fish powder, was used to verify the analytical precision and recovery of the PAHs. Average recovery of the PAHs from the SRM 1941 was between 81 % and 95 %, which is within acceptable analytical limits. Limits of detection (LOD) for the individual PAHs ranged between 0.05 μg/kg and 0.12 μg/kg. The Limit of Quantification (LOQ) for most PAHs was 1.00 μg/kg. As such, values below this quantification threshold were reported by the laboratory as <1.00 μg/kg, in accordance with standard reporting practices. This applied to several key carcinogenic PAHs such as Benzo[a]pyrene (B[a]P), Chrysene (CHR), and Indeno[1,2,3-cd]pyrene (IND), among others, which were detected but fell below the LOQ. Ten replicate sub-samples were analyzed from each composite homogenized fish sample (according to wood age treatment).

Quantification of PAH4 as a marker of potential carcinogenicity in smoked fish

Cancer risk due to dietary exposure to PAHs in smoked fish was assessed using the PAH4 index. The PAH4 was calculated based on the methods described by the Panel on Contaminants in the Food Chain (CONTAM), which provides scientific advice on contaminants and residues of unauthorized substances in food. The panel concluded that PAH4 is a more suitable indicator of PAHs in food (EFSA 2008). PAH4 was evaluated using the sum of four different Polycyclic Aromatic Hydrocarbons, namely Benzo[a]Anthracene (B[a]A),

Benzo[b]Fluoranthene (B[b]FL), Chrysene (Chr) and Benzo[a]Pyrene (B[a]P). The estimated PAH4 index of the fish samples smoked with different ages of red mangrove wood was then compared with the maximum allowable limit (ML) to determine the occurrence and effect of carcinogenic PAHs in the fish samples. The maximum allowable limit (ML) for the PAH4 index in smoked fishery products recommended by the European Union Commission Regulation No 1327/2014 in 2014 is 12.0 µg/kg (European Union Commission Regulation 2014). The PAH4 was calculated using the formula:

$$\text{PAH4 Index (PAH4)} = \sum \text{B[a]A} + \text{Chr} + \text{B[b]FL} + \text{B[a]P}$$

In calculating the PAH4 Index, the values of PAHs that were reported as <1.000 µg/kg were estimated as 0.500 µg/kg for summation purposes, following standard practice in environmental data analysis ([22]; USEPA 2000).

Determination of heavy metals in smoked fish samples

The Kjeldahl digestion method was used to determine the concentration of the heavy metals in the fish samples. The homogenized fish samples were first oven-dried for 24 h and milled into powder. One gram of the samples was weighed into a Kjeldahl digestion tube to test for the heavy metal content. Nitric acid and hydrochloric acid solution (ratio of 1:3) was added to the powdered sample, and the mixture was digested at 450 °C until digestion was complete, which is usually indicated by a change in the solution colour from slightly yellow to whitish. The mixture was decanted into a 100 mL volumetric flask and the solution top-off to the 100 mL mark in accordance with AOAC [23]. The unknown concentrations of this solution were then read using an atomic absorption spectrophotometer (AAS) (PerkinElmer Analyst 800 USA) for the various metals at specified wavelengths to determine the heavy metal content in the smoked fish and control samples. The calibration standards were prepared using the certified reference standards (Merck KGaA, Germany). Blank solutions were used to control for potential contamination. The recovery efficiency was verified using a spiked fish matrix, yielding average recovery rates of 85 – 102 %. LOD values were 0.01 mg/kg for cadmium, 0.02 mg/kg for lead, and 0.02 mg/kg for arsenic. Ten replicate sub-samples were analyzed from each composite homogenized fish sample (according to wood age treatment).

