Vol. 17(51), pp. 1403-1411, 19 December, 2018
DOI: 10.5897/AJB2016.15556
Article Number: 0807D3859629
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of heat sources on the levels of polycyclic aromatic hydrocarbon in selected fish samples from Ogidingbe (Escravos Estuaries)

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Received 2 July, 2016; Accepted 22 November, 2018

The riverine areas of the Niger-Delta region of Nigeria are popularly known for their smoked fish from both fresh and salt water. To the people living in these areas, smoking is a method of preservation as the fish is known to undergo spoilage within 8 h of harvest from its natural habitat; though not without the potential health hazard associated with smoked foods. During smoking, polycyclic aromatic hydrocarbons (PAHs) and its carcinogenic derivatives are produced. Two species of locally consumed fish: *Clarias gariepinus* and *Micropogonias undulantes* were used for this study. The *M. undulantes* were collected from the creeks and estuaries of Ogidigbe and the *C. gariepinus* from a local pond, Ereyi farms, in Benin City. They were both analyzed for PAHs level in both the fresh and dried form. Comparison of the PAHs level was also done for various dried samples using four different drying methods. Extraction of the PAHs were carried out using solvents by ultrasonication, clean-up was done using solid phase extraction and thereafter analyzed for 16 US EPA PAHs using gas chromatography-flame ionization detector (GC-FID). The results show that drying with mangrove wood charcoal gave the highest PAHs level and it was for samples harvested from the Escravos Estuaries.

Key words: Benzo(a)pyrene, *Clarias gariepinus, Micropogonias undulantes,* polycyclic aromatic hydrocarbons (PAHs).

INTRODUCTION

Fresh fishes are known to contain oils and plenty of water and thus will undergo spoilage within certain hours of harvest from their natural habitat unless they are placed in refrigerators or preserved by other methods. In Nigeria, fish preservation is either by smoking or drying with charcoal or wood fire and in the process of preservation by drying, the water and oil content of the fish is reduced. Charcoal or firewood drying is frequently practised in Nigeria as a method of fish preservation either due to unavailability of means of refrigeration, non-availability of light, need for ease of transportation of preserved fish to the market without spoilage or because of the flavor the

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smoke or heat adds to the dried fish product. However, this is not without the health implication as these methods of preservation can have negative impact on the health of those consuming them. Processing fresh fish by subjecting them to heat treatment like drying, smoking, roasting, baking, and frying has reportedly impacted and subsequently increased the level of polycyclic aromatic hydrocarbons (PAHs) in them (Ishizaki et al., 2010; Farhadian et al., 2011). The flames used in the drying process contain PAHs that adhere to the surface of the dried fish product (Cross and Sinha, 2004).

The environment from which the fish is harvested is another source through which the fish can bioaccumulate these PAHs, most especially if they are in areas with a lot of oil exploration and exploitation activities. Years back in Delta State and most of the Niger-Delta areas of Nigeria, there has been numerous petroleum leaks from transport vessels, pipeline, incessant gas flaring and exploration wells. Oil spills from production well accidents and bunkery has also resulted in several spills (Farwell et al., 2009; Nwilo et al., 2000; Olagoke, 1996). Olaji et al. (2010) had reported some levels of PAH in cage reared fish in Deghele area of Delta State. Also, Chimezie and Herbert (2007) reported high levels of PAHs in the fish caught in the Niger Delta area. Thus, PAHs' contamination could be from the aquatic environment where they are harvested from.

Consumption of these contaminated locally harvested river and sea food species is a major route for human exposure to these toxic PAHs (Rotkin-Ellman et al., 2012; Xia et al., 2012). Nonetheless, it is difficult to estimate the contribution of each source of PAHs exposure in different individuals as they are also exposed to them in different ways depending on the nature of their job, environment, type of diet and method of food preparation or a combination of two or more of these factors. Thus, there is need for more data on the levels of PAHs in locally harvested fishes from these areas predisposed to oil exploration activities, in a bid to increase the database of PAHs levels in fishes and sea foods from the Niger-Delta rivers and creeks. It is also necessary to use a reliable, accurate and effective method to qualitatively and quantitatively describe the level of PAHs in these samples. The aim of this study was to determine the effect the source of fish and heat treatment would have on the level of PAHs in the fresh and dried fish samples.

MATERIALS AND METHODS

Sample collection

Escravos Estuary in Ogidigben area of Delta State and Ereyi Farms Escravos Estuary in Ogidigben area of Delta State and Ereyi Farms which contains catfish ponds located in Oredo Local Government Area of Edo State were used as the sampling site. These two locations are within the south-south region or Niger-Delta region of Nigeria. Two species of locally consumed fish samples, viz; *Clarias gariepinus* also known as catfish and *Micropogonias undulates* commonly called croaker fish were used in this study. Croaker fish samples were collected from Ogidigben, in Escravos Estuaries with the aid of the local fishermen while catfish were collected from Ereyi Farms local pond in Benin City. The fishermen go into the estuary with fishing boats and nets mostly at nights and early hours of the day to catch the fishes.

