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Evaluation of Polycyclic Aromatic Hydrocarbons Concentrations in Locally Smoked and Fresh Clarias Gariepinus from Government Assisted Clustered Fish Farm, Ikorodu, Lagos State, Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aim: To evaluate the polycyclic aromatic hydrocarbons (PAHs) concentrations in fresh and smoked *Clarias geriepinus*.
Study Design: Fifty numbers of fresh *Clarias geriepinus* which range in weight between 200.5g and 655.2g were collected from selected clustered farmers and categorized based on their weight into five groups. The tissues were collected from each fresh fish for analysis of PAHs before smoking.

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Place and Duration of Study: The sampled fish were collected from government assisted clustered fish farm estate. The analyses for PAHs were conducted for two weeks in year 2022 at the central laboratory of Nigeria Institute for Oceanography and Marine Research
Methodology: The laboratory procedures and analyses for the detection of sixteen PAHs in the muscles of fresh and smoked on each five grouped of *C. geriepinus* were carried out using Gas Chromatography (Model HP 6890A).
Results: The results shows presence of PAHs in both locally smoked and fresh *Clarias geriepinus* therefore, there is no significant difference in the means concentrations of PAHs in both tissues but significant difference occurs (*p=.05*) in the grouped smoked fish with Indeno (1, 2, 3-c, d) Pyrene mean concentrations higher in both fresh and smoked fish.
Conclusion: PAHs concentrations from both fresh and smoked *C. geriepinus* muscles are below the FAO/WHO threshold limit. It is therefore safe for human consumption but a gas or electric powered smoking kiln is hereby recommended to prevent further accumulation of PAHs in smoked fish which could be carcinogenic to human consumers.

Keywords: Polycyclic aromatic hydrocarbons; Clarias geriepinus; food toxicity; degradation.

1. INTRODUCTION

Food toxicity from harmful substances and the determination of lethal concentrations for consumers are the mainstream of food safety and standards regulatory agencies all over the world. In most cases, the toxic substances in food are usually chemical compounds that are either elemental or complex harmful materials or rather organic toxicants such as polycyclic aromatic hydrocarbons (PAHs) [1].

According to [1] PAHs are a group of organic compounds consisting of two or more fused aromatic rings and a group of lipids soluble compounds that are carcinogenic in nature and their presence have been found in all bionetworks [2]. The formation of PAHs is known by researchers to be through the natural processes of carbonization and subsequent separations into small amount of persistent organic pollutants that usually occur in the entire biospheres [3-5] formed from distance and settled elsewhere or

