Bioaccumulation of PAHs in Fishes from Ogidigben-Escravos Estuaries

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ABSTRACT

Levels of sixteen polycyclic aromatic hydrocarbons (PAHs) in fresh fish oil samples from OgidigbenEscravos Estuaries (Micropogoniasundulantes) and from local ponds in Ereyi farms in Benin City (Clariasgariepinus) were investigated. Gas chromatography analysis with flame ionization dictector (FID) were employed for (PAHs) determination. Observed mean individual PAHs level in the C. gariepinus ranged from below detection limit (BD) to 0.015mg/Kg and BD – 0.022mg/Kg for M. undulantes,the highest average concentration being recorded for indeno(1,2,3)pyrene. The total mean concentration for the four indicators of PAHs for both sampling sites were below EU maximum limits of 2.0-10ug/kg. The research shows that the PAHs contamination in the aquatic environment were both petrogenic and pyrolytic origin for the escravos Estuaries which were affected by seasons of the year and pyrogenic origin for the pond.

Keywords:Escravos Estuaries, *Clariasgariepinus, Micropogoniasundulantes,* Polycyclic Aromatic Hydrocarbons, pyrolytic, petrogenic.

INTRODUCTION

Terrestrial and marine environment which play host to most aquatic animals has been a source of concern since the issue of oil exploration and exploitation activities started in the Niger-Delta region of Nigeria which leaves behind several persistent organic pollutants in our water ways, part of which are as a result of oil spills.Oil spills in Nigeria are a common occurrence. It has been estimated that between 9million to 13million barrels have been spilled since oil drilling started in 1958 to 2000[1]. The government estimated that about 7000 spills occurred between 1970-2000[1] causes include corrosion of pipeline and tankers (account for 50% of all spills), sabotage (28%) and oil production operation (21%) with 1% of the oil spill being accounted for by inadequate non-functional production equipment. These spills have major impact on the aquatic ecosystem. Large tracts on the mangrove forest have been destroyed just to mention a few of the effects.

Polycyclic aromatic hydrocarbons are ubiquitous organic pollutants. It occur in varying amounts in water, soil, air and even in some food products as traces. Due to their lipophilicity, persistence and high toxicity, their residues are readily accumulated in the

tissues of non-target living organisms[2] where they may cause detrimental effects. Associated gas flaring, oil waste dumping, sabotage and other anthropogenic activities in these coastal areas contribute to the PAHs contamination and the activities are often assessed from changes in the physical, chemical[3] and biological components of aquatic ecosystem. The marine organisms like fish are able to accumulate several fold higher concentrations of PAHs than the surrounding water[4-6] conversely since human exposure to PAHs is majorly from dietary intake of food contaminated with PAHs except for smokers and occupationally exposed population[7-8], anyone that ingest these contaminated food substances are thus expose to these PAHs[9].

In the Niger-Delta areas of Nigeria, there are several studies that have been carried out on PAHs in fish harvested from these areas which include 'Assessment of PAHs level in some fish and Sea Food from Different Coastal Waters in the Niger Delta[10], PAHs accumulation in farmed fish samples from Degele Community[11], Aquatic environment and Fish Feed: A Source of PAHs Contamination in Some Selected Fish Samples[12].

This study is aimed at assessment of PAHs in two fish species, one from Escravos estuaries and the other from farm reared fish and to estimate the source of the PAHs in the two sampling sites.

MATERIALS AND METHODS

Materials

1.*Clariasgariepinus* commonly called catfish

2. *Micropogoniasundulantes* commonly called croaker fish

Equipment/Apparatus

The equipment/apparatus used include: water bath, vials and cock, test tubes, round bottom flasks, rotary evaporator, thermometer, silica gel packed cartridges, conical flasks, retort stands, weighing balance, spatula, beakers, volumetric pipets: 1, 5, and 10ml, micro syringes: 10µL, 100µL, 250µL, 500µL, 1000µL, drying oven, dessicator, blender, ultrasonic bath, SPE cartridges with stand, and gas chromatography instrument,.

