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Assessment of Total Hydrocarbon Concentration in Four Fish Species of Degele Community, Nigeria and Their Dietary Intake in the Populace

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Authors' contributions

This work was carried out in collaboration between all authors. Author EDO designed the study, and wrote the protocol. Authors EDO and NAN wrote the first draft of the manuscript, managed the literature searches, and performed the gas chromatography analysis. Authors AFY and COO managed the experimental process and statistical analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess concentration of total petroleum hydrocarbons (TPHs) in four common fish species of Degele community, Nigeria and estimate their dietary intake in the populace through the consumption of studied fish species.

Study Design: Factorial design.

Place and Duration of Study: Degele, a fishing community in Sapele, Niger Delta, Nigeria between April to August, 2010.

Methodology: Four species of fish namely Tilapia (*Oreochromis niloticus*), Catfish (*Clarias gariepinus* and *Heterobranchus longifilis*), and *Liza falcipinnis* were collected from River Aquirinrin by the help of local fishermen. Fish were samples were stored at -20°C until further analysis. The scales (*O. niloticus*) were sloughed off and muscle tissues were dissected. Gas chromatography with flame ionization detector (GC-FID) was used for the evaluation of TPHs. Dietary intake concentration was calculated by multiplying the PAHs concentration measured in each species of fish by the per capita consumption for Nigeria.

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Results: The concentration of aliphatic hydrocarbon in *Clarias gariepinus* ranged from non-detectable level of 0.001 to 46.7±16.7 µg/kg; wet weight.; *Heterobranchus longifilis* (0.001 to 42.7±17.4 µg/kg; wet weight); *Oreochromis niloticus* (0.001 to 1123.70±952.10 µg/kg; wet wt); *Liza falcipinnis* (0.001 to 29.0±1.4 µg/kg; wet wt). The highest level of aliphatic hydrocarbon concentration was obtained in *Oreochromis niloticus*. The less carcinogenic polyaromatic hydrocarbon of lower molecular weight (LMW PAHs) was detected with clearly observed naphthalene and its substituents in all the studied fish species. The more carcinogenic high molecular weight (HMW) PAHs (BaA, BbF, BkF, BaP, and InP) were not detected in the fish samples analysed. There was significant difference ($P = 0.05$) in the Σ Aliphatic and Σ PAHs concentrations among the fish species. However, the levels are below EU recommended limit of 2µg/kg;wet weight for edible fish. The average intake of PAHs through fish consumption was calculated to be 0.02 – 0.94 mg/kg;body weight/day (*O. niloticus*), 0.02–0.12 mg/kg;body weight/day (*C. gariepinus*), 0.12–0.16 mg/kg;body weight/day (*H. longifilis*) and 0.14–0.58 mg/kg;body weight/day (*L. falcipinnis*). *O. niloticus* contributed to the highest intake.

Conclusion: The observed levels of TPH in fish species from this study indicate that River Aquirinrin in Degele community is not highly contaminated with petroleum hydrocarbons. Therefore, Degele community is less exposed to carcinogenic health risks associated with the consumption of the studied fish. However, continuous monitoring programme should be formulated and conducted to ensure that the concentrations of petroleum hydrocarbons is within the baseline levels established in the present study.

Keywords: Cancer; bioaccumulation; PAHS; oil spillage; Niger Delta.

ABBREVIATIONS

BaA - Benzo(a)Anthracene; BbF - Benzo(b)Fluoranthrene; BkF - Benzo(k)Fluoranthrene; BaP - Benzo(a)Pyrene; InP - Indeno(1,2,3-cd)Perylene.