On arrival at the shores, the fish samples which are representative of the sampling area are sorted out. Three croaker fish (*M. undulantes)* samples were collected from the catch at every time of collection (bimonthly) and washed. The samples were kept in polythene bags, labelled properly, kept in coolers with ice and transported to the laboratory where they are stored in refrigerator prior to treatment. Catfish (*C. gariepinus*) were harvested from Ereyi Farms from which three were carefully chosen bimonthly for the study.

Sample preparation

The fresh fish samples from the two sampling sites were washed and re-rinsed with distilled water. They were weighed using top loading balance; the lengths were taken using metre rule and then cut into two parts longitudinally. One part was kept back in the refrigerator to be extracted fresh and the other smoked using African traditional processes of smoking using charcoal from mangrove wood, until the fish samples were properly dried. The time taken was noted, the fresh and smoked portions were separately homogenized using a blender and then stored in a refrigerator at 4°C prior to extraction and analysis. In the last month, the fish samples from the two sampling sites were divided into five parts. One was processed fresh while the other four parts were dried using oven, forest wood charcoal (Owewe), mangrove wood charcoal and wood shavings, respectively.

Fish drying

Drying with charcoal

A metal bowl lined with clay of about two inches thick was packed with charcoal and metal gauze placed over it. The charcoal was lit and allowed to burn to certain point before the fish cuts were placed on the gauze over the fire. It was then dried for a period of 4 to 6 h depending on the size or weight of the fish sample. Weighing was done at intervals before the fish was said to be completely dried. Drying was stopped when there was no more change in weight of the fish samples when weighed using electronic balance after interval of 20 min. The samples were allowed to cool, stored in polythene bags and properly labelled. This method of drying was carried out for 10 months of sampling period.

In the last sampling month, three other drying methods were used, namely: drying using electronic oven, drying using charcoal collected from tropical rain forest (owewe) and drying using wood shavings from saw mill. The wood shavings were the ones from white wood used for planks.

Drying with wood shavings

A drum of about 2 to 3 ft long with wire gauze or mesh over it was used. A small section is cut from the base where the wood shaving is added to refuel the oven as the fire is going down.

Gas chromatography operating procedure

HP gas chromatography system 6890 series was used with flame ionization detector. The basic chromatography parameters used for

Table 1. Average PAHs level for *C. gariepinus.*

Parameter	Pond FF	Pond DF	2Pond FF	2Pond DF	3Pond FF	3Pond DF	4Pond FF	4Pond DF	5Pond FF	5Pond DF
Naphthalene	0.005	0.004	0.005	0.014	0.001	0.024	0.007	0.016	0.008	0.023
Acenaphthalene	0.002	0.001	0.002	0.005	0.007	0.011	0.010	0.019	0.004	0.007
Acenaphthene	0.003	0.004	0.006	0.002	0.009	0.011	0.018	0.008	0.004	0.003
Florene	0.003	0.009	0.003	0.004	0.007	0.010	0.010	0.012	0.004	0.004
Phenathrene	0.007	0.005	0.007	0.005	0.005	0.005	0.009	0.010	0.004	0.005
Anthracene	0.003	0.008	0.006	0.010	0.015	0.011	0.001	0.010	0.004	0.003
Fluoranthene	0.003	0.144	0.006	0.004	0.014	0.009	0.000	0.000	0.002	0.004
Pyrene	0.004	0.018	0.006	0.007	0.005	0.008	0.001	0.000	0.006	0.004
Benzo(a)anthracene	0.003	0.001	0.005	0.002	0.001	0.003	0.012	0.028	0.003	0.003
Crysene	0.001	0.003	0.001	0.003	0.000	0.002	0.002	0.000	0.003	0.002
Benzo(b)fluoranthrene	0.000	0.000	0.000	0.002	0.001	0.001	0.000	0.003	0.003	0.001
Benzo(k)fluoranthrene	0.000	0.023	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000
Benzo(a)pyrene	0.000	0.000	0.000	0.003	0.000	0.009	0.000	0.002	0.000	0.001
Indeno(1,2,3) perylene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i) perylene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total PAH (mg/kg)	0.035	0.221	0.047	0.061	0.067	0.105	0.069	0.108	0.045	0.072

Pond FF, Average PAHs concentrations (mg/kg) for samples in the first sampling month for fresh fish samples collected from pond. Pond DF, average. PAHs concentrations (mg/kg) for samples in the first sampling month for dried fish samples collected from pond. 2pond FF, average PAHs concentrations (mg/kg) for samples in the second sampling month for fresh fish samples collected from pond. 2pond DF, average PAHs concentrations (mg/kg) for samples in the second sampling month for dried fish samples collected from pond. 3pond FF, average PAHs concentrations (mg/kg) for samples in the third sampling month for fresh fish samples collected from pond. 3pond DF, average PAHs concentrations (mg/kg) for samples in the third sampling month for dried fish samples collected from pond. 4pond FF, average PAHs concentrations (mg/kg) for samples in the fourth sampling month for fresh fish samples collected from pond. 4pond DF, average PAHs concentrations (mg/kg) for samples in the fourth sampling month for dried fish samples collected from pond. 5pond FF, average PAHs concentrations (mg/kg) for samples in the fifth sampling month for fresh fish samples collected from pond. 5pond DF, average PAHs concentrations (mg/kg) for samples in the fifth sampling month for dried fish samples collected from pond.

the analysis of PAHs were as follows: Initial temperature of 100°C at a rate of 1:4°C min^{-1} with a detector temperature of 300°C and final temperature of 330°C.