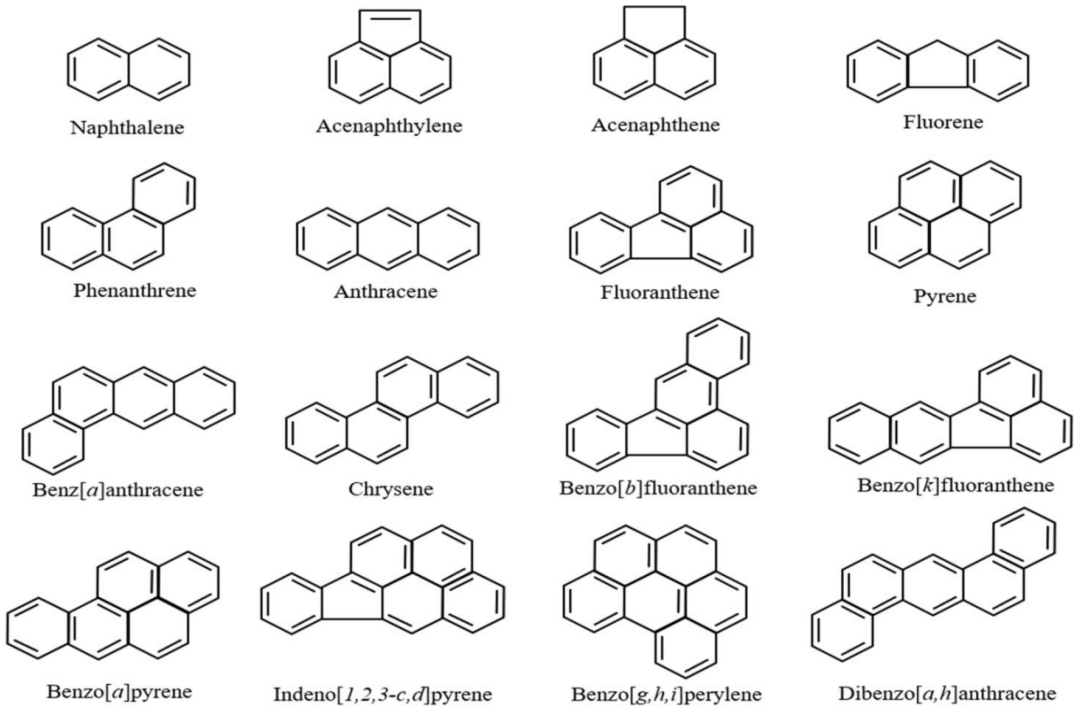

Fig. 1. Chemical structure of 16 PAHs
Source: [10]

formed as a native of a particular place (allochthonous and autochthonous) [6], and it could also be a by-product from incomplete combustion or burning organic matter such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products, with combustion sources predominating during industrial processing or from natural processes such as volcanic eruptions and, various anthropogenic activities [7].

PAHs are dispersed extensively into the atmosphere and this could form another complex compounds with sulfur dioxide, nitrogen oxides, and ozone pollutants under processes known as Photo-Degradation [8]. There are sixteen (16) known PAHs and their sources are but not limited to; coal tar, petroleum fraction, diesel exhaust fumes, spruce needle, tree leaves, grass and plants, vehicle exhaust, processing of organic material, emissions from petroleum refining, incomplete combustion of organic matter, agricultural burning and hazardous waste sites, tobacco smoke, coal tar, soot and certain food products, especially smoked and barbecued foods [9] and [8].

Ifegwu et al. [11] Classified PAHs as ninth among other chemical compounds causing health issues in humans. The health risk factor associated with PAHs could serve as precursor for incurable diseases such as cancer attacks on consumers of such contaminated food items.

In Nigeria, smoked or barbecued fish is one of the consumer's preferences for eating fish, and data shows that out of 30,000 Tons of various freshwater and brackish water fish species produced in Nigeria, about 61% are smoked [12]. Fish smoking is one of the prehistoric technologies of fish processing and preservation that has been assisting fishermen, fish farmers and fish mongers for ages. Smoked fish commands a high market price due to its aesthetic taste and availability for consumption during periods of fresh fish scarcity and most times, some species of fish are considered as a "status food" when smoked. The traditional fish smoking techniques involve subjecting the entire or gutted fish to smoldering wood or charcoal that comes into direct contact with the fish and this process gives opportunity for the accumulation of PAHs on fish with its attendant carcinogenic effect on consumers [13]. The rate of accumulation of PAHs on smoked fish depends on the techniques, combustion temperature and intensity of smoking [14].

Clarias gariepinus, is a cultivable fish species of great economic importance in Nigeria. The species is widely accepted and most times, it is at the center of meal in some households in Nigeria. It is a preferred source of animal protein due to its distinctive taste, flavor and tenderization [15]. Smoking of *C. gariepinus* is one of the most popular methods of processing and preserving the fish as well as improving the taste qualities of the fish [16].

Government assisted clustered fish farm estate in Ikorodu occupies about 34 hectares of land with 250 prospective fish farmers that form clustered groups with a production capacity of over 12 tons of both fresh and smoked fish per annum, mainly *Clarias gariepinus*. This annual tonnage of fish production can support meeting the protein needs of about one-third of Lagos population annually. Hence, the need to conduct a toxicological study on the fish produced considering the number of people that may be predisposed to these harmful substances and make recommendations to appropriate authority.

2. MATERIALS AND METHODS

Clarias garipenus with a weight range between 200.5g and 655.2g were randomly taken from government assisted clustered fish farm estate, Ikorodu, Lagos State for the analyses of sixteen PAHs. Fifty (50) numbers of *C. garipenus* were collected from selected clustered farmers and are separated into 5 groups based on their weight range (Table 1).

Table 1. Grouped wet weight of *C. garipenus* for PAHs determination

Group	Number of fish	Weight Range (g)
1	10	200.5 – 220.1
2	10	315.2 - 325.5
3	10	410.3 – 455.5
4	10	515.4 – 550.3
5	10	620.2 – 655.2

Fish muscles were taken from each of the 50 fresh *C. garipenus* and tagged before smoking accordingly to avoid mix-ups. Likewise, fifty (50) samples of *C. garipenus* muscles from the tagged fish were taken after smoking the same set of fish. The samples collected was subjected to complete randomized experimental design.

