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Original Research Article

Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products

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ABSTRACT

In this study, 108 fish samples of smoke cured fish *Sardinella aurita* (sardines/herrings) collected from 12 major fishing communities along the coastal belt of Ghana were extracted with Soxhlet apparatus using dichloromethane (DCM), and analyzed for sixteen polycyclic aromatic hydrocarbons (PAHs) using the GC/MS (Varian 3800 GC system with 8400 auto-sampler) to determine levels, distribution and the characterization of their sources. The mean total PAHs in the smoked sardines from the various communities ranged from 510.59 μg/kg to 1461.79 μg/kg for all seasons with a mean value of 716.84 μg/kg. The benzo[a]pyrene (BaP) had a maximum mean level of 73.78 μg/kg. The unit risk of carcinogenic PAHs for benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, benzo[a]anthracene, benzo[k]-fluoranthene, benzo[b]fluoranthene, and indenol[1,2,3-cd]pyrene associated with oral ingestion of smoked sardine was calculated to be 6.1×10^{-7} for adults and 1.6×10^{-7} for children. There is a significant correlation between the fish lipid content and the total PAH levels. The average lipid content of the smoked sardine samples collected varied among the various fishing seasons (3 seasons) in Ghana. A significantly high accumulation of PAHs was found in the smoke-cured fish as compared to the non-smoke-cured fish control samples, which showed PAH levels that were below detection (0.10–2.0 μg/kg). This study highlights the increased danger of consuming smoke-cured fish in Ghana as some of the PAHs are known to be carcinogenic. These results indicate that there is a need to find alternative ways of curing fish other than the traditional smoking process.

1. Introduction

Traditional smoking of food such as meat and fish products has been used for millennia in many countries. Smoke-curing is a method of fish preservation carried out through a combination of drying and depositing naturally produced chemicals such as phenols, aldehydes, acetic acids and a range of polycyclic aromatic hydrocarbons (PAHs) resulting from the thermal breakdown of wood (Kramlich et al., 1980; Wilson, 1981; Serden-Basak et al., 2010). The main purpose of smoke-curing is to preserve the food, partly by drying and partly by transferring anti-microbiological compounds such as phenols from the smoke to the food. However, in recent times, it has been used to achieve the characteristic taste and appearance of smoked food, with preservation playing only a minor role. Smoking is still widely used in fish processing, and it involves using either modern controlled methods, or traditional uncontrolled kilns. In Ghana, traditional uncontrolled smoke kilns are still widely being used by fishmongers (Nti et al., 2002).

Practically all species of fish available in Ghana can be smoked, and it has been estimated that about 70–80% of the domestic marine and freshwater catch is consumed in smoked form (Nti et al., 2002). Smoked *Sardinella aurita* (called herrings in Ghana) are the most common staple smoked fish product available throughout the year on the Ghanaian market. This fish product is consumed by both children and adults in preparations of various kinds of Ghanaian dishes.

In Ghana, smoking of fish using traditional kilns is generally carried out at a temperature of between 300 and 700 °C (Nti et al., 2002). The smoke is usually generated by burning hard wood. The kiln is built with bricks and sometimes metal, with a "top lid" of wood in order to generate enough smoke and heat for the rapid smoking of the fish. Generation of wood smoke during curing is a typical example of incomplete combustion, and undoubtedly PAHs are generated and released into the various smoked products (Phillips, 1999; Stołyhwo and Sikorski, 2005). PAH compounds are a very well-known class of ubiquitous ecotoxicants that are harmful to human health, with some known to be carcinogenic (Vazquez Troche et al., 2000; Kishikawa et al., 2003; Janoszka et al., 2004; Davina and Yusty, 2005; Okuda et al., 2006; Tfouni et al., 2007). Food processing methods such as smoking and cooking at high temperatures are

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known major sources of PAH contamination (Guillen et al., 1997; Phillips, 1999).

PAHs are lipophilic in nature and usually accumulate in the fatty tissues of organisms, and as such are produced from the fatty tissues of fish during smoking at higher temperatures. The formation of PAHs is known to occur through pyrolysis of fat at temperatures above 200 °C (SCF, 2002), and it is highly stimulated at temperatures over 700 °C (Bartle, 1991). Pyrolysis of other organic matter such as proteins and carbohydrates might be involved, but the greatest concentrations of PAHs have been shown to arise from fat pyrolysis (Bartle, 1991; Knize et al., 1999). Consequently, if the fish is in direct contact with the flame, pyrolysis of the fat in the fish generates PAHs that become deposited on the fish. It has been reported that, PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat/fish and the proximity of the food to the heat source (Phillips, 1999; Kazerouni et al., 2001).

The presence of PAHs, especially benzo[a]pyrene (used as biomarker) in smoked fish has been extensively reported (Šimko, 2002), but little information is available concerning the influence of the smoking processes. The highest amounts of total and carcinogenic PAH in foods were observed in samples after smoking (Chen and Lin, 1997; Reinik et al., 2007; Lund et al., 2009). The normal content of benzo[a]pyrene in smoked fish is between 0.1 and 1 μg/kg (Gómez-Guillén et al., 2009). Residual PAH concentrations in smoked foods are highly variable and result from the different smoking methods used.

Seven PAHs have been classified by the USEPA as compounds of probable human carcinogens (USEPA, 1994). These are benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene. PAHs known for their carcinogenic, mutagenic and teratogenic properties are benz[a]anthracene chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, coronene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene and ovalene (Luch, 2005). According to the recent classification on carcinogenicity of PAHs by International Agency for Research on Cancer (IARC), it is has been established that benzo[a]pyrene is carcinogenic (group 1), dibenz[a,h]anthracene is probably carcinogenic (group 2A), whereas naphthalene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene and indenol[1,2,3-cd]pyrene are classified as possible human carcinogens (group 2B), (IARC, 2012).

The Codex Alimentarius Commission, at its 29th session on contaminants in food, discussed ways to reduce the levels of PAHs in both dried and smoked foods. In addition, EU Regulation 1881/2006 requires formally setting a new and stricter rule on the content of PAHs in smoked products (EC Regulation, 2006).

Health effects resulting from PAH exposure have recently been discussed extensively in the literature (Shen et al., 2008). These include growth retardation, low birth weight, small head circumference, low IQ, damaged DNA in unborn children and the disruption of endocrine systems, such as estrogens, thyroid and steroids. Skin changes (thickening, darkening, and pimples) and reproductive-related effects such as early menopause due to destruction of ova have also been identified with PAHs (Essumang et al., 2011). It is known that in mammalian cells, PAHs undergo metabolic activation to diol, and epoxides that bind covalently to cellular macro-molecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process (Rodriguez et al., 1997; Schoket, 1999; Lightfoot et al., 2000). Polymorphisms causing glutathione transferase deficiencies (GSTM1) may result in elevated breast cancer, lung cancer and other forms of human cancer risk from PAHs (IARC, 1999; Van der Hel et al., 2003). Infants may be at risk for exposure to PAHs.

The Cancer Control Division of Ghana Health Service (GHS) on February 4, 2011 estimated that 16,600 cases of cancer occur annually in Ghana with an occurrence rate of about 109.5 cases per 100,000 persons. The report stated that most of the cases seen in Ghana and other West African countries identified the disease with younger people, which is the direct opposite of what has been reported in the developed world (GNA, 2011). Another statement by the CEO of the non-governmental organization Breast Care International on March 22, 2011, indicated that breast cancer cases among Ghanaian women are on the rise. It is very possible that high levels of PAHs in Ghanaian diets from smoked fish may be contributing to some of these increased cases of cancer, especially breast cancer among Ghanaian women.

