



# Bioaccumulation of Polycyclic Aromatic Hydrocarbons in Tissues (Gills and Muscles) of (Catfish) *Chrysichthys nigrodidatus* from Crude Oil Polluted Water of Ogoniland, River State, Nigeria

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## Authors' contributions

This work was carried out in collaboration between all authors. Authors MOM, GSI and CLO designed the study and wrote the protocol. Authors GSI and CLO carried out the laboratory analysis. Authors MOM, GSI and CLO managed the literature searches, performed the analysis and wrote the first draft. All authors read and approved the final manuscript.

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## ABSTRACT

The present study of PAHs concentration in gills and muscle of (*Chrysichthys nigrodidatus*) collected from Ogoni-land, Rivers State, Nigeria was determined. Total of 17 PAH was analyzed. 13 of these PAH were found in the gills and 12 in the muscle of catfish from Kaa, also 15 were found in gills and 13 in the muscle from Bodo-city. Fishes were collected at different point and were analyzed for PAH using gas chromatography with flame ionization detector (GC/FID). Out of the two organs analyzed, the gills showed a highest level of PAH (179 µg/kg) in Bodo-city followed by (4.125 µg/kg) in gills of *Chrysichthys nigrodidatus* from Kaa.

The benzo[a]pyrene marker for the occurrence and effect of carcinogenic in foods exceeded the EU recommended limit of 2 µg/kg in the muscle of *Chrysichthys nigrodidatus* from Kaa and Bodo-city respectively. PAH concentrations in tissues decreased in the order gills>muscle. Thus, these organs have the ability to accumulate PAHs at different concentration.

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## 1. INTRODUCTION

Numerous PAHs generating activities take place in Nigeria, Ogoni-land to be precise, without much control. One of such locations is a sampling site (Kaa and Bodo-city) in this study. Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous organic compounds consisting of two or more fused aromatic rings. They are mostly hydrophobic and are capable of bio-accumulating in animal and human tissues [1]. The total emission of all PAHs is quite difficult to estimate, but the global emission of benzo[a]pyrene is estimated at about 8600 tons/year [2]. Fish which is an important source of protein and low fat has the ability to accumulate hydrocarbon and thus good bio-indicator. PAHs compounds are toxic, mutagenic, and carcinogens. benzo (a) pyrene, a known carcinogen compound [3] could also be generated and released into the river. The health effects of these mixtures is in practically all cases confined to their carcinogenic potential, for which there is evidence from a number of epidemiological studies, especially for lung cancer and, in some cases, cancers of the skin and of the urinary bladder. Also several authors have reported the effect of PAHS on fish which includes retardation of growth, several organs and tissue damage etc [4]. Fish accumulate these pollutants through absorption and human are exposed to them via food chain. This can result to acute and chronic effect in human such as cancer, neurotoxicity [5], physical and psychological effect. It is therefore necessary to assess the PAHs in the biota as their bioaccumulation in organs of aquatic biota could serve as a good indication of pollution problem in Ogoni-land.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study area, Bodo-city and Kaa, are located in Gokhana and Khana local government area of Rivers State, Nigeria with geographical coordinates of approximately latitudes 4.05° and 4.20° north and longitude 7.10° and 7.30° east. It has a population of close to 832,000 consisting mainly of the Ogoni people.

### 2.2 Collection of Test Samples

Fresh samples of *Chrysichthys nigrodidatatus* were collected from landing beaches of Bodo

City and Kaa water side in Gokana and Khana Local Government Area Rivers State, Nigeria. At each site, a total of 28 fishes ranging between 350-568 g in weight of *Chrysichthys nigrodidatatus* were collected, at two site, (14 for each site) cleaned and wrapped in aluminum foils, then kept frozen in an ice chest before transported to the laboratory where the gills and muscle were removed for analysis.

### 2.2.1 Processing of seafood

The organs were washed with distilled water and put in Petri dishes to dry at 144°C in oven for three days. They were then ground with blender (National, MX 795N, Japan) and kept in air tight containers prior to extraction process.