Data analysis

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM SPSS, Armonk, NY, USA). Prior to comparative analysis, the normality of the data for heavy metal (As, Cd, Pb) and polycyclic aromatic hydrocarbon (PAH) concentrations in smoked fish was assessed using the Kolmogorov–Smirnov test. The results indicated that the data followed a normal distribution ($p > 0.05$ for all variables), thus meeting the assumptions required for parametric testing. To evaluate the influence of mangrove wood age (8, 11, and 14 years) on the accumulation of heavy metals and PAHs in smoked fish, a one-way analysis of variance (ANOVA) was performed for each contaminant measured. The independent variable was wood age, while the dependent variables were the concentrations of individual heavy metals (As, Cd, and Pb) and selected PAH compounds. For variables that showed statistically significant differences ($p < 0.05$), the Bonferroni *post hoc* test was applied to identify specific differences among the three wood age groups. Mean concentrations and standard deviations for each contaminant were reported, and results were visualized using tables and bar charts to illustrate variation across treatments. Where PAH values were reported as <1.00 µg/kg, these values were below the analytical method's limit of quantification (LOQ). Therefore, standard deviations could not be computed for the values due to non-quantifiable replicates.

Results

Chemical characterization of red mangrove wood at different ages

The chemical constituents in red mangrove wood were significantly different among the age groups (lignin: $p = 0.010$; hemicellulose: $p = 0.002$; cellulose: $p = 0.003$; extractives: $p = 0.000$).

The lignin content increased from 28.1 % at age 8 to 36.9 % at age 14 (Table 1). Hemicellulose similarly increased slightly from 11 % at age 8 to 11.8 % at age 11 but decreased sharply to 7.8 % by age 14. Cellulose also increased from 34.5 % at age 8 to 36.0 % at age 11 and decreased to 31.6 % at age 14. The percentage of extractives after ethanol extraction was highest in the 14-year-old mangrove wood (0.8 %) and lowest in the 11-year-old (0.4 %).

Table 1
Chemical characterization of mangrove wood at different ages (means ± SD).

Chemical constituents (%)	Age of mangrove wood		
	8 years	11 years	14 years
Lignin	28.1 ± 1.2a	31.2 ± 0.9b	36.9 ± 1.8c
Cellulose	34.5 ± 0.5a	36.0 ± 1.2b	31.6 ± 0.8c
Hemicellulose	11.0 ± 0.1a	11.8 ± 0.4b	7.80 ± 0.1b
Extractives	0.70 ± 0.0a	0.41 ± 0.0b	0.84 ± 0.0a

Note: Means on the same row with the same letters are not significantly different ($p > 0.05$).

Types and concentration of PAHs in the Atlantic chub mackerel

Eighteen (18) Polycyclic Aromatic Hydrocarbon (PAH) congeners were identified and quantified at varying concentrations in all smoked samples of Atlantic Mackerel (Table 2). Among these, four, which were Benzo[a]pyrene (BAP), Chrysene (CHR), Benzo[a]Anthracene (BAA) and Benzo[b]Fluoranthene (BBF) have been classified by the United State Environmental Protection Agency (USEPA) as compounds of probable human carcinogens.

For these four compounds, a concentration of <1.00 µg/kg each was recorded for BAP and CHR in the fish samples smoked with the 8-, 11- and 14-year-old wood. The concentration of BAA varied among the wood ages, with 2.13 µg/kg recorded for fish smoked with 8-year-old wood, 2.54 µg/kg for 11-year-old wood and 6.56 µg/kg for the 14-year-old. The control samples had a much lower concentration of 0.02 µg/kg. The differences were, however, not statistically significant ($p = 7.34$). Similarly, the concentration of BBF in the smoked fish samples was <1.00 µg/kg for the 8-year-old wood, 2.52 µg/kg for 11-year-old wood, 4.94 µg/kg for 14-year-old wood and <1.00 µg/kg for the control group. Again, the differences were also not statistically significant ($p = 3.05$).

Quantification of PAH4 as a marker of potential carcinogenicity in smoked fish

The control group (unsmoked fish) had the lowest PAH4 Index (Table 2). This was followed by the fish smoked with 8-year-old red mangrove wood. The highest PAH4 Index was recorded for the fish smoked with 14-year-old red mangrove wood. Despite this, the B[a]P levels across all smoked fish samples remained at <1.00 µg/kg, which is within the acceptable limit of 2.0 µg/kg.