Chemicals and reagents

All reagents and chemicals used were analytical grades and standards and include the following:

1. Acetone
2. Dichloromethane
3. Cyclohexane.

The three solvents were redistilled before use.

4. Sodium sulphate: granular and anhydrous. The sodium sulphate granules were purified by heating at 400^0C for 4hours in a shallow tray and cooled in dessicator

5. Mix 26- the internal standard or surrogate: the mix-26 used as the internal standard comes in 1ml vials and is prepared by diluting the 1ml solution which contains 4000ng/µl in 100ml of dichloromethane. The solution which contains 40ng/100ml will thus have a fluorescent green colour.

Sampling Area

Escravos estuary in Ogidigbenn area of Delta state and Ereyi farms which contains catfish ponds located in Oredo local Government Area of Edo were used as sampling sites.

Sample Collection

Clariasgariepinus also known as catfish and *Micropogonias undulates* commonly called Croaker fish were used for this study. *Micropogonias undulates* were collected from Ogidigbenn, in Escravos Estuaries through the local fishermen. The reason for the two species was because catfish was not available all the year round but available in farm or pond all year round. The pond was to serve as control. The fishermen go into the estuary with fishing boats and nets most of the time at nights and early hours of the day to catch the fishes. The fishes were sorted out when they get to the shores. These fishes were said to be representative of the sampling area. Three croaker fish 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 were stored in refrigerator at 4^0C prior to other treatments. The samplings were carried out from April 2017 to December 2017 so as to capture both the raining and dry seasons.

Clariasgariepinus were harvested from Ereyi farms using harvesting nets from which three were carefully selected bimonthly for the study. These three samples were said to be representative of the sampling area which are the fishes in that particular pond. They were washed and kept in polythene bags, labelled properly, kept in coolers with ice and transported to the laboratory where they were kept in the refrigerator at 4^0C prior to other treatments. The samplings were carried out from April 2017 to December 2017.

Extraction and Clean-Up of Samples
Fish Samples

The fresh 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 samples. 50g of each of the fish samples were mixed with 25g of sodium sulphate and 200ml of 50/50 cyclohexane/acetone mixture in a tight fitted covered bottle and10ml of the internal standard added to each of the bottles. Each bottle containing the sample, solvents mixture, sodium sulphate and the internal standard mix were placed inside the ultrasonic

bath (Astrabroultrasonic cleaner) model 7E for 2hours. The bottles were brought out after every 10mins and shaken. 25ml of the extract was then collected using a pipette and filler and concentrated using rotary evaporator to 5ml and each 5ml concentrate were concentrated to 1ml using a pure stream of nitrogen and clean-up was done using solid phase extractor and cyclohexane was employed as the eluting solvent. The cleaned up sample was concentrated to 1ml using nitrogen and stored in 1ml vials and subjected to GC analysis using FID detector.

Gas Chromatography Operating Procedure

Instrument type: Gas chromatography system 6890 series, Product: HP, Detector type: FID

The basic chromatography parameters for the analysis of polycyclic aromatic hydrocarbons are as follows:

Initial Temperature: 100^0C
Rate 1:4^0C/mins
Final temperature: 330^0C
Detector temperature: 300^0C

Calculation for Sample Analysis

The concentration of each analyte and hydrocarbon range in a sample can be determined by calculating the amount of analyte or hydrocarbon range injected, from the peak response, based upon the analyte/internal standard response ratio.