The smoked *C. garipenus* was subjected to the same smoking temperature between 66^0c and 77^0c for 6 hours in a 1000kg charcoal-powered smoking kiln. From each smoked and fresh *C. garipenus*, 10g of fish muscle was taken respectively at the ventral axis of the fish girth and the samples collected were conveyed to the laboratory using standard chains of custody involved in PAHs sampling and assessment. The analysis of PAHs concentrations in the muscles of fresh and smoked *C. geriepinus* was carried out at the central laboratory of Nigeria Institute for the Oceanography and Marine Research, Lagos.

2.1 Investigated Organics Toxicant

Nephthalene, Acenaphthylene , Fluorene, Phenanthrene, Anthracene, Pyrene, Benz(a) anthracene, Chrysene, Benzo(b) fluoranthene, Acenaphthene, Benzo [k] Fluoranthene, Benzo [a] pyrene, Benzo [g,h,f] Perylene, Indeno [1,2,3–c,d) pyrene, Fluoranthene and Dibanzo [a,h]Anthracene.

2.1.1 Reagents, stock solution preparation, and extraction methods for fish muscle samples

Fish samples were shade-dried, and separately homogenized with a blender. A 5g portion of each sample homogenate was separately saponified with 200 ml methanol/KOH (12% KOH in 95% methanol) solution in an ultrasonic bath at 60 ^{o}C, for 30 min. The extract was cooled and filtered, through glass wool, into a separatory funnel. The filtrate was extracted twice with 25 ml hexane. The extract was washed with methanol/water (4:1) mixture, and then concentrated to 1ml with a rotary evaporator. The concentrate was fractionated through a silica gel column, first eluted with 10 ml hexane to collect the aliphatic hydrocarbon fraction, and then with 15 ml methylene chloride to collect the aromatic hydrocarbon fraction. The aromatic hydrocarbon fraction was dried over anhydrous sodium sulfate, and then concentrated to 1 ml with a rotary evaporator, and saved in GC vials for subsequent determination of PAHs.

2.1.1.1 Analysis protocol, GC set-up conditions for PAHs and quantitation and detection limits

The extracts in the GC vials were transferred to a GC auto-sampler and analyzed by GC/FID following the procedures specified in the appropriate PAHs method. Gas Chromatography model HP 6890A with Column DB 5 MS (30m x 0.25mm x 0.5um) was used in the determination of PAHs. The oven was set to 40^oC and held for 2 min, then ramp 250^oC at 25^oC/min to 265^oC, and at 5^oC/min to 300oC. Then hold 4 min, at 25^oC. Flame Ionization Detector (FID) was set at 350^oC. The Carrier Gas, Hydrogen at 4 ml/min (constant flow Injector: 350^oC). The Injection volume: 1 uL, splitless, (hold 2 min).1µL of the aromatic hydrocarbon fraction was injected into the GC, set-up for the quantitation of PAHs, using the Agilent Chemstation Software. The lowest concentration of Polycyclic Aromatic Hydrocarbons was taken as detection limits that are extractable with good recoveries. The procedures in PAHs determination in smoked and fresh *C. garipenus* is followed with slight modifications by Mohammadi et al. [17].

Applications and Statistical Analysis: Evaluation of polycyclic aromatic hydrocarbon concentrations in smoked and fresh *C. garipenus* from government assisted clustered fish farm estate and its dietary effect on human health. Statistical significance was assessed using a two-way analysis of variance (ANOVA) and the software used is SPSS version 20. The correlation coefficient and differences between groups were considered significant when $p = .05$ (two tailed).

3. RESULTS AND DISCUSSION

The results of levels of concentrations (µg/kg) of PAHs from GC analyses on fresh and smoked fish muscles are shown in Table 2. It indicated that Dibenz (a,h) anthracene had the highest concentration in fresh fish from Group 3 (0.085µg/kg) while smoked fish had the highest concentration of Benzo (g, h, i) Perylene (0.085µg/kg) in group 1. Benzo (a) Pyrene had the lowest concentrations of 0.001µg/kg in both fresh and smoked fish form groups 5 and 1 respectively. Though, Napthalene, Anthracene, Chrysene, Benzo (b) Fluoranthene, Benzo