Concentration of heavy metals in smoked fish samples

The concentrations of cadmium (Cd), arsenic (As), and lead (Pb) were assessed in Atlantic Mackerel smoked with red mangrove wood of different ages and compared to the control (unsmoked) samples (Fig. 1). The cadmium concentration ranged from 0.4 % to 0.6 % in smoked fish with the highest concentration (0.6 %) recorded in fish smoked with the 14-year-old wood, and 11-year-old wood had the lowest (0.4 %). The control group had a significantly lower concentration of 0.1 % ($p = 0.000097$). The arsenic levels across the samples were significantly different ($p = 0.008$). Fish smoked with 11-year-old wood contained the highest arsenic concentration (0.5 %), while those smoked with the 8-year-old wood had the lowest (0.01 %). Lead concentrations were also significantly different ($p = 0.0000185$), ranging from 0.3 % to 0.4 %. The fish smoked with 11-year-old and 14-year-old wood both had 0.4 % lead, while the 8-year-old wood and the control group had 0.3 %.

Discussion

Chemical characterization of red mangrove wood at different ages

Distinct variations were observed in the extractive, hemicellulose, cellulose, and lignin content. The lignin content increased with wood age, with the highest concentration at 36.9 % in the 14-year-old. The result compares with the findings by Huang et al. [24] who

Table 2
Types and Average Concentrations (\pm SD) of PAHs quantified in smoked Atlantic Mackerel.

Types of Polycyclic aromatic hydrocarbons (PAHs)	Concentration of PAH in Atlantic mackerel fish (µg/kg)			
	Control (Unsmoked)	Smoked with 8-year-old wood	Smoked with 11-year-old wood	Smoked with 14-year-old wood
Naphthalene (NAP)	92.1 \pm 10.1	122 \pm 11	124 \pm 14	136 \pm 10
Acenaphthylene (ACE)	<1.00	<1.00	<1.00	<1.00
Acenaphthene (ACA)	<1.00	<1.00	<1.00	<1.00
Fluorene (FLU)	<1.00	10.2 \pm 0.5	13.2 \pm 0.5	15.6 \pm 0.6
Anthracene (ANT)	6.05 \pm 0.1	22.8 \pm 2.1	29.54 \pm 2.8	36.7 \pm 4.1
Phenanthrene (PHE)	<1.00	13.2 \pm 0.6	33.6 \pm 3.1	36.9 \pm 2.1
Fluoranthene (FLT)	<1.00	3.33 \pm 0.1	3.39 \pm 0.1	5.38 \pm 0.1
Pyrene (PYR)	<1.00	4.89 \pm 0.2	7.58 \pm 0.5	12.0 \pm 0.4
*Chrysene (CHR)	<1.00	<1.00	<1.00	<1.00
*Benzo[a]anthracene (BAA)	0.02 \pm 0.0	2.13 \pm 0.1	2.54 \pm 0.1	6.56 \pm 0.2
*Benzo[b]fluoranthene (BBF)	<1.00	<1.00	2.52 \pm 0.1	4.94 \pm 0.2
Benzo[k]fluoranthene (BKF)	<1.00	<1.00	<1.00	<1.00
*Benzo[a]pyrene (BAP)	<1.00	<1.00	<1.00	<1.00
Dibenzo[a,h]anthracene (DAA)	<1.00	<1.00	<1.00	<1.00
Benzo[e]pyrene (BEP)	<1.00	<1.00	<1.00	<1.00
Indeno[1,2,3-cd]pyrene (IND)	<1.00	<1.00	<1.00	<1.00
Benzo[g,h,i]perylene (BGP)	<1.00	<1.00	<1.00	<1.00
Perylene (PYL)	<1.00	<1.00	<1.00	<1.00
PAH4 Index	2.02 \pm 0.0	3.63 \pm 0.01	6.06 \pm 0.02	12.5 \pm 0.1

* Represent four compounds of probable human carcinogens according to EU (2011). All PAH values reported as <1.00 µg/kg were below the analytical method's limit of quantification (LOQ). Standard deviations could not be computed for these values due to non-quantifiable replicates.