This study seeks to determine the levels of PAHs and their distribution in smoked fish (sardines/herrings) in Ghana. The data from the study will also be used to assess cancer risk as well as to characterize the various sources of PAHs in smoke-cured fish products.

2. Materials and methods

2.1. Sample collection

Smoked fish (*Sardinella aurita*/herrings) samples ($n = 3 \times 36 = 108$, including triplicates) were collected from commercial fishmongers from 12 major fishing communities along the coastal belt of Ghana between April and June, 2009 (season 1), July and September, 2009 (season 2) and November, 2009 and February, 2010 (season 3). The bumper catch season for sardine in Ghana is from mid-July to late September, whereas the intense off-season is usually from late November to mid-February. Smoked sardine was chosen for this work because it is a staple fish consumed by the majority of Ghanaians. Each community chosen for sampling was demarcated into four parts, and smoked fish samples (sardines) were randomly collected from the fishmongers. The smoked samples from a particular community were composited, homogenized, kept in amber bottles at 4 °C in the freezer and extracted in triplicates. The average length and mass of the smoked sardines used were 19.8 ± 2.6 cm and 45.8 ± 8.2 g, respectively. Fresh sardine samples were equally taken and analysed in same manner for PAHs in these areas for the three seasons under study.

2.2. Reagents

All reagents and chemicals were of analytical grade and of highest purity possible. Chromatography grade dichloromethane, n-hexane (Purity (GC) ≥ 99.0%, analytical reagent, UN 1208, EC: 203-777-6, lot: K39517278905, product: 103876Q) and dichloromethane (HPLC grade, 99.8% purity, UN1593 EC: 200-838-9) used for the extraction and clean-up were purchased from VWR-BDH Chemicals Limited UK. Sodium sulfate (analytical reagent, 99.4% purity, product: 28114.296, EC label: 231-820-g) and glass wool were obtained from VWR-BDH PROLABO UK. Column chromatography Silica gel (mesh: 70-230, lot no: 0102/073/2, product: 36020) used to clean up the extract was purchased from Auro Avenida Export, Pvt. Ltd. (India). Methanol (100%, grade: analytical reagent, UN1230, Prod: 20847.320) and potassium hydroxide pellet (purity: 86.1%, analytical reagent, UN1813, EC: 2151813, product: 26668.296) used for saponification were purchased from VWR-BDH PROLABO UK. Petroleum ether (40–60 °C) used for crude fat extraction was also obtained from BDH PROLABO UK. A PAH standard mixture containing 16 PAHs compounds (purity: 95.9–99.9%, lot no: LB61945, 47940-U) was purchased from SUPELCO-analytical, Bellefonte, PA, USA. A mixture containing four isotopically labeled PAHs namely D10-acenaphthalene, D10-phenanthrene, D12-chrysene, and D10-pyrene used as an internal

standard were also purchased from Chemservice, Westchester, PA, USA.

2.3. Dry weight determination

Ten grams of the smoked fish powder (blended whole fish with head but without the skin) were homogenized with 10 g of sodium sulfate using a mortar and pestle until a fine, free-flowing dry homogenate was obtained. The homogenate was oven dried at 105 °C overnight. The oven-dried homogenate was cooled in a desiccator at room temperature for 6 h and then weighed, and the mass was used for calculating dry weight using the following formula:

$$\%\,\text{moisture} = \frac{[(\text{mass of sample and } Na_2SO_4, \text{g}) - (\text{mass of dried sample, g})] \times 100\%}{(\text{mass of sample, g})}$$ (AOAC, 1990).

2.4. Determination of crude fat content

PAHs are lipophilic, which implies the amount of PAHs in a particular fish species would partly depend on the amount of fat or lipids contained in the cells or tissues of the fish species. Fat content of fish is also an important parameter for determining the quality of a smoked fish product. Soxhlet extraction is the method usually recommended by the Association of Official Analytical Chemists (AOAC) as the standard method for crude fat analysis. About 10 g of finely ground fish fillet was homogenized with 10 g of sodium sulfate (pre-dried in oven at 105 °C overnight) in a mortar until free-flowing powder was obtained. The homogenate was transferred into an extraction thimble and the end was plug with glass wool. The extraction thimble with the sample was placed in a Soxhlet apparatus attached to a 500 mL round bottom flask fitted into a temperature control heating mantle. About 300 mL of petroleum ether was added to the flask and about 3 pieces of boiling chips were also added to the content. The empty flask and the chips were pre-weighed.

Soxhlet extraction was carried out for 6 h. The extract was removed and allowed to cool to room temperature. It was then concentrated using the Rotavap to about 5 mL. The 5 mL extract was further concentrated using a heating mantle to about 1 mL. The 1 mL extract was placed in oven at 105 °C for 2 h. The flask and its content were cooled and reweighed. Weighing was repeated until a constant weight was obtained (AOAC, 1990).

The percentage crude fat was calculated using the following formula:

$$\%\,\text{crude fat content} = \frac{X - F \times 100\%}{W}$$

where X = weight of flask with fat and chips, F = weight of flask and chips, and W = weight of sample.

2.5. Extraction of PAHs

A Soxhlet apparatus consisting of 500 mL round bottom flask, an extraction chamber, condenser and water circulators were mounted on temperature-controlled heating mantles for the extractions. A quantity of 10 g of the smoked fish powder was homogenized in a mortar with about 10 g of Na_2SO_4 until a completely dry homogenate was obtained. The homogenate was carefully transferred into the extraction thimble made from cellulose. The cellulose thimble containing the homogenate was then placed in the extraction chamber of the Soxhlet extractor, and 50 mL of methanol–KOH mixture prepared by dissolving 6 g of KOH in 12 mL distilled water and making it up to the mark with methanol in 100 mL volumetric flask was added to the homogenate in the extraction chamber. Soxhlet extractions were carried out using 300 mL dichloromethane (DCM). Solvent circulation cycles were at an average of 4 cycles per h and extraction of each sample was done for 24 h. The extract was cooled to room temperature. The aqueous layer containing the stearate was separated by addition of 100 mL methanol–water mixture (2:8) using a separatory funnel. The organic layer was washed twice with 50 mL distilled water to removal all remaining stearates from the organic extracts. The extract was concentrated using Rotavapor R-114 at a temperature of 45 °C to about 5 mL. The extracts were further concentrated to about 1 mL using a stream of an inert nitrogen gas (USEPA method 3540C; Telli-Karakoç et al., 2002).

2.6. Post-extraction clean-up

The 1 mL concentrated extract was loaded onto a packed silica gel column. The column used was prepared by loading 10 g of activated silica gel into a chromatographic column (all the columns used had uniform internal diameter of 1 mL). About 1 g of anhydrous sodium sulfate was added to the top of the column. Both ends of the packed column were plugged with glass wools. The packed column was then preconditioned with 5 mL:15 mL (1:3) dichloromethane/hexane mixture. The 1 mL concentrated extract was then applied on top of the column and eluted first with 20 mL hexane to remove n-hydrocarbons and the darkest part of the samples. This step was followed with 20 mL dichloromethane/hexane (1:3) mixture and repeated. The remaining traces of lipids, being very polar, were adsorbed at the top of the column (Anyakora and Coker, 2007). Prior to GC/MS analysis, 5 μL of 0.5 mg/mL of 4 internal standards in dichloromethane was added to each of the sample extract and its triplicates and the volume reduced to 1 mL using a stream of an inert nitrogen gas (USEPA method 3540C; Telli-Karakoç et al., 2002).