(k)Fluoranthene, and Benzo (a) Pyrene concentrations from fresh and smoked fish in both Group 3 are below the detection level (BDL) while Acenaphthylene concentration was below the detection level in Group 1 of fresh fish muscle but detected in all grouped smoked fish muscle. Furthermore, other listed PAHs in Table 2 and their detected concentrations are predominant in all the grouped wet and dried weights of fish. The mean concentrations of PAHs (∑PAHs) present in smoked fish are higher than that in fresh fish, smoked fish muscles had mean total of 0.0014176µg/kg while fresh fish had mean total of 0.0009496µg/kg but Indeno (1, 2, 3-c, d) Pyrene had the highest mean PAHs concentrations in smoked fish while Dibenz (a, h) Anthracene had the highest mean PAHs concentrations in fresh fish as indicated in Table 2. Nevertheless, the mean PAHs concentrations did not increase with increase in the weight of fresh and smoked fish but statistical analysis shows no significant difference in the means concentrations of PAHs in both fresh and smoked fish muscles but significant difference occurs ($p=.05$) in the grouped smoked fish. However, Indeno (1, 2, 3-c, d) Pyrene mean concentrations was higher in both fresh and smoked fish.

The overall concentrations of PAHs shows that PAHs in smoked fish is higher than that of fresh fish in Groups 1 to 4, but in group 5, the overall concentrations of PAHs in fresh fish is higher than that of smoked fish as indicated in the multiple bar chart in Fig. 2. The sixteen types of PAHs concentration between and within the grouped fresh fish in the experiment shows that there is no significant difference among the means concentrations while PAHs concentration between and within the grouped smoked fish also shows that there is no significant difference among the mean concentrations of sixteen types of PAHs but the concentration of each of PAHs type in grouped smoked fish showed that there is significant difference among the mean concentration ($p = .05$). Moreover, positive correlation in PAHs concentrations exists between fresh fish and smoked fish. The dependent variable for all the analyses in the experiment is the concentrations of Polycyclic Aromatic Hydrocarbons in both fresh and smoked fish muscles.

The dietary effect of food on human growth, performance and health has been the major concern of the stake holders saddled with the responsibility of public health, food security and safety. The focus of food growers and food processing industries should be on food items that are consumed to be holistic devoid of toxicants and food poisoning substances that can cause ill-health in human but with high nutritive values. However, the presence of toxicant in some food items has been identified to cause adverse health effects in human consumers with various clinical manifestations and symptoms [19].

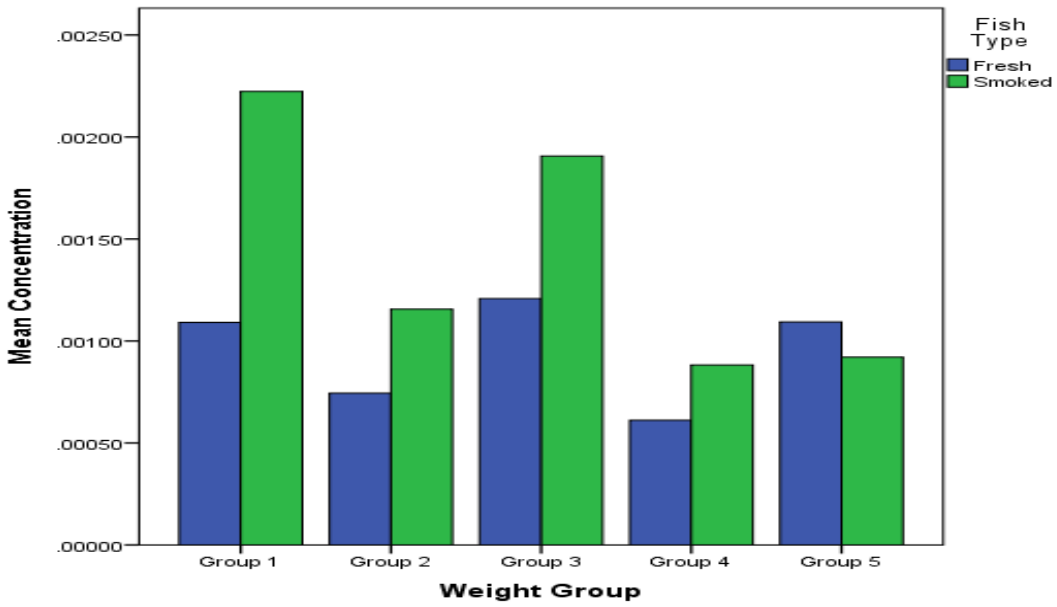

Fig. 2. Overall concentrations of PAHs in smoked and fresh fish

Table 2. Showing mean concentrations of polycyclic aromatic hydrocarbons in fresh and smoked muscle of *Clarias gariepinus*