Extraction and clean-up of samples

The fresh and dried fish samples were separately ground using a blender (Mikachi meat grinder). The blender was washed, rinsed and re-rinsed with distilled water after using it for each fish sample. 50 g of the ground fish sample was mixed with 25 g of sodium sulphate and 200 ml of 50/50 cyclohexane/acetone mixture in a tight fitted covered amber coloured bottle and 10 ml of the internal standard was added to each bottle. Each bottle containing the sample, solvents mixture, sodium sulphate and the internal standard mix were placed inside the ultrasonic bath (Astrabro ultrasonic cleaner) model 7E for 2 h. The bottles were brought out after every 10 min and shaken.

The extract (25 ml) was then collected using a pipette, filtered and concentrated using rotary evaporator to 5 ml. Each 5 ml concentrate was further concentrated to 1 ml using nitrogen gas. Clean up was done using solid phase extraction with SPE cartridges designed by Biotage and named Isolute*SI* with sorbent Si, sorbent mass 500 mg, 3 ml volume and cyclohexane was employed as the eluting solvent using 10 ml of the eluting solvent. The cleaned up sample was concentrated to 1 ml using nitrogen gas, stored in 1-ml vials and subjected to GC analysis using FID detector.

Statistical analysis

One-way analysis of variance was used to statistically test for the variability of PAHs in fish samples collected from the Escravos Estuaries and pond.

RESULTS AND DISCUSSION

Results obtained (Table 1) show that the average concentration of PAHs for fish samples (*C. gariepinus)* collected from the pond ranged from 0.035 to 0.069 mg/kg, while for dried fish sample, it was from 0.061 to 0.221 mg/kg. Similar trend was observed for fresh and dried fish (*M. undulantes*) collected from Escravos Estuaries ranging from 0.0014 to 0.073 mg/kg and 0.025 to 0.129 mg/kg. Results obtained from dried and fresh sample were not as high as those reported by Nnaji and Ekwe (2018) on smoked fish from Michael Okpara University of Agriculture, Umudike in Abia State of Nigeria. Concentration of benz[a]anthracene and benzo[a]pyrene obtained in fresh and dried fish for *C. gariepinus* ranged from 0.001 to 0.012 and 0.000 to0.000 mg/kg for fresh fish as well as 0.001 to 0.028 and 0.001 to 0.009 mg/kg for dried fish from pond; whereas