The contribution from the solvent and the surrogate compound were excluded from the total area of the sample. The concentration of sediment samples were calculated using the given formulae

$$CF = Area(p) \times R_f \times V_f/W_i \times D_f \times 1000$$

Where

CF = final sample concentration (µg/L)
Area(p) = measured area of peak (peaks)

W_i = initial weight extracted (g dry weight)

V_f = final extract volume (mL)

D_f = dilution factor of sample or extract

R_f = response factor from the calibration standard calculation

Aqueous Samples

$$CF = Area(p) \times R_f \times V_f/V_i \times D_f \times 1000$$

Where:

CF = final sample concentration (µg/L)
Area (p) = Measured area of peak (peaks)

V_i = initial volume of extract (ml)

V_f = final extract volume

D_f = dilution factor of sample or extract

R_f= response factor from calibration standard calculation.

Where an initial standard is used:

$$\text{Conc.}(\square g/L) = (Ax)(Is) \times \frac{1}{2a \quad (Ais)(RRF)(Ws) \quad SF}$$

Where:

Ax = area of compound to be measured
Is = amount of internal standard injected in nanograms (ng)
Ais = area of specified internal standard
RRF = relative response factor from the internal calibration curve.

Ws = weight of samples extracted in grams (g)
SF = split factor (fraction)

$$\% \text{ surrogate recovery} = \frac{\text{c surrogate}}{\text{C spike}} x\ 100$$

Where :

c surrogate = calculated surrogate concentration from sample analysis

C spike = concentration of surrogate spiked into the sample.

RESULTS AND DISCUSSIONS

Table 1 shows average PAHs concentration in *Clariasgariepinus* with mean total PAHs ranging from 0.035-0.067mg/Kg. Levels of Individual PAHs ranged from below detection limits (BD) of analytical instrument to 0.015mg/Kg with the highest (0.015mg/Kg) recorded for anthracene harvested from pond in the month of august 2017. The ratio of BaA/(BaA+Chry) for all the samples analysed were > 0.35 which indicate that the PAHs were from pyrogenic sources[10] while those less than 2 has been attributed to petrogenic sources. This value were in conformity with by Nwaichi& Ntorgbo[10] for *L. littorea*from different rivers in Niger Delta area. The pyrogenic sources of PAHs could be from the feed type as most of the feeds were roasted to keep them afloat on top of the ponds to avoid polluting the water[12]. The CONTAM Panel[13] recommended that groups of PAHs termed PAH4 (Benzo (a) pyrene, Chrysene, Benzo (a) anthracene and Benzo (b) fluoranthene) were better indicators of PAHs occurrence than Benzo (a) pyrene on its own in food. From this study the PAH4 were between BD-0.003 for all the samples which is below the EU regulatory limits of Maximum levels of 30 $\mu g\ kg^{-1}$ wet wt. for PAH4 in Commission Regulation (EU) No 835/2011

Table 1. Average PAHs Concentration in the Fresh *C. garienpinus* oil extract

PARAMETER	Average pond FF	Average 2pond FF	Average 3pond FF	Average 4pond FF	Average 5pond FF
Naphthalene	0.005	0.005	0.001	0.011	0.008
Acenaphthalene	0.002	0.002	0.007	0.003	0.004
Acenaphthene	0.003	0.006	0.009	0.002	0.004
Florene	0.003	0.003	0.007	0.010	0.004
Phenathrene	0.007	0.007	0.005	0.004	0.004
Anthracene	0.003	0.006	0.015	0.004	0.004
Fluoranthene	0.003	0.006	0.014	BD	0.002
Pyrene	0.004	0.006	0.005	0.003	0.006
Benzo(a)anthracene	0.003	0.005	0.001	0.002	0.003
Crysene	0.001	0.001	BD	0.003	0.003
Benzo(b)fluoranthrene	BD	BD	0.001	0.002	0.003
Benzo(k)fluoranthrene	BD	BD	0.002	BD	BD
Benzo(a)pyrene	BD	BD	BD	BD	BD

Indeno(1,2,3) perylene	BD	BD	BD	BD	BD
Dibenzo(a,h)anthracene	BD	BD	BD	BD	BD
Benzo(g,h,i) perylene	BD	BD	BD	BD	BD
BaA:BaA+Chrysene	0.750	0.833	1.000	0.400	0.500
TOTAL PAH (mg/kg)	**0.035**	**0.047**	**0.067**	**0.045**	**0.048**