### 2.2.2 Extraction

Extraction process was carried out accounting to Texas Natural Resource Conservation Commission (TNRCC) [6]. Five of sample was weighed into a clean extraction container (50 ml beaker) and 10 ml of extraction solvent (dichloromethane) was added into the sample and mixed thoroughly and allowed to settle. The sample was carefully filtered into clean solvent rinsed extraction bottle, using filter paper fitted into Buchner funnels. The extract was concentrated to 2 ml and then transferred for cleanup/separation.

### 2.2.3 Cleanup/separation

1 cm of moderately packed glass wool was placed at the bottom of 10 mm ID \* 250 mm Loup chromatographic column. Slurry of 2 g activated silica in 10 ml methylene chloride was prepared and placed into the chromatographic column. To the top of the column was added 0.5 cm of sodium sulphate 2 g. The column was rinsed with additional 10 ml methylene chloride and pre-eluted with 20 ml of dichloromethane. This was allowed to flow through the column at a rate of about 2 minutes until the liquid in the column was just above the sulphate layer. Immediately 1 ml of the extracted samples was transferred into the column. The extraction bottle was rinsed with 1ml of dichloromethane and added to the column as well. The stop clock of the column was opened and the element was collected with a 10ml graduated cylinder. Just prior to exposure of the sodium sulphate layer to air, dichloromethane was added to the column in 1 – 2 increments.

Accurately measured volume of 8 – 10 ml of the eluent was collected and labeled.

#### **2.2.4 Gas chromatography analysis**

The concentrated aliphatic fractions were transferred into labeled grass vials with rubber clip cap for gas chromatography analysis. 1 µl of the concentrated sample was injected by means of hypodermic syringe through a rubber septum into the column. Separation occurred at the vapor constituent partition between the gas and liquid phase. The sample was automatically detected as it emerges from the column (at constant flow rate) by the FID detector whose response is dependent upon the composition of the vapor.

#### **2.2.5 Chromatographic conditions**

The gas chromatography was Hewlett Packed 5890 series II, gas chromatography apparatus, coupled with flame ionization detector (FID) (Hewlett Packard, Wilmington, DE, USA), powered with HP chemstation Rev. A 09:01 (10206) software to identify and quantify compounds. The GC operating conditions were as specified by the procedural manual.

### **2.3 Human Health Risk Assessment of Polycyclic Aromatic Hydrocarbons**

In estimating the carcinogenic risk from exposure to PAHs in fish, the USEPA guideline, as described by [7], was followed. By this method, B[a] P is used as a marker for the occurrence and effect of carcinogenic PAHs in foods and, therefore, the overall carcinogenic health risk from the measured PAHs was estimated based on toxic equivalency factors (TEFs) [8,9] derived from the cancer potencies of individual PAH compounds relative to the cancer potency of B[a] P. Screening value (SV) which is the threshold concentration of total PAHs in fish tissue that is of potential public health is given as  $SV = [(RL/SF) \times BW]/CR$ . Where SV = screening value (µg/g), RL = maximum acceptable risk level (dimensionless), set to  $10^{-5}$  (USEPA, 2000) so that the maximum risk would be one additional cancer death per 100000 person, SF = USEPA oral slope<sup>-1</sup> factor (7.30 µg/g day) used to determine an upper bound probability of an individual developing cancer as a result of longtime exposure to carcinogenic PAHs. BW = body weight set at (60000 g) [10], CR = consumption rate which is set at (68.5 g/day).

### **2.4 Statistical Analysis**

Statistical significance was assessed using a one-way analysis of variance (ANOVA). One-way analysis of variance (ANOVA) was performed to compare PAHs levels between the organs of the two sites with the statistical package SPSS 14.0.2 (SPSS Inc., Chicago, USA).