1. INTRODUCTION

Nigeria is a major oil producer and the sixth largest oil producing country in the world. The Nigeria economy depends heavily on the oil sector where majority of the oil industries are located in the Niger Delta. Niger Delta fishing communities are adversely affected by petroleum production activities. Oil spillage and petroleum products are the major anthropogenic source of total hydrocarbon in the environment [1]. Oil spill is a regular occurrence in Nigeria. This usually results because of corrosion of pipelines and tanks, sabotage, accidents, mishandling, and oil production operation [2]. Oil spillage has led to contamination of aquatic and terrestrial environment. It is estimated that over ten million tonnes of crude oil enters the environment each year from accidental spills associated with routine petroleum operation [3]. Small quantities of crude oil mixed with seawater have been shown to affect the feeding behaviour of fish and shellfish [4]. Crude oil may also reduce growth, tissues, and organ damage in fish. Fish is a low fat, high protein food and constitutes more than 60% of the protein intake of adults in rural areas [5]. Despite the numerous benefits of fish as fish diet, the potential health risk arising from frequent consumption of fish is a great concern. All fish ingest petroleum hydrocarbons directly or indirectly from contaminated water as food and sediments leading to massive destruction of aquatic biota [6]. Both of the aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by most finfish and shellfish because of their high lipid solubility and are bioconcentrated in them [7].

Humans are exposed to total petroleum hydrocarbons through air, water, food, or soil. However, dietary intake has been shown to be a major route for human exposure to petroleum hydrocarbons (PAHs) [8,9]. Several studies have reported the negative effects of petroleum hydrocarbon to human health [10,11,6]. Recently, studies have shown that most human cancers such as prostate and lung cancer can be attributed to dietary sources [8,12].

In Nigeria, there are studies on the presence of petroleum hydrocarbons in Lagos lagoon [13,11], Cross River [6], fish community river of Areba, Niger Delta [14] and waters of Ogoniland [10]. However, there is paucity of information on the presence of petroleum hydrocarbon in Degela community, dietary intake of TPHs and the corresponding risk arising from fish consumption. This study was aimed at assessment of TPH in four common fish species of Degele community of Niger Delta, Nigeria and estimation the dietary intake of PAHs through fish consumption.

2. MATERIALS AND METHODS

2.1 Study Area

Degele is a fishing community in Sapele Local Government of Niger Delta, Nigeria. It is on latitude 5°44'– 50°46' N and longitude 005° 42'– 005°41'E. Figure 1 shows the map of the studied area.



Fig. 1. Map of Nigeria showing Sapele Local Government Area where Degele community is situated. Source: Google Maps 2014

2.2 Fish Sample Collection

Four species of fish namely Tilapia (*Oreochromis niloticus*), Catfish (*Clarias gariepinus* and *Heterobranchus longifilis*) and *Liza falcipinnis* were collected between April and August 2010 from river aqurinrin by the local fishermen with the use of drag net (Mesh size 1.27 cm, thickness 9 ply). The fish samples were collected and wrapped in sterile aluminium foil and stored at -20°C until further analysis.

2.3 Fish Sample Processing and TPH Extraction

Fish samples were removed from the deep freezer, thawed, and cleaned very well in tap water to remove any dirt. Dissection was performed on thawed fish using aseptic instrument and glass dishes. The scales (*O. niloticus*) were sloughed off and muscle tissues were dissected. Each fish sample was cut into pieces and crushed in a mortar with pestle. Ten grams of individual fish sample was weighed using analytical balance into a 100ml beaker and 60ml of TPH extraction mixture (acetone and dichloromethane 1:1 v/v) was added. The fish TPH contents were extracted by a shaking method based on Schwab et al. [15]. The beaker with the content was placed on magnetic stirrer/ heater and shaken for about 10 minutes at 70°C. The extract was decanted into a clean round-bottom flask. 30ml fresh solvent was added and the process repeated. The extracts were combined and 5g of anhydrous sodium sulphate was added to remove water. The extract was concentrated to 3ml with rotary evaporator maintained at 20°C [16]. 1.5ml of the concentrated extract was loaded on a silica gel column. The silica gel column was prepared by loading a 2g glass wool followed by 30g chromatography silica gel, onto a chromatography column (2cm internal diameter and 10cm long).

Each of the bed was conditioned with 40ml HPLC-hexane to remove any organic contaminant. The 1.5ml concentrated extract was loaded and eluted with 30ml HPLC hexane into a labelled 100ml beaker to get the aliphatic hydrocarbon components in the sample. After the hexane had almost eluted through the column, but before completely letting the column dry, 30 mL of dichloromethane was added to elute the aromatic hydrocarbons contents into another labelled 100ml beaker. 2g of anhydrous sodium sulphate was added to remove any traces of water left in the extract. These were re-concentrated using rotary evaporator to about 2ml. 1ml of the extract was transferred into a well labelled chromatography vial ready for gas chromatography analysis. The samples were stored at 4°C until GC analysis.