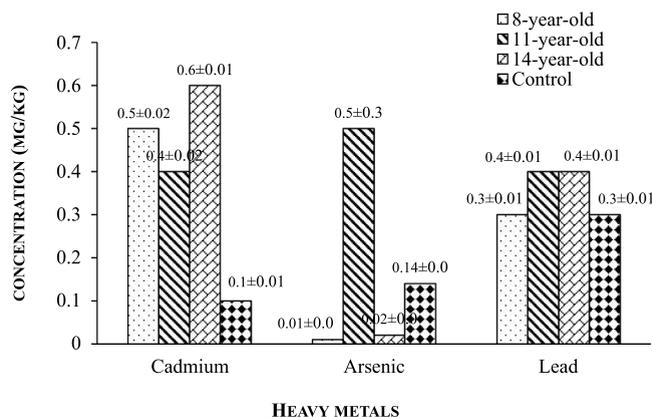


Fig. 1. Heavy metal concentration in fish smoked with mangrove wood at different ages.

suggested that lignification intensifies as trees grow to increase their environmental resilience and mechanical strength. Lignin is a key determinant of wood combustion efficiency and could lead to the production of PAHs [25]. The high concentration recorded in the older mangrove wood could have influenced the formation and accumulation of PAHs during the fish smoking process. Likewise, the 14-year-old wood contained the highest extractive content. Variations in the extractive content of wood with age are typically an adaptive response to the environmental and metabolic changes during tree growth [24]. The extractives influence wood combustion properties and smoke flavour. Adamová et al. [26] explained that the portion of extractives that are organic volatile compounds are released during combustion. Thus, the higher extractives in older wood could influence the PAH and heavy metal accumulation in smoked fish. Conversely, it was observed that the hemicellulose content decreased with wood age. This trend is consistent with the structural modifications that often occur in growing trees, where the hemicellulose gradually gives way to lignin to promote cell wall reinforcement [24]. Hemicellulose is known to ignite more readily than lignin and therefore contributes to the efficiency of wood as fuel [27]. So, the reduction in the hemicellulose content in the older wood could favour incomplete combustion, which potentially leads to increased PAH emissions. In a similar manner, we observed that the cellulose content declined in the 14-year-old wood. This observation aligns with the suggestion by Ivakov et al. [28] that the synthesis of cellulose often swings with tree maturity due to the metabolic shifts, which prioritize structural reinforcement over tree growth. The pattern observed may suggest that wood with higher levels of cellulose than lignin could contribute to combustion stability, which improves the chemical composition of smoke deposits on fish.

Types and concentration of PAHs in the smoked fish

The existence of PAHs in smoked fish is a well-documented food safety concern, and this study documented detectable levels in the smoked fish. The findings align with previous research [29,30,5], which has confirmed a strong linkage between wood combustion, the formation of PAH and food contamination. Most of this earlier research also recorded a number of the eighteen PAH congeners that were found in the fish smoked with the red mangrove wood of varying ages. These studies have reported that the wood species used for fish smoking has an enormous influence on the levels of PAHs, which are often deposited on food products. However, there is currently no research that establishes the effect of wood age on the types and levels of PAH deposited on food products. A trend of increasing PAH concentrations with older red mangrove wood was observed in this study. This can be attributed to the older wood's higher lignin content. Lignin-rich wood often burns at higher temperatures and undergoes incomplete combustion, which leads to greater PAH emissions [31]. This supports earlier findings that the chemical composition of biomass fuels affects the formation of toxic compounds during pyrolysis [32]. Ma et al. [33] concluded that hardwoods that have high lignin content (e.g., older mangrove wood) release more PAHs compared to softwoods. Furthermore, the rapid increase in the levels of PAH in fish smoked with the 14-year-old wood may indicate greater deposition of tar and soot, as noted in other studies, which were conducted on the traditional methods of fish smoking [30].