2.7. GC/MS analysis

A varian GC/MS (3800 GC) system with 8400 auto-sampler (mass data type: centroid) was used for the analysis. The system was also equipped with 40 m × 0.25 mm × 0.25 μm VF-5 ms fused capillary column. Helium gas was used as the carrier gas. The column head pressure was maintained at 10 psi for 15 min with a constant flow rate of 1.0 mL/min. The front injector line was maintained at 250 °C. Injection volumes were 2.0 μL in the splitless mode. The column temperature was initially held at 50 °C for 1 min, and ramped to 320 °C at a rate of 20 °C/min, and then held at 320 °C for 20 min. The mass spectrometer was operated in the ionization mode and spectra were acquired using a mass range of 45–450 m/z and automatic gain control. SIM acquisition was carried out by comparison of the base peak of each targeted PAH.

2.8. Analytical quality control

The analytical precision and recovery of the 16 PAHs was checked first with NIST standard reference material (1941b), which is marine sediment collected at the mouth of the Baltimore Harbour intended for use in evaluating analytical methods for the determination of selected PAHs, PCBs congeners and chlorinated pesticides in marine sediments and similar matrices like smoked fish fillet (or powder). Further analysis of variance for each triplicate of smoked fish samples was conducted. To evaluate the extraction efficiency for the target compounds, recovery studies were carried out using 4 deuterated PAHs, namely acenaphthene-d10 (for naphthalene, acenaphtylene, acenaphthene and fluorene), phenanthrene-10 (for phenanthrene, and anthracene), pyrene-d10

(for fluoranthene, pyrene and benz[a]anthracene), and chrysene-d12 (for chrysene and the remaining 6).

2.9. Calculation of carcinogenic risk

Human health evaluation computerized software-RISC 4.02 (USEPA, 1989) was used in the evaluation of the cancer and non-cancer risk assessment. Carcinogenic risks are estimated as the incremental probability of an individual developing cancer over a lifetime as a result of exposure to the potential carcinogen. This risk is referred to as the individual excess lifetime cancer risk (IELCR, or just carcinogenic risk). Published values of chemical carcinogenic toxicity (slope factor) are used to calculate risk from the lifetime average daily dose (LADD):

$$\mathrm{IELCR}_{ij} = \mathrm{SF}_{ij}\mathrm{LADD}_{ij}, \quad (1)$$

where IELCR_{ij} = individual excess lifetime cancer risk for chemical i exposure route i [dimensionless], SF_{ij} = slope factor for chemical i exposure route j [mg/kg-d] − 1, and LADD_{ij} = lifetime average daily dose for chemical i exposure route j [mg/kg-d].

2.10. Calculation of hazard index

Human health evaluation computerized software-RISC 4.02 (USEPA, 1989) was used in the evaluation of both cancer and non-cancer risk assessment. The potential for non-carcinogenic effects was evaluated by comparing an exposure level over the exposure duration (maximum of 7 years) with a reference dose derived for a similar exposure period. This ratio of exposure to toxicity for an individual pathway and chemical is called a hazard quotient. The hazard quotients are usually added across all chemicals and routes to estimate the hazard index. However, some will argue that it is more appropriate to sum only the hazard quotients for chemicals that affect the same target organ (e.g. liver or blood). The non-cancer hazard quotient assumes that there is a level of exposure below which it is unlikely that even sensitive populations would experience adverse health effects (USEPA, 1989).

3. Results and discussion

3.1. Quality control result

There were no statistical significant differences in the PAHs results for triplicates ($n = 3$) of each sample at the 95% confidence level in a particular season. The recovery of each triplicate of the internal standards analysis was in the range of 72–102% and the average recovery was 88%. The PAH standard mix was run to calibrate the instrument; it was also run with the sample to ensure accurate reading and results. The limit of detection (LOD) and limit of quantitation (LOQ) for the individual PAHs ranged from 0.10 to 2.00 μg/kg and 0.30 to 6.00 μg/kg, respectively. The regression coefficient (R^2) of the PAH standard mix calibration curves over concentration range of 1–10 μg/mL ranged from 0.9872 to 1.00, as presented in Table 1 (see also Appendix A, Figs. A1 and A2).

Table 1
GC/MS calibration parameters of PAH standards > 1.00–10.00 μg/mL range.

Compound	Slope	Intercept	Regression coefficient (R^2)
Naphthalene	4.33E+00	7.94E−02	0.9999
Acenaphthyelene	4.00E+00	0.00E+00	1
Acenapthene	4.41E+00	−1.04E−01	0.9999
Fluorene	4.22E+00	−2.65E−01	1
Anthracene	3.14E+00	−8.10E−02	0.9998
Phenanthrene	4.36E+00	−3.46E−01	0.9998
Fluoranthene	3.92E+00	1.83E−01	1
Pyrene	3.81E+00	−1.61E−01	0.998
Benz[a]anthracene	2.37E+00	−6.31E−01	0.9998
Benzo[k]fluoranthene	1.52E+00	−7.13E−01	0.9951
Indeno[1,2,3-cd]pyrene	2.95E−01	5.52E−01	0.9958
Benzo[b]fluoranthene	1.55E+00	−1.39E−01	0.9951
Chrysene	2.63E+00	−3.69E−01	0.9998
Benzo[a]pyrene	1.03E+00	−4.26E−01	0.9951
Benzo[g,h,i]perylene	3.34E−01	7.94E−02	0.9782
Dibenz[a,h]anthracene	2.20E−01	7.35E−01	0.9898

Table 2
Results of the recovery studies based on NIST 1941B standard reference material.

PAH	Mass fraction expected (μg/kg)	Mass fraction extracted (μg/kg)	Recovery (%)
Naphthalene	848 ± 95	597 ± 79	70
Acenaphthyelene	53 ± 6.4	36 ± 5.3	67
Acenapthene	38 ± 5.2	22 ± 2.4	56
Fluorene	85 ± 15	50 ± 10	59
Anthracene	184 ± 18	190 ± 14	103
Phenanthrene	406 ± 44	438 ± 31	108
Fluoranthene	651 ± 50	670 ± 47	103
Pyrene	581 ± 39	486 ± 33	84
Benz[a]anthracene	335 ± 25	189 ± 22	56
Benzo[k]fluoranthene	225 ± 18	240 ± 30	107
Indeno[1,2,3-cd]pyrene	341 ± 57	340 ± 42	100
Benzo[b]fluoranthene	453 ± 21	271 ± 13	60
Chrysene	291 ± 31	180 ± 26	62
Benzo[a]pyrene	358 ± 17	300 ± 13	84
Benzo[g,h,i]perylene	307 ± 45	300 ± 51	98
Dibenz[a,h]anthracene	53 ± 12	42 ± 15	79

The second recovery study conducted using the NIST reference material showed good recovery PAH values ranging from 56% to 108% with an average PAH recovery value of 81% (Table 2). The values obtained could be used to establish the reliability of the extraction system as well as the efficiency of the GC/FID/MS instrument. In fact, the NIST–1941B reference material was used to establish the reliability of the extraction system as well as the elution efficiency of the GC/MS/FID instrument since there was no certified reference material for the sample matrix under study at the time of the analysis.