2.4 Gas Chromatography Analysis

Each extract transferred to 1.5ml vial was loaded into a gas chromatography system 6890 series model G1530 A, with flame ionization detector (FID), and cold on-column injection. 1µl portion of the sample was injected and analysed for TPH (C₉–C₃₆). A HP-5 (cross-slinked PH ME siloxane) column having the dimensions 30m x 0.25mm 1.d with a stationary phase thickness of 0.25µm was used for analytical separation. The carrier gas was purified nitrogen held at a flow rate of 5ml/min. The operating temperature program was started at 60°C for 2mins and then increase at a rate of 10°C/min to 300°C for 10min [17]. The injector and detector temperature were maintained at 250°C and 300°C respectively. The oven temperature was 60°C. The minimum detection limit for all the compounds analysed was 0.1 µg/kg wet weight.

2.5 Estimation of Dietary Intake of TPH through Fish

Dietary intake concentration was calculated by multiplying the PAHs concentration measured in each species of fish by the per capita consumption. Fish consumption rate was set at 68.5 g/day from the annual per capita fish consumption of 25 kg for Nigeria [10].

2.6 Statistical Analysis

The obtained data were analysed with SPSS package (version 16.0), expressed as mean \pm standard deviation and the significant difference among the group was assessed using one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Concentration of TPH among Fish Species

Concentration of individual aliphatic and aromatic hydrocarbon contents in the fish species is shown in Table 1 and 2 respectively. The concentration of aliphatic hydrocarbon in *Clarias gariepinus* ranged from non-detectable level of 0.001 to 46.7 ± 16.7 $\mu\text{g}/\text{kg}$; wet weight; heterobranchus *longifilis* (0.001 to 42.7 ± 17.4 $\mu\text{g}/\text{kg}$; wet wt); *Oreochromis niloticus* (0.001 to 1123.70 ± 952.10 ($\mu\text{g}/\text{kg}$; wet wt); *Liza falcipinnis* (0.001 to 29.0 ± 1.4 $\mu\text{g}/\text{kg}$; wet wt) (Table 1). The highest level of aliphatic hydrocarbon concentration was obtained in *Oreochromis niloticus*. Nkpaa et al. (2013) showed that *Tilapia guineensis* accumulated significantly higher concentrations ($P = 0.05$) of petroleum hydrocarbons (PAHs) compared to *Liza falcipinnis*. The observed difference in aliphatic hydrocarbon concentrations among the fish species may be attributed to differences in feeding preference and general habit. Tilapia in the wild are omnivorous, feeding on a variety of natural food organisms such as plankton, aquatic macrophytes, larval fish, detritus and decomposing organic matter [18]. Olaji et al. [19] showed that TPH in aquatic plants in Degele community and environs ranged from 44.0 to 79.0 $\mu\text{g}/\text{kg}$ wet wt. It is important to note that aquatic invertebrates that are lower in the food chain tend to bio-concentrate petroleum hydrocarbons more in their tissues since they lack the capacity for effective biotransformation [20,21].

The less carcinogenic polyaromatic hydrocarbon of lower molecular weight (LMW PAHs) was detected with clearly observed naphthalene and its substituents in all the studied fish species (Table 2). This is in accordance to the findings of other researchers [11,22]. The more carcinogenic high molecular weight (HMW) PAHs (BaA, BbF, BkF, BaP, and InP) were not detected in the fish samples analysed. This shows that the source of PAHs in the river was mainly from petrogenic sources. The ratio of high molecular weight PAHs (HMW-PAHs) to low molecular weight PAHs (LMW-PAHs) has been used to characterize the origin of PAHs in the environment [23]. According to Rocher et al. [24], petrogenic sources of PAHs show characteristically higher proportion of LMW-PAHs such as naphthalene and acenaphthenes while pyrogenic PAHs have characteristically higher proportion of HMW-PAHs such as pyrene and benzo[a]pyrene. The absence or below detection level of most PAHs may be because of the ability of fish to transform PAHs. According to GESAMP [25], fish tend to concentrate PAHs in their tissues when exposed to petroleum, but they do not retain it indefinitely, leading to these compounds not accumulating in very high concentration in edible tissues [26]. The accumulation and depuration of PAHs in fish can be influenced by various factors including route and duration of exposure, lipid content of tissues, environmental factors, differences in species, age, and sex, and exposure to other