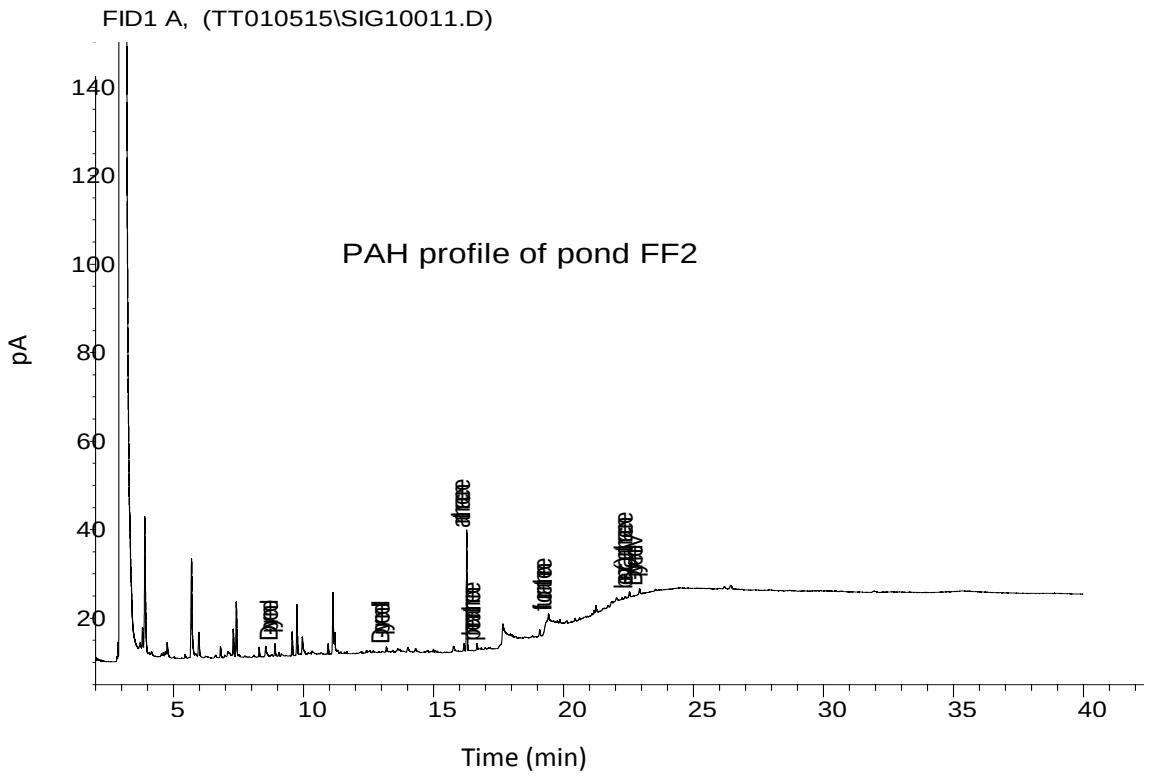

Figure 1. PAHs profile of fresh pond catfish oil sample 2 in the first sampling month.

those from Escravos Estuaries ranged from 0.003 to 0.0029 and 0.00 to 0.029 mg/kg for fresh fish as well as 0.001 to 0.012 and 0.001 to 0.029 mg/kg for dried fish (*M. undulantes*). This value is far more than that reported by Pierre et al. (2012) on smoked fish samples sold in Abobo Abidjan. Benzo[a]pyrene may be bio transformed in humans and animals to 3-OH benzo[a]pyrene (3-OH-B[a]P) (Rey-Salgueiro et al., 2009). 3,4-Benzopyrene, found in smoked products, serves as an indicator of the possible presence of other PAHs and has been repeatedly used as a quantitative index of chemical carcinogens in foods. It has also been reported by the International Agency for Research on Cancer (IARC) that benzo[a]pyrene and benz[a]anthracene and are probably carcinogenic to humans.

Figure 1 shows a gas chromatogram of PAHs' profile of oil extracted from fresh catfish harvested from pond (Pond FF2) with total PAHs concentration of 0.019 mg/kg which is in the range of 0.035 to 0.069 mg/kg as stated earlier in Table 1. Figure 2 shows gas chromatogram of PAHs profile of oil extracted from fresh croaker fish harvested from Escravos Estuaries (ESC FF2) with a total PAHs concentration of 0.009 mg/kg which agrees with the range stated in Table 2. Table 4 gives average PAHs concentration for *M. undulantes* from Escravos using different drying methods and samples dried using the mangrove wood charcoal were found to have the highest average concentration of 0.457 mg/kg; The ones dried using oven and wood shavings were almost having the same values of 0.244 and 0.221 mg/kg, respectively; For individual PAHs 0.136 mg/kg was recorded for pyrene in mangrove wood charcoal dried samples as the highest and is found to be the reason why the mangrove wood charcoal dried samples have the unusually high PAHs concentration when compared with the rest samples.

Figures 3, 4 and 5 give a PAHs profile of oil extract of catfish harvested from the pond but dried using different drying methods namely wood shavings, oven and mangrove wood charcoal, respectively. Figure 6 is a gas chromatogram of oil extracted from croaker fish harvested from the Escravos Estuaries and dried using mangrove wood charcoal.

The results for test of variability using one way analysis of variance show that there was no significant difference in concentration of various PAHs except for *M. undulantes* samples collected from Escravos Estuaries dried using the four different drying methods mentioned in this study [drying with Mangrove wood charcoal, forest wood charcoal (Owewe), oven and wood shaven)]. This result showed that there was significant variation among the values of PAHs in the samples tested as $F_c > F_{t\alpha}$ (3.69>2.45). This may be due to difference in combustion rate of the drying method. According to Rey-Salgueiro et