3.2. PAHs in the fish samples

The PAHs levels in fresh sardine fish samples used as control were all below the detection limit used (0.10–2.00 μg/kg). This may imply that the sea in Ghana which is the source of fresh sardine fish used was not polluted with detectable amount of PAHs during the seasons of study. This is in conformity with the statement made by Stołyhwo and Sikorski (2005) that fish and marine invertebrates may naturally contain small or undetectable amounts of different PAH absorbed from the environment. The statement made by Rainio et al. (1986) in similar work, that the edible parts of fish from unpolluted seas generally do not contain detectable amounts of BaP, also affirms the low detection limit observed, and the fact that the source of fresh sardine fish used in this work was not polluted with detectable levels of PAHs. The total PAH concentrations in the smoked fish (sardines/herrings) ranged from 573.77 μg/kg at Komantsi and its environs (KAM) in the Central region to 971.05 μg/kg at Teshie (TES) in Greater Accra region of Ghana, for samples collected between April and June, 2009 (season 1); 510.59 μg/kg at Tema (TMA) to 926.32 μg/kg at Teshie (TES), both in the Greater Accra region of Ghana, for samples collected between July to September, 2009 (season 2); and 824.08 μg/kg at Komantsi and its environ (KAM) to 1461.79 μg/kg (dry weight) at Teshie (TES) for samples collected between November, 2009 to February, 2010 (season 3) (Table 3). The overall total mean PAH concentration in the smoked sardines was found to be 833.21 μg/kg (Table 4).

Table 3
Mean total PAH concentration (μg/kg) in smoked herrings (sardines) on the Ghanaian market for the three seasons (triplicate analysis; $n = 3$) in μg/kg (dry weight).

Town (community)	Seasons		
	∑[PAHs S1]	∑[PAHs S2]	∑[PAHs S3]
CCM	671.50	845.53	825.77
ELM	940.20	889.00	955.42
ADA	700.95	578.57	1011.73
KAM	573.77	573.77	824.08
SHA	806.84	885.38	904.45
AGN	702.09	898.66	939.97
TDI	759.97	534.95	973.00
JMT	810.97	849.08	1006.57
WIN	791.03	596.49	917.74
TES	971.05	926.32	1461.79
TMA	954.52	510.59	1143.10
SEK	885.83	513.76	851.18

Notes: S1, S2, S3 represents concentrations (μg/kg) for seasons 1, 2 and 3 respectively.
Where CCM = Cape Coast; ELM = Elmina; ADA = Ada town; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; and SEK = Sekondi.

The mean percentage lipid composition of the smoked sardines for the 3 seasons was 19.56 ± 4.64%, with ranges from 16.05 ± 0.89% to 19.94 ± 1.00% in season 1; 8.85 ± 0.26% to 18.72 ± 1.04% in season 2; and 18.06 ± 0.43% to 31.08 ± 1.44% in season 3 (Table 5). The results showed that PAHs levels in smoked fish samples increased with respect to increase in lipid contents of the fish. The presence of lipid in the fish when it undergoes pyrolysis leads to the formation of PAHs which return to the fish being smoked, as has already been observed in other studies (Phillips, 1999; Kazerouni et al., 2001).

It was noticed that fish with high lipid content recorded the highest amount of PAHs; this factor also depends on the season when the fish are caught. During the wet season (mid-June–September) the sea becomes more diluted; as a result, the water content of the fish rises, which invariably results in low lipid content of the sardines. The results demonstrate this, as most of the sardines harvested during the wet period had relatively low PAH concentrations after smoking, for all 12 towns. However, the dry season (November–May) fish showed high lipid content and relatively high PAH concentrations.

In their 2002 report, the Scientific Commission on Food stated that the formation of PAHs occurs through pyrolysis of fat, particularly in temperature ranges of 500–900 °C, and especially above 700 °C (Bartle, 1991). From the results in Table 5 it could therefore be said that fishmongers in Ghana tend to smoke fish with high lipid content at higher temperatures, namely 500–900 °C, where pyrolysis of the fat is stimulated. Moreover, smoking is generally carried out for a long period to improve the quality and shelf-life of the smoked fish products. This may therefore increase the levels of PAHs in the smoked fish with higher lipid contents as stated by Knize et al. (1999) (Tables 3 and 5).

Duedahl-Olesen et al. (2006), in a similar study on Danish smoked fish, found maximum total PAH ($n = 27$) in herring products prepared by direct smoking to be 1387 μg/kg, which is lower than the maximum mean total amount of 1461.79 μg/kg (Table 3) obtained in this work. This may mean that consumers of smoked fish are exposed to higher levels of PAHs. It is important to note that the maximum mean total PAHs 1119.72 μg/kg (Table 4) from this study was quite below the total maximum value reported by Duedahl-Olesen et al. (2006). The maximum mean total PAH level of 1461.79 μg/kg (Table 3) obtained in this work was also notably less than the maximum mean total PAH level of 2058.1 μg/kg in smoked fish obtained by Silva et al. (2011) in similar work.

Table 4
Mean concentration (μg/kg) of individual PAHs in smoked herrings from the communities in Ghana for the seasons ($n = 9$ for each).

PAH	CCM	ELM	ADA	KAM	SHA	AGN	TDI	JMT	WIN	TES	TMA	SEK	Mean	STDEV	VAR
Naphthalene	7.22	4.26	3.27	3.66	3.27	3.40	12.09	3.51	1.69	3.70	10.89	3.01	5.00	3.30	10.88
Acenaphthyelene	8.46	15.48	21.60	20.70	23.27	25.54	49.25	9.00	3.30	2.15	16.05	10.40	17.10	12.72	161.75
Acenapthene	12.91	19.56	28.72	32.50	34.97	19.36	24.61	45.78	8.21	12.07	11.32	16.78	22.23	11.37	129.18
Fluorene	230.04	166.66	215.53	345.77	241.16	184.00	144.47	208.62	204.33	241.76	212.42	166.62	213.45	51.76	2679.15
Anthracene	9.54	14.21	13.79	19.86	16.03	16.62	44.82	26.31	7.82	3.10	7.99	6.49	15.55	11.23	126.11
Phenanthrene	50.97	71.16	65.93	80.24	74.14	56.91	30.73	87.09	25.81	17.40	25.68	34.64	51.72	24.18	584.86
Fluoranthene	95.58	157.01	91.90	38.30	64.56	131.33	65.30	80.16	167.57	470.78	264.18	131.91	146.55	118.70	14090.37
Pyrene	228.56	314.74	206.36	74.85	352.78	336.74	225.07	312.49	278.15	133.75	168.02	206.83	236.53	85.58	7323.69
Benz[a]anthracene	10.53	29.50	27.80	11.12	29.74	26.87	51.96	24.81	13.65	22.77	15.21	69.58	27.79	17.34	300.59
Benzo[k]fluoranthene	24.50	7.08	11.19	4.58	4.04	1.00	1.65	7.18	6.37	6.79	5.18	16.28	7.99	6.61	43.74
Indeno[1,2,3-cd]pyrene	3.06	2.24	2.71	0.82	0.27	11.54	3.21	1.56	6.25	0.90	0.72	8.50	3.48	3.51	12.32
Benzo[b]fluoranthene	32.57	39.93	25.22	2.21	3.93	4.95	14.64	29.03	3.49	75.99	50.57	25.05	25.63	22.32	498.25
Chrysene	35.14	35.22	17.81	2.71	8.40	17.59	33.44	21.71	28.36	47.90	42.66	32.53	26.95	13.57	184.23
Benzo[a]pyrene	18.49	33.76	20.12	17.55	3.36	5.40	21.22	25.11	7.31	52.00	28.35	9.20	20.15	13.77	189.67
Benzo[g,h,i]perylene	10.12	14.89	7.32	0.37	5.44	5.25	32.63	6.15	2.56	15.79	9.52	6.08	9.68	8.51	72.47
Dibenz[a,h]anthracene	3.24	2.48	4.47	5.32	0.20	0.42	0.88	0.36	3.55	12.88	0.66	6.33	3.40	3.65	13.32
TOTAL (∑PAHs)	780.93	928.21	763.75	660.54	865.56	846.91	755.97	888.87	768.42	1119.72	869.40	750.26	833.21		

CCM = Cape Coast; ELM = Elmina; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; and SEK = Sekondi.