xenobiotics [27]. Johnson et al. [28] in their study to evaluate the effects of PAHs on fisheries resource within Kitimat discovered that even in sole collected from sites near the smelter where sediment PAH concentrations are high, PAH levels in edible muscle tissue ranged low to undetectable. Rose et al. [11] did not detect BaP in any of the fish and invertebrate samples from Lagos lagoon despite the high concentrations (881.240ng/g dry weight) obtained in water. The levels of concentration of contaminants in fish reflect the state of contamination of the environment [29] and therefore the observed levels of TPH in fish species from this study indicate that river aquirinrin in Degele community is not highly contaminated with petroleum hydrocarbon. Therefore, Degele community is less exposed to carcinogenic health risks associated with the consumption of the studied fish.

Table 1. Individual Aliphatic Hydrocarbons Content ($\mu\text{g}/\text{kg}$;wet weight) in fish from River Aquirinrin

Component	Fish species			
	<i>C. gariepinus</i>	<i>H. longifilis</i>	<i>O. niloticus</i>	<i>L. falcipinnis</i>
Nonane	3.30 \pm 5.80	12.00 \pm 3.60	16.00 \pm 3.60	5.50 \pm 0.7
Decane	1.30 \pm 2.30	1.00 \pm 1.70	1.30 \pm 2.30	29.00 \pm 1.400
Dodecane	1.13 \pm 1.18	35.00 \pm 13.10	28.30 \pm 2.10	ND
Tetradecane	10.70 \pm 2.10	2.70 \pm 4.60	11.70 \pm 1.20	ND
Hexadecane	11.30 \pm 11.00	10.7 \pm 7.20	16.00 \pm 11.30	ND
Octadecane	7.30 \pm 3.20	7.00 \pm 1.00	4.30 \pm 7.50	ND
Nonadecane	7.00 \pm 6.10	4.00 \pm 3.50	3.70 \pm 3.20	ND
Eicosane	15.30 \pm 4.20	11.70 \pm 3.80	31.00 \pm 6.60	ND
Docosane	46.70 \pm 16.70	42.70 \pm 17.40	1123.70 \pm 952.10	ND
Tetracosane	29.30 \pm 30.90	33.30 \pm 16.30	26.00 \pm 45.00	ND
Hexacosane	44.30 \pm 76.80	35.30 \pm 61.20	53.00 \pm 91.80	ND
Octacosane	ND	ND	ND	ND
Triacosane	ND	ND	ND	ND
Hexacosane	ND	ND	ND	ND

ND = not detected (below detectable limits)

Table 2. Individual Aromatic Hydrocarbons Content ($\mu\text{g}/\text{kg}$;wet weight) in fish from River Aquirinrin

Component	Fish species			
	<i>C. gariepinus</i>	<i>H. longifilis</i>	<i>O. niloticus</i>	<i>L. falcipinnis</i>
Naphthalene	4.3 \pm 0.6	1.7 \pm 0.6	1.7 \pm 0.6	2.0 \pm 0.0
2-methylenaphthalene	0.3 \pm 0.6	0.3 \pm 0.6	2.3 \pm 1.2	ND
Acenaphthalene	3.0 \pm 2.6	0.3 \pm 0.6	1.7 \pm 0.6	ND
Acenaphthene	1.0 \pm 1.7	0.3 \pm 0.6	ND	ND
Florene	3.0 \pm 2.6	1.7 \pm 0.6	ND	4.5 \pm 0.7
Phenathrene	2.7 \pm 2.3	ND	ND	8.5 \pm 0.7
Anthracene	13.7 \pm 2.3	ND	ND	4.0 \pm 0.0
Fluoranthene	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND
Benzo(a)anthracene	ND	ND	ND	ND
Crysene	ND	ND	ND	ND
Benzo(b)fluoranthrene	ND	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND
Benzo(k)fluoranthrene	ND	ND	ND	ND
Indeno(1,2,3)perylene	ND	ND	ND	ND
Benzo (g,h,i) perylene	ND	ND	ND	ND
Benzo(a,h)anthracene	ND	ND	ND	ND