S/N	PAHs	PAHs Concentrations in Fresh Fish Muscle						PAHs Concentrations in Smoked Fish Muscle					
		Grp. 1 (µg/kg)	Grp. 2 (µg/kg)	Grp. 3 (µg/kg)	Grp. 4 (µg/kg)	Grp. 5 (µg/kg)	Mean (STD±)	Grp. 1 (µg/kg)	Grp. 2 (µg/kg)	Grp. 3 (µg/kg)	Grp. 4 (µg/kg)	Grp. 5 (µg/kg)	Mean (STD±)
1	Napthalene	0.004	0.004	BDL	0.004	0.004	0.003±0.000^{b}	BDL	0.006	BDL	BDL	0.004	0.002±0.001^{d}
2	Acenaphthylene	BDL	0.003	BDL	0.003	0.005	0.002±0.001^{b}	0.004	0.006	BDL	0.008	0.004	0.004±0.002^{d}
3	Acenaphthene	0.011	0.006	0.011	0.011	0.023	0.013±0.006^{b}	0.044	0.028	0.028	0.024	0.017	0.028±0.010^{a,b,c}
4	Fluorene	0.008	0.007	0.008	0.007	0.008	0.008±0.001^{b}	0.011	0.010	0.011	0.011	0.012	0.011±0.001^{b,c,d}
5	Anthracene	0.013	0.012	BDL	0.011	0.004	0.008±0.004^{b}	0.006	0.015	0.008	0.016	0.013	0.012±0.004^{b,c,d}
6	Phenanthrene	0.004	0.004	0.012	0.004	0.004	0.006±0.004^{b}	0.011	0.006	0.013	0.006	0.003	0.008±0.004^{c,d}
7	Fluoranthene	0.009	0.006	0.008	0.006	0.004	0.007±0.002^{b}	0.014	0.008	0.011	0.010	0.007	0.01±0.003^{b,c,d}
8	Pyrene	0.004	0.004	BDL	0.004	0.004	0.003±0.000^{b}	0.004	0.006	0.011	0.009	0.005	0.007±0.003^{c,d}
9	Benz (a) Anthracene	0.010	0.005	0.011	0.007	0.006	0.008±0.003^{b}	0.011	0.013	0.012	0.007	0.006	0.010±0.003^{b,c,d}
10	Chrysene	0.005	0.005	BDL	0.005	0.004	0.004±0.001^{b}	0.004	BDL	BDL	BDL	0.004	0.002±.000^{d}
11	Benzo (b)Fluoranthene	0.007	0.006	BDL	0.008	0.008	0.006±0.001^{b}	0.008	0.008	BDL	0.008	0.007	0.006±0.001^{c,d}
12	Benzo (k)Fluoranthene	0.007	0.005	BDL	0.006	0.006	0.005±0.001^{b}	0.007	BDL	BDL	BDL	0.005	0.002±0.001^{d}
13	Benzo (a) Pyrene	0.002	0.012	BDL	0.007	0.001	0.004±0.005^{b}	0.001	0.026	BDL	BDL	0.021	0.010±0.013^{b,c,d}
14	Dibenz (a, h) Anthracene	0.071	0.026	0.085	0.005	0.042	0.046±0.033^{a}	0.051	0.043	0.071	0.012	0.024	0.040±0.023^{a}
15	Indeno (1,2,3 – c,d)Pyrene	0.015	0.007	0.031	0.005	0.015	0.015±0.010^{b}	0.095	0.006	0.073	0.033	0.015	0.044±0.038^{a}
16	Benzo (g, h, i) Perylene	0.004	0.004	0.027	0.004	0.036	0.015±0.015^{b}	0.085	BDL	0.068	BDL	BDL	0.031±0.012^{a,b}
	Total PAHs (µg/kg)	0.174	0.116	0.193	0.097	0.174		0.356	0.181	0.306	0.144	0.147	
	Mean of PAHs (∑PAHs)	0.011^{a}	0.008^{a}	0.012^{a}	0.007^{a}	0.011^{a}		0.024^{a}	0.014^{ab}	0.031^{ab}	0.013^{b}	0.010^{b}	
	STD ±	0.018	0.006	0.026	0.002	0.012		0.031	0.012	0.028	0.008	0.007	