Table 5
Mean percentage crude fat content of smoked sardines ($n = 3$) on the Ghanaian market for the 3 seasons.

Town (community)	Season 1 %Fat	Season 2 %Fat	Season 3 %Fat	% Mean for 3 seasons
CCM	18.86 ± 0.19	18.55 ± 0.51	24.51 ± 1.13	20.64 ± 3.36
ELM	18.85 ± 0.84	17.75 ± 0.38	24.53 ± 1.04	20.38 ± 3.64
ADA	19.94 ± 1.00	17.08 ± 0.21	29.89 ± 1.30	22.30 ± 6.73
KAM	18.05 ± 0.34	12.78 ± 0.29	24.08 ± 0.88	18.30 ± 5.66
SHA	17.20 ± 0.53	8.85 ± 0.26	21.53 ± 1.15	15.86 ± 6.45
AGN	16.05 ± 0.89	12.67 ± 0.12	18.06 ± 0.43	15.59 ± 2.72
TDI	17.31 ± 1.10	15.10 ± 0.31	21.78 ± 0.86	18.06 ± 3.41
JMT	19.06 ± 1.14	18.72 ± 1.04	27.69 ± 1.12	21.82 ± 5.08
WIN	18.61 ± 0.46	12.84 ± 0.16	24.72 ± 1.03	18.61 ± 5.94
TES	19.68 ± 0.62	16.92 ± 0.51	31.08 ± 1.44	22.56 ± 7.51
TMA	19.76 ± 1.36	18.70 ± 0.62	29.86 ± 1.05	22.77 ± 6.16
SEK	17.97 ± 0.57	17.03 ± 0.44	18.16 ± 0.16	17.72 ± 0.60
% Mean for all towns	18.44 ± 1.17	15.58 ± 3.14	24.66 ± 4.36	19.56 ± 4.64

Where CCM = Cape Coast; ELM = Elmina; ADA = Ada town; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; and SEK = Sekondi.

Levels as high as 200 μg/kg in food have been found for some individual PAHs in smoked fish and meat products (EC-SCF, 2002; Yang et al., 2006). The mean individual PAH levels in the smoked sardines on the Ghanaian market from all the sampling sites ($n = 108$) ranged from 3.40 to 236.53 μg/kg for all seasons. The low levels of the total PAH as well as the individual concentrations in season 2 (Tables 3 and 4), could be attributed to the fact that the sardines were caught during the wet season, with lower body fat content, which might have reduced the levels of the PAHs. The smoked sardine samples obtained also showed heavy scales covering the edible part; scales may serve as a barrier preventing the penetration of PAH-carrying smoke into the fish (Ova and Onaran, 1998; Serden-Basak et al., 2010). The maximum mean individual PAH levels obtained in this work were far below the individual mean PAH levels obtained by Silva et al. (2011) in catfish and sole fish samples smoked with fire wood and sawdust using traditional kilns.

The mean concentrations (triplicate analysis) of the individual PAHs in smoked fish from the individual communities for the three seasons ($n = 36$) ranged from 0.20 μg/kg of dibenz[a,h]anthracene at Shama (SHA) to 470.78 μg/kg fluoranthrene at Teshie (TES) (Table 4). The total mean concentrations of the individual PAHs ranged from 660.54 to 1119.72 μg/kg in KAM and TES samples, respectively. The mean of the individual concentrations in the samples from the communities ranged from 3.40 to 236.53 μg/kg of dibenz[a,h]anthracene and pyrene, respectively (Table 4). The presence of high levels of individual PAHs as well as the total PAHs especially BaP in the smoked sardines is an indication of high contamination of smoked sardine in the Ghanaian communities. This may imply an increased risk of carcinogenic and mutagenic hazards in the Ghanaian population.

The safety of smoked fish has been carefully controlled by many scientific institutions in most countries by measuring BaP levels. The European Commission has limited the maximum acceptable concentration of BaP at 5.0 μg/kg for smoked fish and smoked fishery products, excluding bivalve mollusks. The Turkish Codex Regulation (2008) limits the maximum acceptable concentrations of BaP to 2.0 μg/kg in smoked fish. A research conducted by Wretling et al. (2010) on smoked fish, found BaP levels exceeding 5.0 μg/kg in six samples, with concentrations ranging from 8.4 to 14.4 μg/kg. Akpambang et al. (2009) also stated that samples that were smoked using traditional wood fire systems were heavily contaminated with BaP at levels ranging from 2.4 to31.2 μg/kg. The normal content of BaP in smoked fish as stated by Kant. Laboratorium (2005) is between 0.1 and 1.0 μg/kg. However, concentration of up to 200 μg/kg may result from uncontrolled smoking procedure. In this work, the maximum concentrations of (BaP) in the smoked fish (sardines/herrings) taken from the communities for the three seasons ranged from 8.50 ± 0.67 to 73.78 ± 11.44 μg/kg (Table 6).

The mean concentration of BaP in the smoked sardines for the three seasons also ranged from 3.36 ± 4.52 to 52.00 ± 18.88 μg/kg (Table 6). The maximum BaP concentrations for the various communities for the three seasons were all above the maximum allowable limits of 2.0 and 5.0 μg/kg from the Turkish Codex and European Commission regulations, respectively. The mean BaP concentrations for the communities were all above these limits except for Shama, which recorded a mean BaP concentration of 3.36 μg/kg. Stołyhwo and Sikorski (2005) stated that the outer parts of heavily smoked fish from traditional kilns may contain up to 50 μg/kg of BaP (wet weight).

The results obtained in this work indicate that the fish have been heavily smoked and are quite comparable to those obtained by Stołyhwo and Sikorski (2005). The comparatively high levels of BaP in smoked sardines from most communities may be contributing to the elevated levels of cancer and cancer-related cases in Ghana, because BaP is widely known for its carcinogenicity and mutagenicity; further epidemiological studies may be required to prove this conclusion, however.

3.3. Sources of PAHs in smoked fish

PAH ratios of selected compounds are generally considered to be a good indicator of the pollution and the mechanism of PAH

Table 6
Mean benzo[a]pyrene concentration (μg/kg) in smoked sardines on the Ghanaian market from the various communities (triplicate analysis).