Although the regulatory importance of Benzo[a]pyrene as a benchmark PAH is not in doubt, the European Commission [7] has suggested that assessing the PAH4 Index, which is the sum of key carcinogenic PAHs provides a more comprehensive assessment of health risks. In this study, while BAP levels remained within the EU limits, the PAH4 Index exceeded the safety threshold in the fish smoked with the 14-year-old wood. This highlights the cumulative risk, which is posed by multiple PAH compounds. This finding buttresses the concerns that were espoused by Baumard et al. [34], who reported that although individual PAHs can fall within safe limits, their combined effects could pose long-term health risks. Taking into consideration the health risks linked to dietary PAH exposure, the present study has highlighted the urgent need for improved smoking practices and regulatory interventions. The use of wood with lower lignin content and the promotion of indirect smoking techniques would minimize the deposition of PAHs. The optimization of the smoking temperature and standardization of the smoking procedures, which may include the adoption of low-emission smoking technologies to reduce the incidence of PAH deposition, could also be useful ([35]; Racovita 2020; [30]). There is also the need for awareness creation among fish processors about the impact that wood age has on food safety.

Quantification of PAH4 as a marker of potential carcinogenicity in smoked fish

The European Union (EU) has established safety thresholds of 2.0 µg/kg for B[a]P and 12.0 µg/kg for the PAH4 Index [7]. We, therefore, assessed the carcinogenic risk associated with PAH exposure in the smoked fish by comparing the quantified concentrations of B[a]P and PAH4 Index with the EU Maximum Allowable Limits (MLs) for smoked food. The present study found that B[a]P concentrations were <1.00 µg/kg across all smoked fish samples. Thus, the level of B[a]P stayed within the regulatory limits. Gómez-Guillén et al. [15] reported that B[a]P levels between 0.1 µg/kg and 1.0 µg/kg are considered normal in smoked fish products. However, the PAH4 Index showed a progressive increase with wood age: the fish smoked using 14-year-old red mangrove wood exceeded the EU limit (12.5 µg/kg). As has been discussed already, the increase in PAH4 levels with older wood can be attributed to the higher lignin content, which produces byproducts including PAHs from incomplete combustion [31]. These findings are consistent with those of Anyakora and Coker [29], who reported that wood with higher lignin content tend to generate more PAHs in smoked food. Asamoah et al. [30] also emphasized that the traditional wood-smoking methods, which do not have controlled combustion conditions often lead to elevated PAH4 levels. This increases the carcinogenic risk. Based on our findings, it could be stated that the fish smoked with 8-year-old and 11-year-old red mangrove wood pose no significant carcinogenic risk because they met the EU safety limits. On the other hand, since the fish smoked with the 14-year-old wood exceeded the PAH4 Index limit based on EU standards, we can conclude that it is unsafe for consumption. According to Baumard et al. [34], cumulative exposure of consumers to PAHs would lead to long-term ingestion which is associated with cancer risk. These findings underscore the need for strict control over wood selection in traditional fish processing.

Concentration of heavy metals in smoked fish samples

The cadmium concentrations in all the fish samples, including the control in the current study, exceeded the EU maximum permissible level (0.05 mg/kg) [36]. Das et al. [37] similarly reported that cadmium contamination in fish often exceeds the safety thresholds due to environmental pollution and bioaccumulation. The observed trend in the present research, where cadmium levels increased with wood age, could be linked to variations in the chemical content of wood and combustion efficiency. It can be concluded that older wood, such as the 14-year-old red mangrove used in our work, has higher lignin and extractive content, which influences the release and deposition of heavy metals during smoking (Bartlett et al. 2014). Based on the findings of Das et al. [37], we can explain that the higher cadmium levels in the fresh fish sample could indicate cadmium contamination from water sources. The earlier authors found that fish naturally accumulate cadmium from polluted aquatic environments. Cadmium exposure is associated with bone demineralization, kidney damage, and carcinogenic effects [38,39]. Thus, its presence in smoked fish above the regulatory limits shows the potential health risks associated with frequent consumption. Our findings stress the need for stricter monitoring of cadmium levels in both smoked and fresh fish to ensure that the food is safe.