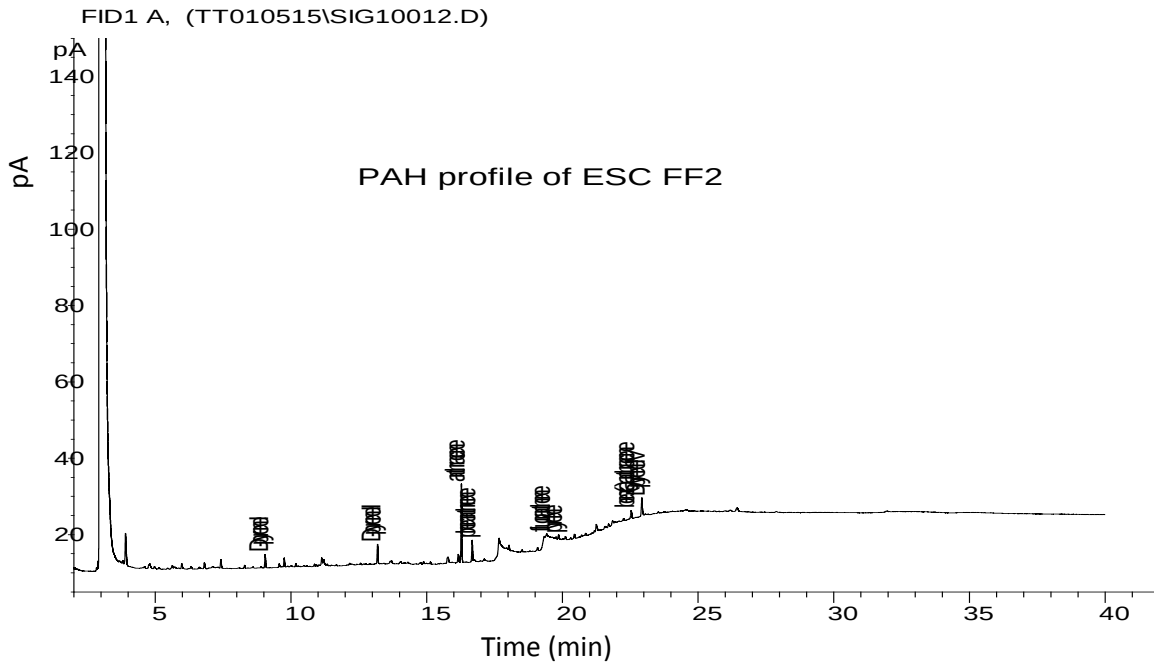

Figure 2. PAHs profile of fresh Escravos fish oil sample in the first sampling month.

Table 2. Average PAHs level of *M. undulantes.*

Parameter	ESC FF	ESC DF	2ESC FF	2ESC DF	3ESC FF	3ESC DF	4ESC FF	4ESC DF	5ESC FF	5ESC DF
Naphthalene	0.000	0.005	0.000	0.003	0.001	0.021	0.016	0.016	0.005	0.039
Acenaphthalene	0.001	0.005	0.013	0.012	0.007	0.023	0.016	0.019	0.008	0.016
Acenaphthene	0.002	0.001	0.006	0.007	0.002	0.005	0.018	0.008	0.022	0.027
Florene	0.001	0.004	0.007	0.006	0.006	0.014	0.010	0.012	0.005	0.014
Phenathrene	0.001	0.002	0.011	0.010	0.009	0.009	0.009	0.010	0.004	0.001
Anthracene	0.000	0.000	0.006	0.025	0.004	0.014	0.001	0.010	0.001	0.004
Fluoranthene	0.000	0.001	0.000	0.007	0.002	0.006	0.000	0.000	0.000	0.006
Pyrene	0.002	0.000	0.000	0.000	0.002	0.027	0.001	0.000	0.000	0.007
Benzo(a)anthracene	0.003	0.000	0.029	0.029	0.006	0.003	0.012	0.028	0.013	0.008
Crysene	0.000	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.004	0.001
Benzo(b)fluoranthrene	0.000	0.002	0.000	0.000	0.003	0.002	0.000	0.003	0.000	0.000
Benzo(k)fluoranthrene	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(a)pyrene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000
Indeno(1,2,3) perylene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i) perylene	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Total PAH (mg/kg)	0.014	0.025	0.073	0.099	0.041	0.129	0.084	0.108	0.063	0.124

ESC FF, Average PAHs concentrations (mg/kg) for samples in the first sampling month for fresh fish samples collected from Escravos. ESC DF, average PAHs concentrations (mg/kg) for samples in the first sampling month for dried fish samples collected from Escravos. 2ESC FF, average PAHs concentrations (mg/kg) for samples in the second sampling month for fresh fish samples collected from Escravos. 2ESC DF, average PAHs concentrations (mg/kg) for samples in the second sampling month for dried fish samples collected from Escravos. 3ESC FF, average PAHs concentrations (mg/kg) for samples in the third sampling month for fresh fish samples collected from Escravos. 3ESC DF, average PAHs concentrations (mg/kg) for samples in the third sampling month for dried fish samples collected from Escravos. 4ESC FF, average PAHs concentrations (mg/kg) for samples in the fourth sampling month for fresh fish samples collected from Escravos. 4ESC DF, average PAHs concentrations (mg/kg) for samples in the fourth sampling month for dried fish samples collected from Escravos. 5ESC FF, average PAHs concentrations (mg/kg) for samples in the fifth sampling month for fresh fish samples collected from Escravos. 5ESC DF, average PAHs concentrations (mg/kg) for samples in the fifth sampling month for dried fish samples collected from Escravos.