Town (community)	Mean maximum [BaP]	Mean [BaP] for 3 seasons
CCM	34.59 ± 3.37	18.49 ± 13.99
ELM	47.09 ± 2.41	33.76 ± 11.93
ADA	38.69 ± 2.01	20.13 ± 19.20
KAM	21.29 ± 1.19	17.55 ± 6.48
SHA	8.50 ± 0.67	3.36 ± 4.52
AGN	11.59 ± 0.53	5.40 ± 5.84
TDI	32.09 ± 1.51	21.23 ± 16.48
JMT	34.13 ± 1.42	25.11 ± 8.23
WIN	9.25 ± 0.53	7.31 ± 2.58
TES	73.78 ± 11.44	52.00 ± 18.88
TMA	37.96 ± 1.81	28.35 ± 15.82
SEK	14.44 ± 1.22	9.20 ± 5.77

Where CCM = Cape Coast; ELM = Elmina; ADA = Ada town; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; and SEK = Sekondi.

distribution in foods. Usually, PAH ratio calculations are said to be restricted to PAHs within a given molecular mass in order to minimize complicating factors such as differences in volatility, water solubility, and adsorption (Yunker et al., 2002). Yunker and coworkers have summarized the literature on PAH ratios for petroleum, single-source combustion and some environmental samples. Their conclusions were that for mass 178, an anthrancene to anthracene plus phenanthrene [An/(An + Phen)] ratio < 0.10 usually is an indication of petroleum while a ratio > 0.10 indicates dominance of combustion. For mass 202, a fluoranthene to fluoranthene plus pyrene [Fl/(Fl + Py)] ratio of 0.40 seems to be the petroleum/wood combustion transition point, though not definite. Ratios between 0.40 and 0.50 are more characteristic of liquid fossil fuel (vehicle and crude oil) combustion, whereas ratios > 0.5 are characteristic of grass, wood or coal combustion. For mass 228, a benzo[a]anthracene to benzo[a] anthracene plus chrysene [BaA/(BaA + Chry)] ratio < 0.20 implies petroleum, 0.20–0.35 indicates either petroleum or combustion (mixed), and >0.35 implies combustion. The ratio of benzo[a]pyrene to benzo[g,h,i] perylene [BaP/(BghiP)] ratio > 0.6 is characteristic of traffic sources (Park et al., 2002), and 1.2–5.0 indicates wood or coal combustion sources (Maher and Aislabie, 1992; Maliszewska-Kordybach et al., 2008; Yin et al., 2008); the ratio of indeno[1,2,3-cd]pyrene to indeno[1,2,3-cd]pyrene plus benzo[g,h,i]perylene [Ind/(Ind + BghiP)] > 0.5 also indicates grass/coal/wood combustion sources (Maliszewska-Kordybach et al., 2008; Yin et al., 2008).

The ratio of [An/(An + Phen)] in this study ranged from 0.15 to 0.59. This indicates a predominance of combustion as a source for PAHs (i.e. ratio > 0.1) in the smoked fish from the various communities in Ghana. The ratio of [Ind/(Ind + BghiP)] for the communities ranged from 0.05 to 0.71. The higher ratio of [Ind/(Ind + BghiP)], i.e. >0.5 for KAM (0.69), AGN (0.69) and WIN (0.71), affirms that, on the average, wood or coal combustion is the major source of PAHs in the smoked sardines in these communities, whereas the rest of the communities with [Ind/(Ind + BghiP)] ratio < 0.5 might have pyrolytic sources (especially pyrolysis of fats). This pyrogenic source could be attributed to the high levels of fat in the samples for season 3 of this work (Phillips, 1999; Kazerouni et al., 2001). The [Fl/(Fl + Py)] ratio in this work also ranged from 0.35 to 0.82. The levels from communities such as SHA (0.41), TDI (0.4), JMT (0.4) and WIN (0.42), with ratios between 0.4 and 0.5, imply some amount of fossil fuel combustion sources (fat, vehicular and crude oil) of PAHs. A majority of the communities had [Fl/(Fl + Py)] ratio > 0.5, which is an indication of wood or coal combustion as a source of the PAHs in the smoked fish samples. The results from the [BaA/(BaA + Chry)] ratio again confirms that there is no absolute petroleum source for PAHs contamination i.e. ratio >0.2 in the smoked fish samples from the communities, but a mixed petroleum and combustion source was found only for CCM, TMA, TES, and WIN.

The [BaP/(BghiP)] ratio for the communities were all >0.6, which is an indication of contamination from petroleum sources (traffic source). This could be attributed to the fact that some of these communities are situated near highways where vehicular traffic is commonly found and motor vehicle fuel might have contributed to the petrogenic PAHs deposited on the surface of the fish. Nevertheless, ratios from all the communities except SHA and AGN having [BaP/(BghiP)] > 1.2 confirm wood combustion as the primary and major source of PAH contamination in smoked fish from these communities. These results are shown in Tables 7 and 8. The communities most affected by these ratios with mixed petroleum and combustion sources were TDI, JMT, CCM, TMA, and WIN. This could be attributed to PAH emissions from motor vehicle exhaust and other natural organic combustible sources like oils.

Table 7
Source characterization and assessment of PAHs, reference table.

PAH ratios	Petroleum	Wood combustion	In this study	Reference
[An/(An + Phen)] 178	<0.10	>0.10	0.15–0.59	Budzinski et al. (1997), Zhang et al. (2006), Pies et al. (2008) and Plachá et al. (2009).
[Fl/(Fl + Py)] 202	0.40	>0.5	0.35–0.82	Yunker et al. (2002), Zhu and Wang (2003) and Plachá et al. (2009)
[BaA/(BaA + Chry)] 228	<0.20	1.2–5.0	>0.2	Maher and Aislabie (1992), Gilbert et al. (2006), Zhang et al. (2006), Pies et al. (2008) and Plachá et al. (2009)
[BaP/(BghiP)]	>0.6	1.2–5.0	>0.6	Maher and Aislabie (1992), Park et al. (2002), Maliszewska-Kordybach et al. (2008) and Yin et al. (2008)
[Ind/(Ind + BghiP)]	<0.5	>0.5	0.05–0.71	Maliszewska-Kordybach et al. (2008) and Yin et al. (2008)

Where An = anthracene, Phen = phenanthrene, Fl = fluoranthene, BaA = benz[a]anthracene, Chry = chrysene, BaP = benzo[a]pyrene, BghiP = benzo[g,h,i]perylene, and Ind = indeno[1,2,3-cd]pyrene.

Table 8
Sample sites and PAHs ratio for source assessment in smoked fish in Ghana.

Town (community)	An/(An + Phen)	Fl/(Fl + Py)	BaA/(BaA + Chry)	BaP/(BghiP)	Ind/(Ind + BghiP)
CCM	0.158	0.502	0.231	1.826	0.232
ELM	0.166	0.346	0.456	2.267	0.131
ADA	0.173	0.511	0.610	2.751	0.270
KAM	0.198	0.822	0.804	47.253	0.688
SHA	0.178	0.406	0.780	0.617	0.047
AGN	0.226	0.353	0.604	1.027	0.687
TDI	0.593	0.391	0.608	0.651	0.090
JMT	0.232	0.400	0.533	4.082	0.202
WIN	0.232	0.424	0.325	2.858	0.710
TES	0.151	0.644	0.322	3.293	0.054
TMA	0.237	0.558	0.263	2.978	0.070
SEK	0.158	0.446	0.681	1.512	0.583

Where CCM = Cape Coast; ELM = Elmina; ADA = Ada town; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; SEK = Sekondi. For PAHs ratios, An = anthracene; Phen = phenanthrene; Fl = fluoranthene; BaA = benz[a]anthracene; Chry = chrysene; BaP = benzo[a]pyrene; BghiP = benzo[g,h,i]perylene; and Ind = indeno[1,2,3-cd]pyrene.

Table 9
Fluoranthene-to-pyrene ratio for season 3 and mean PAH levels for the communities during all seasons.

Town (community)	(FL/Pyr)	
	Season 3	Mean (PAH)
CCM	0.171	0.418
ELM	0.252	0.499
ADA	0.653	0.445
KAM	0.360	0.512
SHA	0.233	0.183
AGN	0.107	0.390
TDI	0.175	0.290
JMT	0.067	0.257
WIN	0.499	0.602
TES	5.699	3.520
TMA	3.751	1.572
SEK	1.167	0.638

Where CCM = Cape Coast; ELM = Elmina; ADA = Ada town; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; and SEK = Sekondi. For the ratio, FL = fluoranthene and Pyr = pyrene.