The contamination of fish with arsenic is a public health concern. This is because inorganic arsenic is highly toxic and associated with cancer, neurological disorders, and cardiovascular diseases [40]. Significant differences were recorded in the arsenic levels across the smoked fish samples: the fish smoked using the 11-year-old wood had the highest arsenic concentration, which was 0.5 %. The differences in arsenic volatilization during the smoking could be responsible for the variation. The level of arsenic in the fresh fish/control sample suggests some degree of arsenic bioaccumulation from water sources, which has previously been discussed by Pei et al. [41]. It has been shown that arsenic levels in seafood are often linked to environmental pollution from industrial runoff and the activities of the mining and agricultural industries [42]. Since the organic arsenic compounds in fish are generally less toxic than inorganic arsenic, it is important to distinguish between the two forms in future studies to better assess the health risks [43]. There is no specific EU limit for arsenic in fish. Therefore, it is incumbent on regulatory authorities to reflect on establishing safety guidelines for arsenic contamination in smoked fish products. Also, an improvement in wood selection and control of combustion conditions could contribute to reducing arsenic exposure through smoked foods.

There is no known safe exposure level of lead in humans, although lead is a cumulative toxicant that affects multiple body systems [44]. The study found significant differences in lead concentrations across smoked fish samples: the highest levels were recorded in fish smoked with 11-year-old and 14-year-old red mangrove wood. The EU safety limit for lead in fish (0.30 mg/kg) [45] was exceeded in the samples smoked with 11- and 14-year-old wood. The higher lead concentrations in fish smoked with older wood could be due to the greater chemical components of aged wood, which leads to increased lead release during combustion [46]. Lead can bind to other smoke components during smoking. Its bioavailability in smoked fish could also reduce through volatilization. Lead exposure is linked to cardiovascular diseases, developmental issues in children, and neurological disorders [45,47]. Therefore, it is important to monitor lead levels in smoked fish. Also, the findings suggest that the use of younger wood for smoking may contribute to reducing lead contamination.

Conclusion

This study investigated the safety risks related to traditional smoking of Atlantic Mackerel fish using red mangrove wood at varying ages. We found that the age of wood significantly influences the levels of PAHs and heavy metals in the smoked fish. The older wood (14 years) contributed to increased heavy metal contamination and higher carcinogenic PAH4 levels of smoked fish. Although the B[a]P concentrations were within the EU regulatory limits, the PAH4 Index exceeded the safety threshold in fish smoked with the older wood. This made the fish unsafe for consumption. The levels of cadmium in all fish samples and lead in the fish samples smoked with 11- and 14-year-old wood similarly exceeded the EU limits, which raises additional concerns over food safety. To ensure the safety of

smoked fish products, our study recommends the importance of restricting the use of older wood for fish smoking while adopting indirect smoking methods and enforcement of routine food safety monitoring. Efforts that promote consumer awareness, regulatory interventions, and improved smoking technologies would moderate PAH and heavy metal exposure. These will also minimize the health risks and preserve the economy of smoked fish. By addressing these pertinent issues with materials for fish smoking, food safety authorities, policymakers, and fish processors can work together to ensure that smoked fish remains a nutritious, safe, and culturally significant food source for consumers.

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Data availability

All relevant data are within the manuscript.

CRediT authorship contribution statement

Kwadwo Boakye Boadu: Conceptualization, Methodology, Supervision, Formal Analysis, Writing – review & editing. **Sylvia Bordoh:** Investigation, Methodology, Data curation, Writing – review & editing. **Michael Ayim:** Investigation, Methodology, Data curation. **Caleb Arku:** Investigation, Methodology, Data curation. **Ofori Attah Nkansah:** Investigation, Methodology, Data curation. **Rogerson Anokye:** Formal Analysis, Writing – review & editing. **Michael Ansong:** Conceptualization, Methodology, Supervision. **Kwasi Adu Obirikorang:** Conceptualization, Formal Analysis, Writing – review & editing. **Richeal Akuorkor Turkson:** Conceptualization, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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