## **3. RESULTS AND DISCUSSION**

The Table 1 shows the distribution of polycyclic aromatic hydrocarbons (PAHs) in gills and muscle of *Chrysichthys nigrodidatus* collected from study site. Total mean PAH concentrations (µg/kg.), potency equivalent concentration (PEC), [11]Low Molecular Weight PAH, High Molecular Weight-PAH ratio and diagnostic ratio BaA/(BaA + Chry) ratios [12] in catfish gills and muscle are shown in Table 1. Total of 13 PAHs were detected in the gills and muscle of catfish from kaa and Bodo-city while 15 in the gills from Bodo- City and 12 in the muscle from Kaa. In Kaa, the highest average concentration range from 0.001 to 0.875 and 4.125 µg/kg which were recorded for pyrene in the muscle and gills while in Bodo-City, the highest average concentration range from 0.001 to 5.61 and 179 µg/kg in the muscle and gills of *Chrysichthys nigrodidatus* for pyrene. Total PAHs concentrations in the muscle and gills of catfish sample were 5.03 and 6.08 in Kaa while that of Bodo-City were 9.94 and 181.83µg/kg respectively. The calculated potency equivalent concentration (PEC), were 0.19 and 1.93 in the gills and muscle of *Chrysichthys nigrodidatus* from Kaa and 0.63 and 2.35 in the gills and muscle of *Chrysichthys nigrodidatus* from Bodo- City respectively. In Table 1 low molecular weight –PAH and High molecular weight-PAH ratios in gills and muscle of *Chrysichthys nigrodidatus* were 0.18 and 0.15 at Kaa, while that of Bodo-City 0.005 and 0.08 in the gills and muscle respectively. However, from these result, it was observed that in the two sites the ratios for all the organs were < 1 indicating that the sources of these PAHs in the fish analyzed are as a result of fossil fuel utilization. BaA/ (BaA + Chry) diagnosis ratio in the gills of *Chrysichthys nigrodidatus* was 0.37. The ratios in gills of catfish from Bodo City were > 0.35 while that of Kaa was below. B[a] P concentrations in muscle of *Chrysichthys nigrodidatus* were 3.18 in Kaa and 2.82 in Bodo-city. Because of its serious public health effect, the permissible limit of B[a] P (2 µg/kg in fish) [13] among all the PAHs is determine by the European Union.

Table 1. PAH concentrations in catfish gills and muscle from the study areas (Kaa, and Bodo City). Value are mean  $\pm$ S.E.M (n=14)