To confirm whether the high PAH concentrations obtained in season 3 for some communities is from fat pyrolysis (pyrolytic source), a fluoranthene-to-pyrene ratio was calculated (SCF, 2002). The result is represented in Table 9. The FL/Pyr ratio ranged from 0.067 to 5.699 in season 3. The high ratio values obtained for TES, TMA and SEK are an excellent indication of fat pyrolysis as a major contributor to PAH level in the samples taken from these communities in season 3. This was further affirmed by the ratios for the mean PAHs obtained for the three seasons, as TMA and TES showed higher ratios (Table 9).

These PAH ratios reveal that the primary source of PAHs in the smoked fish for all the seasons is the wood combustion with fat pyrolysis (pyrolytic source) as only a cofactor in fatty samples taken in season 3, and vehicular traffic source contributing a comparatively insignificant amount in almost all the samples taken throughout the seasons.

3.4. PAH interrelationships

To assess the interrelationships of PAH concentration in the smoked fish samples from all communities and their possible sources, a correlation analysis was conducted using Pearson Product correlation method, which describes the strength of linear dependence between two variables being tested, given a value between +1 and −1. It is known that where two compounds have a common source, there is more likelihood of there being a correlation between their concentrations (Gilbert et al., 2006). In this work, strong positive correlations were found among almost all the communities with respect to their PAH levels at both 0.01 and 0.05 significant level (2-tailed), except for TES/KAM (0.435), TES/SHA (0.425), TES/TDI (0.452), and TES/JMT (0.468) (Table 10).

Statistical values for correlation interpretation show that correlation coefficient of ±(1.0–0.5) is strong, ±(0.5–0.3) is medium, ±(0.3–0.1) is small and ±(0.09–0.00) shows no correlation. Hence there was a medium correlation in PAH levels among TES and the communities KAM, SHA, TDI, and JMT. It could therefore be said that PAHs in smoked fish from the communities were basically from the same source (wood combustion with fat pyrolysis being significant only in fish with high fat content), although there might be additional sources as to the strong correlation observed among the communities.

To test whether statistically there are no significant differences at the 95% confidence level ($P > 0.05$) in the PAH levels in the smoked sardines from the communities for the various seasons, analysis of variance (ANOVA) was employed. The results from the ANOVA in a given season showed no statistical significant difference in the mean PAH levels ($P > 0.05$) for the various communities through the seasons (i.e. $P = 0.963$ for season 1, $P = 0.612$ for season 2 and $P = 0.959$ season 3). This suggests that fishmongers throughout Ghana basically employ the same smoking procedure in their fish-smoking processes. Thus the non-statistical differences seen in the physical levels of PAHs in the samples might have originated from differences in type of firewood, non-absolute temperature ranges, the type of kiln or the proximity of a community to a vehicular traffic source.

3.5. Cancer and non-cancer risk assessment of PAHs in smoked fish

Risk assessment studies were conducted for the 7 PAHs considered by the United States Environmental Protection Agency (USEPA) as probable human carcinogens and the other 5 PAHs known to be hazardous (i.e. acenaphthene, anthracene, fluoranthene, fluorene, and pyrene), by taking the averages of the means of the individual PAHs in the smoked sardines from the 12 communities. These were calculated by employing the central tendency exposure (CTE), in accordance with the USEPA's *Risk Assessment Guidance for Superfund* (RAGS) (USEPA, 1989). Unit risks were estimated considering a lifetime of 70 years for adults and 2 years for children using the human health evaluation computerized software RISC 4.0. From the results (Table 4), the total carcinogenic unit risk for ingestion was calculated to be 6.1×10^{-7} for adults and 1.6×10^{-7} for children. This mean that approximately 6 out of every 10,000,000 adults

Table 10
Correlation of PAH levels in smoked sardines among the 12 communities in Ghana.

	CCM	ELM	ADA	KAM	SHA	AGN	TDI	JMT	WIN	TES	TMA	SEK
CCM	1											
ELM	0.928**	1										
ADA	0.989**	0.924**	1									
KAM	0.780**	0.526*	0.806**	1								
SHA	0.953**	0.939**	0.959**	0.670**	1							
AGN	0.936**	0.986**	0.937**	0.562*	0.972**	1						
TDI	0.915**	0.933**	0.920**	0.593*	0.967**	0.963**	1					
JMT	0.957**	0.958**	0.962**	0.656**	0.992**	0.973**	0.953**	1				
WIN	0.958**	0.972**	0.948**	0.628**	0.928**	0.973**	0.922**	0.934**	1			
TES	0.602*	0.632**	0.599*	0.435	0.425	0.561*	0.452	0.468	0.711**	1		
TMA	0.824**	0.823**	0.817**	0.609*	0.685**	0.777**	0.698**	0.715**	0.889**	0.946**	1	
SEK	0.942**	0.953**	0.945**	0.638**	0.913**	0.950**	0.916**	0.918**	0.975**	0.706**	0.877**	1

The abbreviations for the towns have their usual meaning of representative towns, where CCM = Cape Coast; ELM = Elmina; KAM = Komantsi and environ; SHA = Shama and environ; AGN = Agona, Discov & environ; TDI = Takoradi; JMT = James Town (Chokor); WIN = Winneba; TES = Teshie; TMA = Tema; and SEK = Sekondi.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 11
Summary of PAH carcinogenic risk units for smoked fish consumption in Ghana.

PAH	Adult	Child
Benz[a]anthracene	5.3E−08	1.4E−08
Benzo[a]pyrene	1.5E−07	4E−08
Benzo[b]fluoranthene	6.6E−09	1.7E−09
Benzo[k]fluoranthene	4.9E−09	1.3E−09
Chrysene	5.1E−10	1.3E−10
Dibenz[a,h]anthracene	3.8E−07	1E−07
Indeno[1,2,3-cd]pyrene	6.5E−09	1.7E−09
Total	6.1E−07	1.6E−07

Table 12
Summary of PAH hazard quotients for smoked fish consumption in Ghana.

PAH	Adult	Child
Acenaphthene	7.50E−06	8.80E−06
Anthracene	1.10E−06	1.20E−06
Fluoranthene	7.50E−05	8.70E−05
Fluorene	1.10E−04	1.30E−04
Pyrene	1.60E−04	1.90E−04
Total	3.50E−04	4.10E−04

and 2 out of every 10,000,000 children in Ghana may suffer from cancer and cancer-related diseases during their lifetime through the ingestion of carcinogenic PAHs (Table 11) from smoked sardines in their diets.

The hazard quotient calculated (Table 12) shows a total of 3.5×10^{-4} for adult and 4.1×10^{-4} for children. These results suggest that approximately about 4 out of 10,000 adults and at least 4 out of 10,000 children in Ghana may also suffer from some non-cancer-related ailments in their lifetime through the ingestion of these hazardous PAHs (Table 12) from smoked fish in their diets.

4. Conclusion

From the results discussed above, it may be concluded that smoked sardines on the Ghanaian markets showed elevated levels of polycyclic aromatic hydrocarbons (PAHs), and this may be a contributing factor to the recent increases in prevalence rates in cases of cancer and cancer-related ailments in Ghana; further studies are required to confirm this conclusion. The high levels of PAHs in smoked sardines is a result of uncontrolled fish-smoking practices that burn wood at higher temperatures, coupled with thermal pyrolysis of fat in fatty fish at higher temperatures to give the fish a longer shelf-life, but which also promotes PAH production. There is also a risk of higher levels of PAH during the extreme off-season (dry season) of the species, when lipid content of the fish is comparatively higher. This study found that fish smoking practices employed by fishmongers in Ghana are similar throughout the nation. There is therefore a need to educate fishmongers about safe smoking practices, and also most importantly to adopt a fish-smoking procedure that would reduce considerably the levels of toxins in fishes smoked with traditional kilns in order to ensure not only the health safety of consumers but also that of fishmongers exposed to smoke during fish-smoking processes.

