Risk Assessment of Consumption of Pah-Contaminated *Papyrocranus Afer* from Banegbe River

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ABSTRACT

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| **Aims:** Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds with two or more fused aromatic rings. They are present in the environment mainly as a result of incomplete combustion, such as in forest fires, internal combustion engines, wood stoves, and coal coking This study aims to determine the concentration of polycyclic aromatic hydrocarbons (PAHs) in river water and *Papyrocranus afer* samples from the Banegbe River.  **Study design:** To assess the potential health risks associated with PAH contaminants due to consumption of *Papyrocranus afer* fish from the Banegbe river, the concentrations of sixteen polycyclic aromatic hydrocarbons were measured.  **Place and Duration of Study:** Fish samples were collected from different points of the Banegbe River Latitude 5o 14N and longitude 5o 22E, Latitude 5o 28N and longitude 5o 10E and Latitude 5o 43N and longitude 5o 14E.  **Methodology:** Extraction and analysis of polycyclic aromatic hydrocarbon in fish and water samples were done. The analysis was carried out using gas chromatography/ Flame Ionization Detector (GC/FID). The human health risk impact resulting from consumption of contaminated fish from Banegbe river and Ekpan pond were estimated.  **Results:** The bioassay results indicated that the middle-stream point of the Banegbe River water samples had the highest total PAHs concentration of 4.695mg/L when compared to downstream and upstream, with the value of 4.442mg/L and 1.340mg/L, respectively. Varying levels of PAH congeners were observed in the fish tissues, with fish samples from the middle-stream having the highest total concentration of PAHs during the dry and rainy seasons. PAH levels were higher during the dry season than during the rainy season. Mean hazard indexes were all below 1 (<1) for all the sampled points (below an acceptable cumulative threshold). Risk assessment conducted using benzo(a)pyrene carcinogenic and mutagenic toxicity equivalency factors (TEF and MEF) showed risk for middle-stream, down-stream, up-stream, and Ekpan pond, respectively. The cancer risk associated with the consumption of fish from the Banegbe River were all above the USEPA guideline (1.0× 10 -6) for potential cancer risk, 0.0328, 0.1648, 0.1096 and 0.5958 for TEF and 0.33128, 0.40564, 0.26809 and 0.02179 for MEF during the dry season for up-stream, middle-stream, down-stream and Ekpan respectively. During the rainy season the cancer risk associated with the consumption of fish were, 0.00222, 0.29698 and 0.01442 for TEF and 0.00329, 0.2179 and 0.2888 for up-stream, middle-stream and down-stream respectively.  **Conclusion:** The study suggests that fish from the Banegbe River are not safe for human consumption. |

*Keywords: Polycyclic aromatic hydrocarbons, Risk assessment, carcinogenic, mutagenic*

1. INTRODUCTION

“Polycyclic aromatic hydrocarbons (PAHs) are a significant group of persistent organic pollutants (POPs) that are commonly found in aquatic environments”. (Qu *et al.,* 2020). “PAHs are widespread environmental pollutants characterized by their structure of two or more fused aromatic rings. They have relatively low solubility in water but are highly lipophilic. pahs are formed and released into the environment through natural and anthropogenic sources” (grimmer, 1983). Natural sources include volcanoes and forest fires (harvey, 1997). “PAHs are organic pollutants that are widely distributed in the environment; they are harmful and very persistent in the environment” (gao and zhou, 2004).

“Hundreds of these compounds exist in the ecosystem, and of which 16, (Naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, chrysene, benzo(a)anthracene, pyrene, benzo(*k*)fluoranthene, benzo(*b)*fluoranthene, benzo(*a)*pyrene, dibenzo(*a, h*) anthracene, dibenzo(*b, c)*fluoranthene and benzo (*g, h, i*) perylene) are with greater toxicities and have been selected by the US Environmental Protection Agency (USEPA) as priority pollutants to be regulated”. (Bojes and Pope, 2007; Sun *et al*., 1998).