PAH compound $\mu\text{g}/\text{kg}$	Kaa		Bodo city	
	Gills	Muscle	Gills	Muscle
Naphthalene	0.086 $\pm$ 0.029	0.201 $\pm$ 0.430	0.650 $\pm$ 0.281	0.354 $\pm$ 0.057
Acenaphthylene	BDL	BDL	BDL	BDL
Acenaphthene	BDL	BDL	BDL	BDL
Fluorene	0.840 $\pm$ 0.035	0.035 $\pm$ 0.004	0.169 $\pm$ 0.025	0.095 $\pm$ 0.003
Anthracene	0.010 $\pm$ 0.011	0.012 $\pm$ 0.001	0.040 $\pm$ 0.009	0.052 $\pm$ 0.017
Phenanthrene	BDL	BDL	0.026 $\pm$ 0.015	0.008 $\pm$ 0.001
Fluoranthene	0.173 $\pm$ 0.031	0.015 $\pm$ 0.009	0.436 $\pm$ 0.004	0.045 $\pm$ 0.007
Pyrene	4.125 $\pm$ 3.164	0.875 $\pm$ 0.021	179.0 $\pm$ 0.096	5.61 $\pm$ 4.898
Benz[a]anthracene	BDL	BDL	0.004 $\pm$ 0.001	BDL
Chrysene	0.040 $\pm$ 0.006	0.026 $\pm$ 0.002	0.068 $\pm$ 0.004	0.058 $\pm$ 0.005
Benzo[b]Fluoranthene	0.390 $\pm$ 0.002	BDL	0.657 $\pm$ 0.011	BDL
Benzo[k]Fluoranthene	0.148 $\pm$ 0.005	0.140 $\pm$ 0.009	0.248 $\pm$ 0.047	0.173 $\pm$ 0.003
Benzo[a]Pyrene	0.020 $\pm$ 0.002	3.18 $\pm$ 0.001	0.026 $\pm$ 0.001	2.82 $\pm$ 0.002
Indenol[1,2,3-cd]Pyrene	0.125 $\pm$ 0.016	0.093 $\pm$ 0.001	0.289 $\pm$ 0.036	0.143 $\pm$ 0.020
Dibenz[a, h]anthracene	0.020 $\pm$ 0.006	0.380 $\pm$ 0.003	0.066 $\pm$ 0.001	0.460 $\pm$ 0.002
Benzo[g, h, 1]perylene	0.042 $\pm$ 0.004	0.042 $\pm$ 0.001	0.060 $\pm$ 0.005	0.046 $\pm$ 0.001
2-Methylnaphthalene	0.060 $\pm$ 0.012	0.037 $\pm$ 0.007	0.090 $\pm$ 0.004	0.075 $\pm$ 0.002
<b>Total PAHs</b>	<b>6.08<math>\pm</math>3.32</b>	<b>5.03<math>\pm</math>0.49</b>	<b>181.83<math>\pm</math>1.54</b>	<b>9.94<math>\pm</math>5.02</b>
<b>PEC</b>	<b>0.19</b>	<b>1.93</b>	<b>0.63</b>	<b>2.35</b>
<b>LMW-PAH/HMW-PAH ratio</b>	<b>0.18</b>	<b>0.15</b>	<b>0.0049</b>	<b>0.08</b>
<b>BaA/(BaA + Chry) ratio</b>	<b>0.00</b>	<b>0.00</b>	<b>0.37</b>	<b>0.00</b>

*BDL implies below detection limits of 0.0001  $\mu\text{g}/\text{kg}$*

Data in Table 1 show that B[a]P exceeded the permissible limits in the muscle of *Chrysichthys nigrodidatus* from both Kaa and Bodo-city respectively as such this can be harmful to the human health while that of gills were below detection limit. This finding also confirms the findings of Nayarko et al. [15] who assessed the sources of PAH in coastal water of Ghana. The PEC calculated value for *Chrysichthys nigrodidatus* in the gills and muscle from Kaa were 0.19 and 1.93 while that of Bodo-city were 0.63 and 2.35. The calculated screening value (SV) for polycyclic aromatic hydrocarbons in fish was 0.0012. PEC values of fish species from all sites were above the calculated SV (0.0012), about 1–1958 times higher in the gills and muscle of both Kaa and Bodo-city respectively. This indicates high levels of PAHs in the organs of *Chrysichthys nigrodidatus*. The result from this study shows that PEC value exceeded the SV in all the tissue of *Chrysichthys nigrodidatus* analyzed, indicating that consumption of catfish at a rate of 68.5 g/day can have adverse health effects. Thus, these fish species could be an important source of PAHs exposure among the Ogoni-Land where, fish constitutes a major source of protein [14] in the diet. The people who tend to consume larger quantities of fish could be at a greater risk.

#### 4. CONCLUSION

Though high molecular weight PAHs were found to bio-accumulate more than the lower ones, also the total PAH concentration was high in the gills of *Chrysichthys nigrodidatus* from Bodo-City then in the muscle. This study suggests that fish is a good bio-indicator of Polycyclic Aromatic Hydrocarbons. The result also shows that the levels of PAHs detected in *Chrysichthys nigrodidatus* are high and the level of the most hazardous compound B[a]P were recorded high in the muscle of *Chrysichthys nigrodidatus* from both sites, thus, consumption of these fishes may pose public health risk to the people who consume this fish species.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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