** Grp = Group* *** [18] Threshold limit =10µg/kg)* **** BDL = below detection level*

Fresh *C. geriepinus* that was taken from rearing pond from fish farm estate had some levels of PAHs concentrations in their muscle which may probably be gotten from ingestion of fish feed because they were not exposed to direct carbonization during rearing. The contamination of food items could be from the raw ingredients before harvesting or during storage or processing for fish food [20]. Other route by which PAHs can bioaccumulate in the muscle of fresh fish is through PAHs contaminated water that enters into fish pond and permeate through their skin. This process is referred to as "atmospheric deposition" where water is polluted through atmospheric pollutants and such pollutants are detected in fish [21]. Most fish farmers from fish farm estate depends on deep bore-hole water for their source of water and overhead tanks for water storage and the atmospheric carbon can find their way into the storage tank. The levels PAHs concentrations from fresh fish muscle is significantly lower than that of USEPA threshold limit for fish possibly, because the fish are raised under the manageable production system of aquaculture.

The assessment of PAHs concentrations in smoked farmed *C. geriepinus* muscles are also below the USEPA threshold limit but, comparatively it has higher concentrations than that of fresh fish. The elevated concentrations in smoked fish muscle might be due to the charcoal used in smoking the fish. This observation agreed with the scientific publication of [13] on the effect of traditional smoking techniques on quality of smoked fish using charcoal or wood. Moreover, some PAHs elements that are in fresh fish but not detected in the same fish when smoked may perhaps be due to volatility properties of some PAHs types that are initially bioaccumulated in fresh fish but when subjected to heat during smoking becomes volatile [22] as cited by Hussein et al. [19] on the volatility properties of PAHs under certain heat conditions.

All sixteen (16) PAHs tested were found in both fresh and smoked fish muscles of *C. geriepinus,* although some that were found in fresh fish may not occur in smoked fish and vice versa because farmed *C. geriepinus* are known to be rich in saturated fatty acids, monounsaturated fatty acids, and omega-6 polyunsaturated fatty acids [23] PAHs are described lipophilic in nature which result in a strong bonding between the fatty acid deposits and PAHs concentrations in fish [2] and [24]. It is the fatty acid content, properties of the PAHs and methods of rearing and processing the fish that determines the level of PAHs concentrations in their muscles. However, the weight of the fish plays no significant roles in the concentration of PAHs in their muscles.

In addition, it is sufficient to mention the adverse effects of consuming smoked and fresh fish that have accumulated PAHs in their muscles on human health. The health risks involved in ingesting fish laden with PAHs that is above the agency's regulatory permissive limit range from carcinogenic to complex physiological health issues [14]. The presence of Pyrene and benzo (a) pyrene in fresh and smoked *C. geriepinus* muscles could be fatal to human health when consumed. These two types of PAHs have the ability to undergo number of phase transformation and DNA methylation in humans when ingested in food and the concentrations are above threshold limits and when this occur, it could lead to dangerous and unsolicited gene editing or genetic mutagenization in humans as reiterated by Ezike et al. [24] on the effect of using different wood in smoking fish and PAHs concentrations.

4. CONCLUSION

PAHs concentrations from both fresh and smoked *C. geriepinus* muscles from the government assisted clustered fish farm are below the USEPA threshold limit and therefore safe for human consumption but the long-term effect of bioaccumulation and subsequent bio-magnification of it in consumers over time should not be overlooked. The need to reduce the accumulation of PAHs on fish items during processing, preservation and storage is very important. It is therefore, recommended that periodic sensitization programs from The National Agency for Food and Drug Administration and Control in conjunction with, Standard Organization of Nigeria be embarked on to those in the fish production line from this teaming fish farmers from government assisted fish farm estate on the essentiality of quality assurance and quality control using standard operating procedures in fish production, processing, preservation, and storage.

The Government should also endeavour to introduce to the clustered fish farmers the use of gas or electric powered smoking kilns instead of wood or charcoal which will promote PAHs accumulation on the smoked fish. The food and safety regulatory agencies and extension workers from the Ministry of Agriculture should

also visit the clustered fish farmers on a regular basis to educate them and promote new technology in fish processing, preservations and storage that will improve the quality of fish produced. Above all, bush burning should be discouraged and the use of environment-friendly materials should be encouraged.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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