Key

Ave Pond FF = Average PAHs concentration for April 2017 fresh fish samples
Ave 2Pond FF = Average PAHs concentration for June 2017 fresh fish samples
Ave 3Pond FF = Average PAHs concentration for Aug 2017 fresh fish samples
Ave4pond FF = Average PAHs concentration for Oct 2017 fresh fish samples
Ave 5pond FF = Average PAHs concentration for Dec 2017 fresh fish samples

Table 2- Average PAHs' Concentrations in Fresh Croker fish from Escravos Estuaries

PARAMETER	Average ESC FF	Average 2ESC FF	Average 3ESC FF	Average 4ESC FF	Average 5ESC FF
Naphthalene	BD	BD	0.001	0.016	0.005
Acenaphthalene	0.001	0.013	0.007	0.016	0.008
Acenaphthene	0.002	0.006	0.002	0.018	0.022
Florene	0.001	0.007	0.006	0.010	0.005
Phenathrene	0.001	0.011	0.009	0.009	0.004
Anthracene	BD	0.006	0.004	0.001	0.001
Fluoranthene	BD	BD	0.002	BD	BD
Pyrene	0.002	BD	0.002	0.001	BD
Benzo(a)anthracene	0.003	0.029	0.006	0.012	0.013
Crysene	BD	BD	BD	0.002	0.004
Benzo(b)fluoranthrene	BD	BD	0.003	BD	BD
Benzo(k)fluoranthrene	0.002	BD	BD	BD	BD
Benzo(a)pyrene	BD	BD	BD	BD	BD
Indeno(1,2,3) perylene	BD	BD	BD	BD	BD
Dibenzo(a,h)anthracene	BD	BD	BD	BD	BD
Benzo(g,h,i) perylene	BD	BD	BD	BD	BD
BaA/(BaA+Chry)	0.003	0.029	0.006	0.857	0.765
TOTAL PAH (mg/kg)	**0.014**	**0.073**	**0.041**	**0.084**	**0.063**

Key
Ave ESC FF = Average PAHs concentration for April 2017 fresh fish samples
Ave 2ESC FF = Average PAHs concentration for June 2017 fresh fish samples
Ave 3ESC FF = Average PAHs concentration for Aug 2017 fresh fish samples
Ave 4ESC FF= Average PAHs concentration for Oct 2017 fresh fish samples
Ave 5ESC FF= Average PAHs concentration for Dec 2017 fresh fish samples

Table 2 shows mean PAHs concentration for *Micropogoniasundulantes* from Escravos estuaries. Average levels of individual PAHs ranged from below detection limit to 0.029mg/Kg with the highest recorded for Benzo(a)anthracene for fish harvested in the month of June 2017. BaA/(BaA+Chry) calculated were less than 0.2 for samples harvested in the first three sampling months which shows that they were all of petrogenic origin[10]while the last two sampling months gave values for BaA/(BaA+Chry) > 0.3 indicating pyrogenic origin. This could be as a result of seasonal variation from the wet season to the dry season of Oct-Dec. predominance of the pyrolitic origin may also be linked to intense illegal great number of linear and diffused combustion sources [10]. The PAH4 for samples in this aquatic media were between BD-0.029 which below the EU regulatory levels of 30ug/Kg wet wt.

CONCLUSION

The research shows that the PAHs contamination in the aquatic environment were both petrogenic and pyrolytic origin for the escravos Estuaries which were affected by seasons of the year and pyrogenic origin for the pond.The carcinogenic PAHs concentration for samples from both aquatic media were still below the EU permissible standards for maximum PAHs Level of 30ug/Kg wet wt. close monitoring of these aquatic media is therefore advised as this may not be the case at certain time of the year because of the activities in these water ways.

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