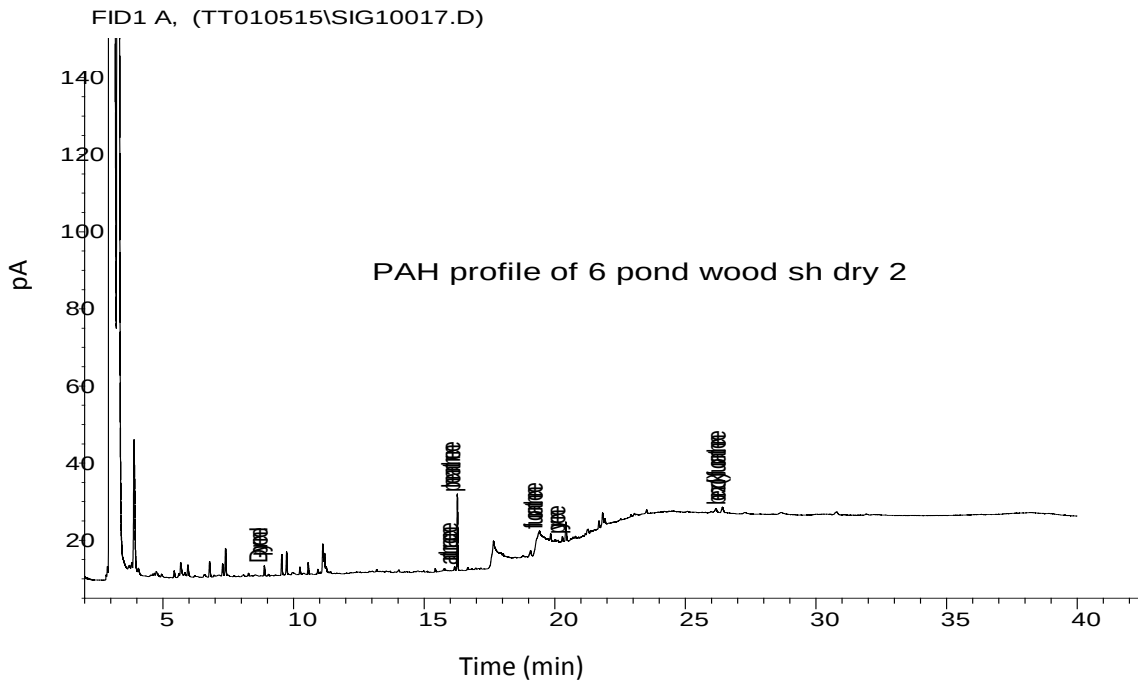

Figure 3. PAHs profile of pond catfish sample dried using wood shavings in the 6th sampling month.

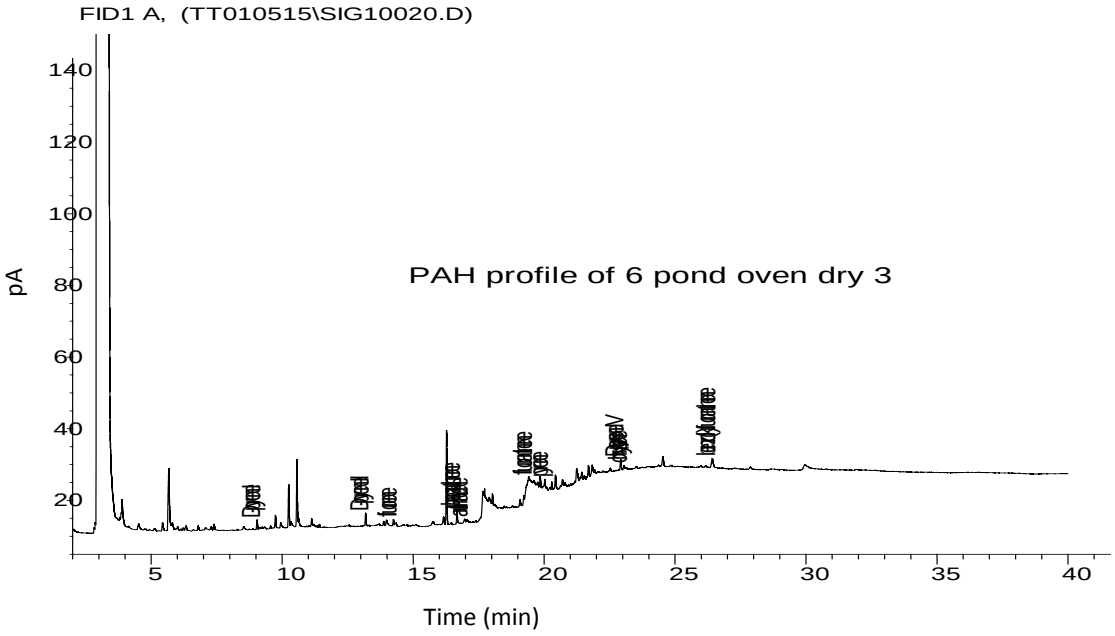

Figure 4. PAHs profile of oven dried pond catfish oil sample in the 6th sampling month.

al., (2004) and Garcia-Falcon and Simal-Gandara (2005), the PAH levels in smoke depend on heat source temperature, flame intensity in flame combustion, and particulate material generated during combustion. The combustion temperature during the generation of smoke seems particularly critical and PAHs are formed during incomplete combustion processes (Muthumbi et al., 2003). According to Zohair (2006), if phenathrene to anthracene ratio is less than 10, combustion is a major source of the PAHs contamination, but if greater than 10