ND = not detected (below detectable limits)

Table 3 shows the concentration of total aliphatic hydrocarbons (Σ Aliphatic) and different total PAHs (Σ PAH) in the studied fish species. The sum PAHs of fish from different water bodies in Niger Delta, Nigeria averaged 100 μ g/kg [30]. There was significant difference ($P = 0.05$) in the Σ Aliphatic and Σ PAHs concentrations among the fish species. However, the levels are below EU recommended limit of 2 μ g/kg; wet weight for fish [10].

Table 3. Summation of total petroleum hydrocarbons in selected fish species (mg/kg; wet weight)

Component	Fish species				P value
	<i>C. gariepinus</i>	<i>H. longifilis</i>	<i>O. niloticus</i>	<i>L. falcipinnis</i>	
Σ Aliphatic	0.188 ^a \pm 0.122	0.196 ^b \pm 0.059	1.315 ^a \pm 0.808	0.034 ^b \pm 0.001	$P = 0.05$
Σ PAH	0.038 ^b \pm 0.025	0.003 ^b \pm 0.002	0.005 ^b \pm 0.004	0.019 ^a \pm 0.001	$P = 0.05$

NOTE: $P = 0.05$ Highly Significant

3.2 Dietary Intake of PAH through Fish Consumption

Table 4 shows the estimated dietary intake concentration of PAHs through the consumption of the studied fish species. The average intake of PAHs through fish consumption was calculated to be 0.02–0.94 mg/kg; body weight/day (*O. niloticus*), 0.02–0.12 mg/kg; body weight/day (*C. gariepinus*), 0.12–0.16 mg/kg; body weight/day (*H. longifilis*) and 0.14–0.58 mg/kg; body weight/day (*L. falcipinnis*). *O. niloticus* contributed to the highest intake. Thus, the consumption of *O. niloticus* at the rate of 68g/day with time may induce adverse health effects. There are limited literatures in Nigeria regarding dietary intakes of PAHs through fish consumption. Nkpaa et al. [10] observed that the potency equivalent concentration (PEC) value exceeded Screening Value (SV) in all the fish analysed in their study on assessment of polycyclic aromatic hydrocarbons (PAHs) levels in two commercially important fish species from crude oil polluted waters of Ogoniland and their carcinogenic health risks. The calculated amount of average dietary intake of PAHs in humans through fish consumption in Degele community is lower to that in other countries. An estimated dietary intake of 1.77–10.7 ng/kg body weight/day was reported for Mumbai, India [8]; 626–712 ng/d for Spain [31] and 13.8–16.7 ng/kg; body weight/d for Korea [32].

Table 4. Estimated dietary intake concentration of PAHs through consumption of sample fishes

PAHs component	Dietary intake (mg/kg body; weight/day)			
	<i>C. gariepinus</i>	<i>H. longifilis</i>	<i>O. niloticus</i>	<i>L. falcipinnis</i>
Naphthalene	0.29	0.12	0.12	0.14
2-methylenaphthalene	0.02	0.02	0.16	ND
Acenaphthalene	0.21	0.02	0.12	ND
Acenaphthene	0.07	0.02	ND	ND
Florene	0.21	0.12	ND	0.31
Phenathrene	0.18	ND	ND	0.58
Anthracene	0.94	ND	ND	0.27

ND = not detected (below detectable limits)

4. CONCLUSION

The observed levels of TPH in fish species from this study indicate that river aquirinrin in Degele community is not highly contaminated with petroleum hydrocarbons. Therefore, Degele community is less exposed to carcinogenic health risks associated with the consumption of the studied fish. However, continuous monitoring programme should be formulated and conducted to ensure that the concentrations of petroleum hydrocarbons is within the baseline levels established in the present study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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