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Appendix A. Standard and instrumental calibration standards

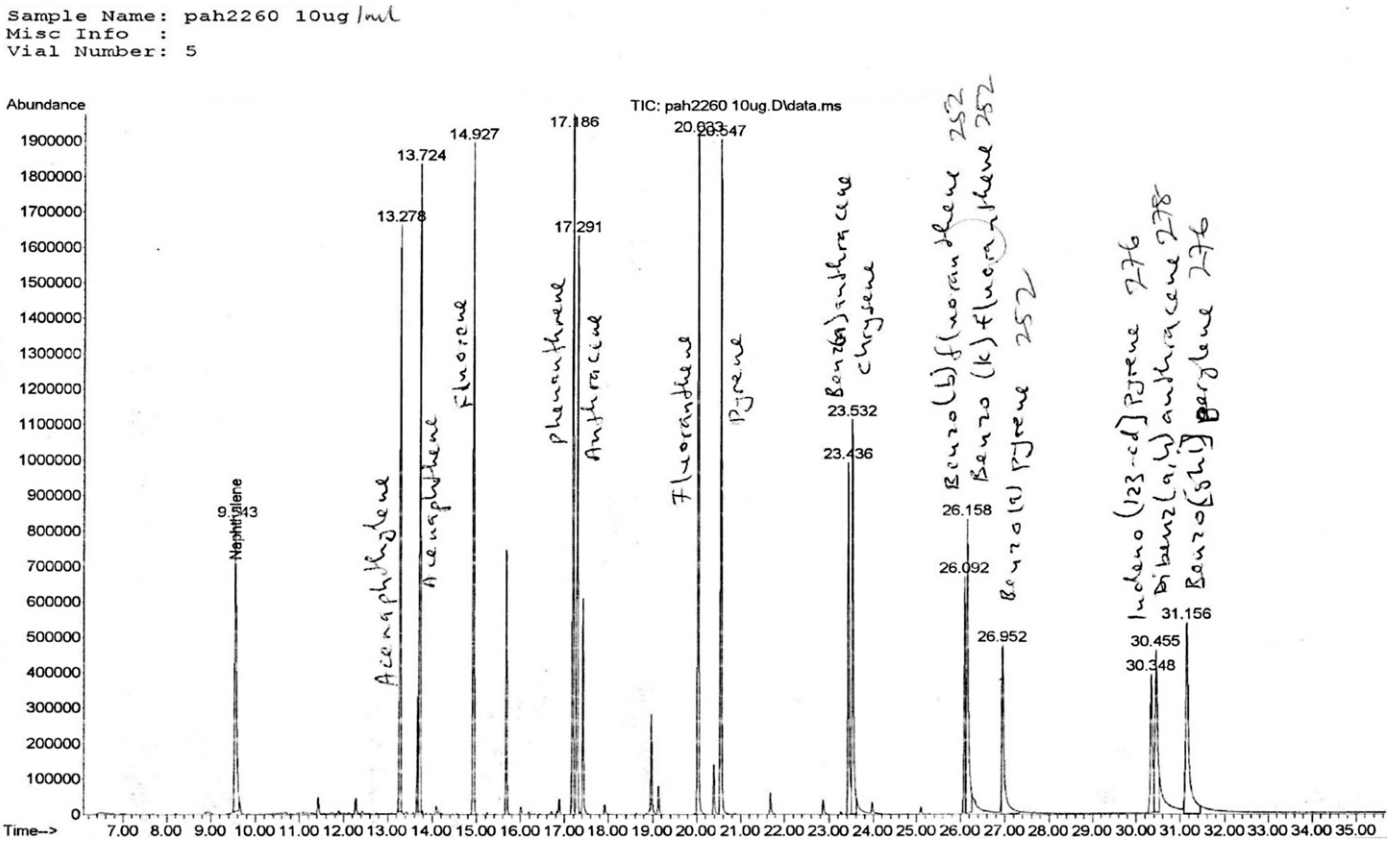

Fig. A1. GC/MS chromatograph showing peaks of the PAHs identified in the calibration standard mix for this study.

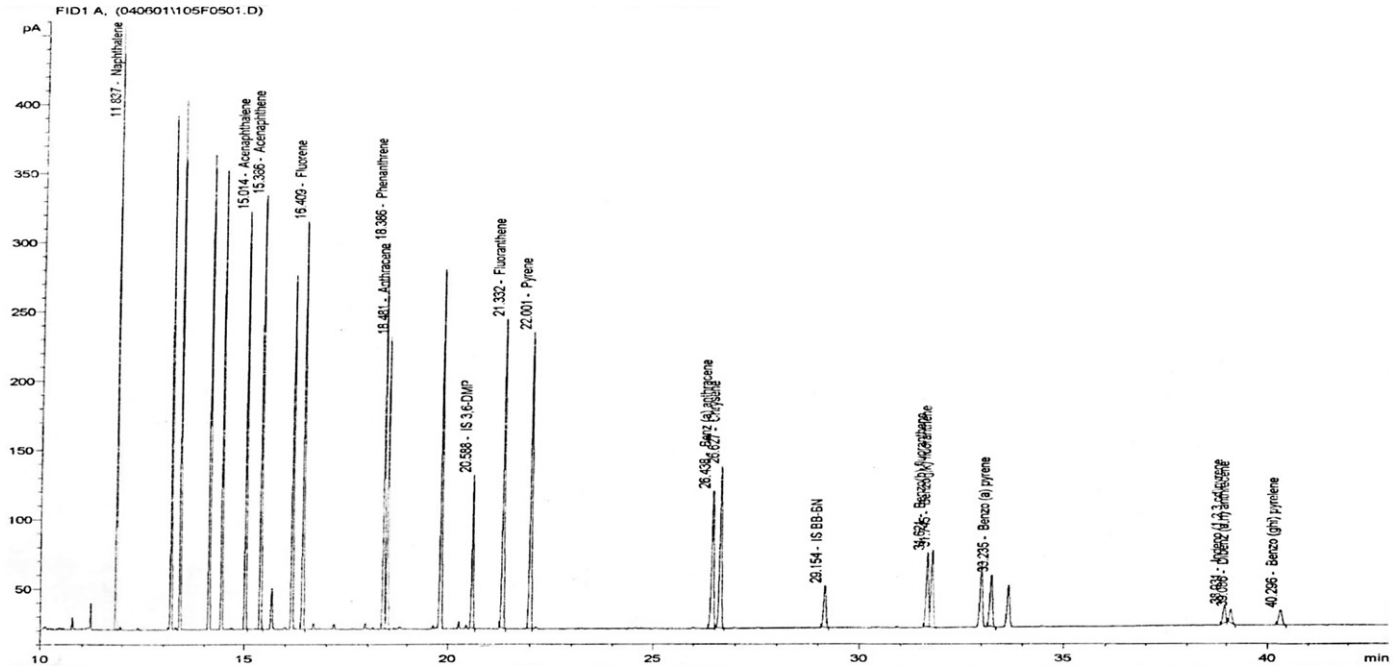

Fig. A2. Chromatogram showing peaks for PAH calibration of standard mix with good baselines during instrumental calibration for this study.

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