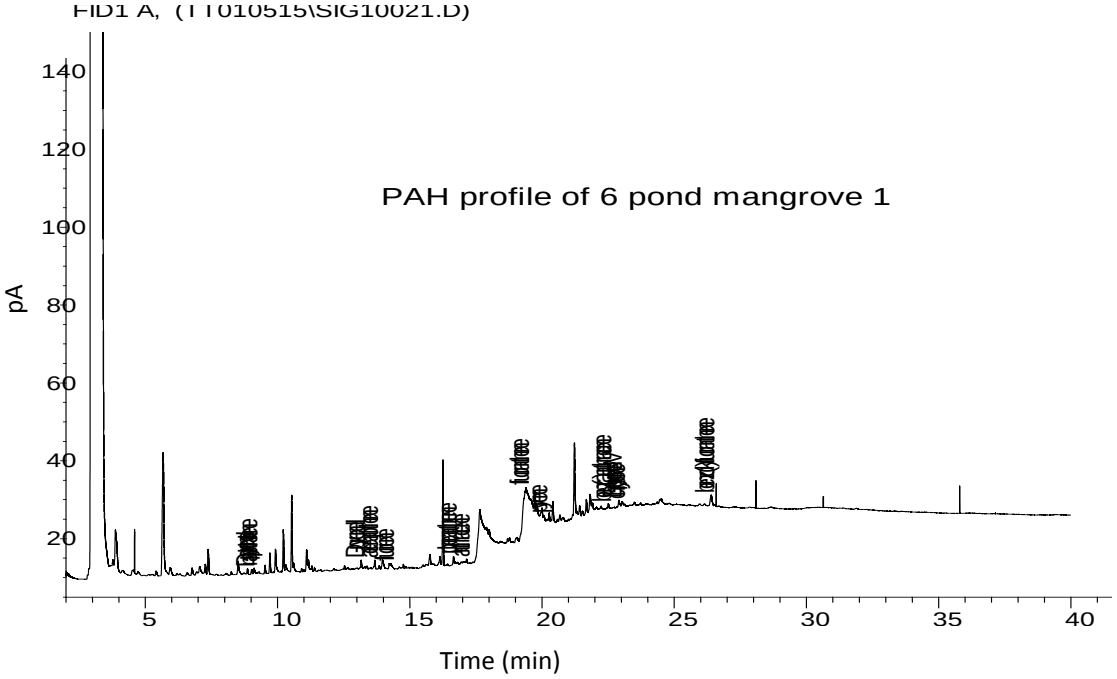

Figure 5. PAHs profile of Mangrove wood charcoal dried pond catfish oil sample in the 6th sampling month.

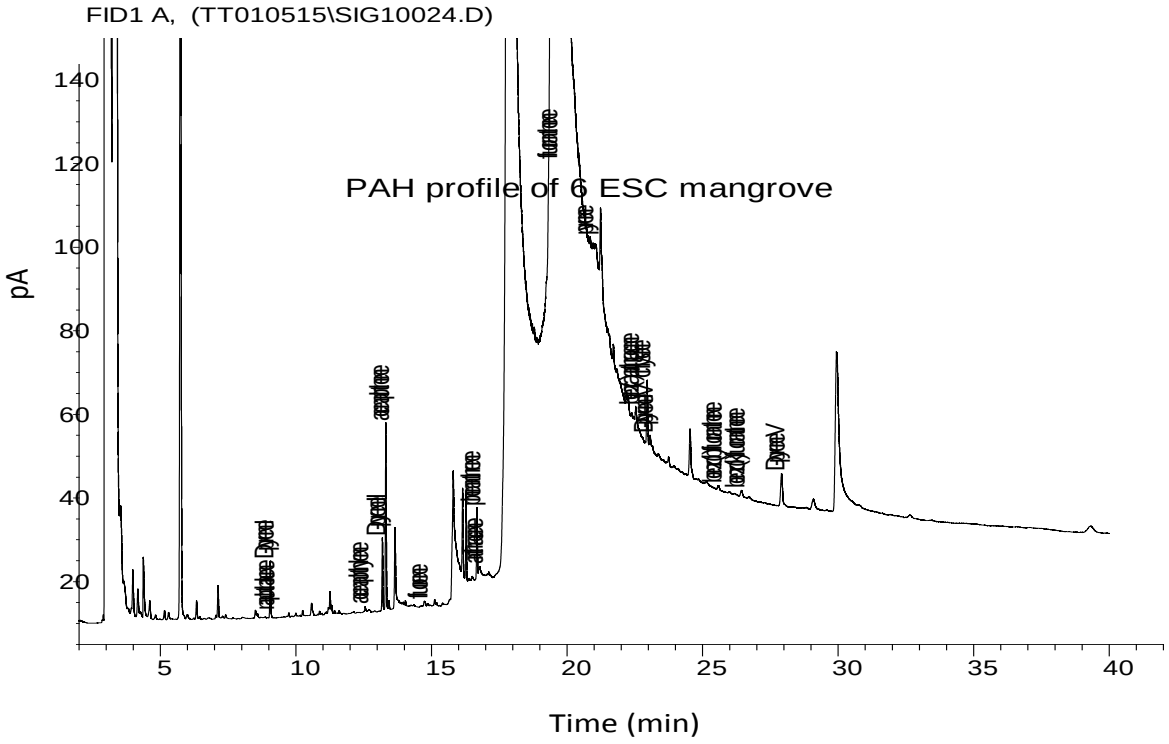

Figure 6. PAHs profile of mangrove wood dried Escravos sample 1 in the 6th sampling month.

it implies that it is petrogenic. From Tables 3 and 4, it was observed that regarding different drying methods for both fish species, the phenathrene to anthracene ratio is less than 10 implying combustion process of the drying source

Table 3. Average PAHs level of *C. gariepinus* using the different drying methods.

Parameter	6pond FF (m/kg)	6pond forest DF (m/kg)	6pond wood shavings DF (m/kg)	6pond mangrove DF(m/kg)	6pond oven DF(m/kg)
Naphthalene	0.007	0.007	0.002	0.013	0.010
Acenaphthalene	0.010	0.000	0.002	0.008	0.007
Acenaphthene	0.018	0.008	0.006	0.012	0.007
Florene	0.010	0.003	0.014	0.006	0.008
Phenathrene	0.009	0.009	0.029	0.009	0.004
Anthracene	0.001	0.001	0.003	0.003	0.007
Fluoranthene	0.000	0.024	0.058	0.086	0.035
Pyrene	0.001	0.001	0.011	0.008	0.014
Benzo(a)anthracene	0.012	0.019	0.003	0.001	0.000
Crysene	0.002	0.000	0.025	0.009	0.005
Benzo(b)fluoranthrene	0.000	0.000	0.006	0.000	0.002
Benzo(k)fluoranthrene	0.000	0.000	0.004	0.004	0.003
Benzo(a)pyrene	0.000	0.000	0.000	0.000	0.000
Indeno(1,2,3) perylene	0.000	0.000	0.000	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i) perylene	0.000	0.000	0.000	0.000	0.000
Total PAH (mg/kg)	0.069	0.073	0.165	0.159	0.101

6pond FF, Average PAHs concentrations for fresh fish from pond in the sixth sampling month. 6pond forest DF, Average PAHs concentrations for forest wood charcoal dried fish from pond in the sixth sampling month. 6pond wood shavings DF, Average PAHs concentrations for fish dried using wood shavings from pond in the sixth sampling month. 6pond mangrove DF, Average PAHs concentrations for fish dried using mangrove wood from pond in the sixth sampling month. 6pond oven DF, Average PAHs concentrations for oven dried fish from pond in the sixth sampling month.

Table 4. Average PAHs level of *M. undulante* using the different drying methods.

Parameter	6ESC FF (m/kg)	6ESC Oven DF (m/kg)	6ESC forest DF (m/kg)	6ESC mangrove DF (m/kg)	6ESC wood shavings DF (m/kg)
Naphthalene	0.029	0.051	0.005	0.013	0.024
Acenaphthalene	0.003	0.034	0.007	0.011	0.007
Acenaphthene	0.002	0.022	0.006	0.044	0.082
Florene	0.002	0.038	0.026	0.001	0.008
Phenathrene	0.005	0.020	0.012	0.021	0.004
Anthracene	0.003	0.013	0.011	0.064	0.026
Fluoranthene	0.001	0.007	0.000	0.056	0.020
Pyrene	0.003	0.011	0.000	0.136	0.022
Benzo(a)anthracene	0.002	0.031	0.002	0.078	0.009
Crysene	0.008	0.002	0.000	0.028	0.008
Benzo(b)fluoranthrene	0.000	0.000	0.000	0.001	0.011
Benzo(k)fluoranthrene	0.000	0.000	0.000	0.004	0.000
Benzo(a)pyrene	0.000	0.000	0.000	0.000	0.000
Indeno(1,2,3) perylene	0.000	0.015	0.001	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i) perylene	0.000	0.000	0.000	0.000	0.000
Total PAH (mg/kg)	0.059	0.244	0.069	0.457	0.221

6ESC FF, Average PAHs concentrations for fresh fish from Escravos in the sixth sampling month. 6ESC Oven DF, average PAHs concentrations for oven dried fish from Escravos in the sixth sampling month. 6 ESC Forest DF, average PAHs concentrations for forest wood charcoal dried fish from Escravos in the sixth sampling month. 6ESC Mangrove DF, average PAHs concentrations for Mangrove wood charcoal dried fish from Escravos in the sixth sampling month. 6ESC Wood Shavings DF, average PAHs concentrations for fish dried using wood shavings from Escravos in the sixth sampling month.

as an agent of petroleum hydrocarbon. Effect of combustion from drying method followed the sequence, wood shaving > forest wood charcoal > mangrove wood > oven for *C. gariepinus* and oven > forest wood charcoal > mangrove > wood shaving for *M. undulante*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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