“Aquatic bodies are the main recipients of almost all anthropogenic discharges, especially domestic and industrial wastewater discharges, oil spills, tire wear debris, asphalt particles, atmospheric transport, dispersion, and deposition of industrial stack emissions” (Binet *et al.* 2002; Feng *et al.* 2009). “In aquatic animal species, fish are the specie that cannot escape from the detrimental effects of these contaminants” (Dickman and Leung, 1998). Also, “they may have the ability to bioaccumulate harmful substance such as PAH and other lipophilic chemicals, which ultimately affect not only the productivity and reproductive abilities of the organisms, but also the health of the human beings that depend on the organisms as an essential source of protein” (Berman and Lal, 1994). “PAHs can enter all the tissues of an animal's body that contain fat. They tend to be stored mostly in the kidneys, liver, and fat. Smaller amounts are stored in the spleen, adrenal glands, and ovaries. The main exposure route is dietary, drinking water, and swallowing food, apart from smokers, soil, or dust particles that contain PAHs. Occupationally exposed populations are other routes of exposure of these chemicals to enter the body” (Nwaichia and Ntorgbo, 2016).

“Banegbe River (Ekakpamre River) is one of the tributaries of Warri River, a major navigable channel of the Niger Delta, southern Nigeria. It takes its origin from around Utagba Uno and flows through zones of freshwater swamps, mangrove swamps, and coastal sand ridges” (Tetsola, 1988). Some of the industries in Ughelli are a few meters along the Banegbe River. A large number of local people, especially farmers, depend on the River Benegbe for navigation, fisheries, and a source of drinking water. The effluents produced from these industries are released into the river and increase the level of pollution of the river.

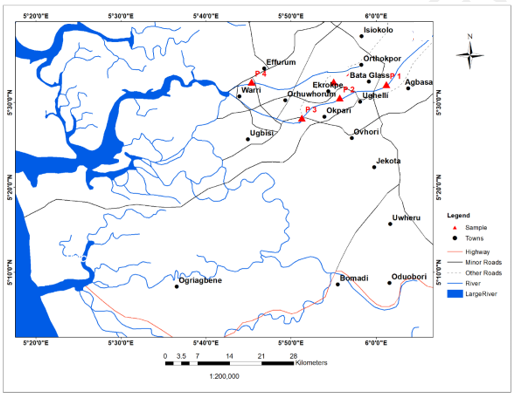
The growing concern for food safety is juxtaposed with the alarming presence of PAHs in fish, exceeding safe limits and posing significant risks to public health (Tongo *et al.*, 2017). In spite of the health risks associated with PAHs, public awareness on their nature, accumulation, toxicity and presence in the Nigeria's environment especially water bodies are still very low. Therefore, the study is aimed at assessing the levels of Polycyclic Aromatic Hydrocarbons (PAHs) in surface water and fish of Banegbe river and also to investigate the risk associated with consumption of the fish from the river.

To date, no previous studies have evaluated the carcinogenic risk to human health associated with ingestion exposure to PAHs from Papyrocranus *afer in Banrgbe river*. Therefore, the main aim of this study was to assess the risk to human health from exposure to PAHs present in the fish. Specifically, the purpose of this study was to evaluate the distribution patterns of PAHs during the rainy and dry season

2. material and methods

**2.1 Collection of fish samples**

Fish samples were collected from different points (Fig. [1](file:///C:\Users\JOY%20OGANA\Desktop\PHD\Heavy%20metals%20in%20fish%20and%20earthworm\JOY12.htm#Fig1)) of the Banegbe River Latitude 5o  14N and longitude 5o 22E, Latitude 5o 28N and longitude 5o 10E and Latitude5o 43N and longitude 5o 14E. Fishes were collected with the help of professional fishermen while they were fishing in the river. Individuals of the same species were of similar size and weight. A total number of 24 papyrocranus afer were used for this study, the weight and length of the fish sample were taken. Then the samples were immediately preserved in air sealed plastic bags and transported to the laboratory.



**Figure 1:** Map showing Sample sites.

Note: Where P1= up-stream,

P2 = effluent discharge point,

P3 = down-stream,

P4 = Ekpan pond**.** (Ogana *et al.*, 2023)

**2.2 Extraction and analysis of polycyclic aromatic hydrocarbon**

**2.2.1 Aqueous Sample Extraction**

Extraction and analysis of polycyclic aromatic hydrocarbon in fish and water samples were done according to USEPA 8270 and 8015, 2004 method.

For the water samples, a litre each of the sample and reagent water was transferred to a 2-liter different separatory funnel. For all samples and blanks, 1 ml of the concentrated surrogate spiking solution was added directly to the separatory funnel. The pH was noted and adjusted to pH <2. 60 mL of DCM was added to the separatory funnels. The separatory funnel was sealed and shaken vigorously for at least three (3) minutes with periodic venting to release excess pressure. Then the organic layers were allowed to separate from the water phase for a minimum of 5 minutes. The solvent extracts were then collected in an Erlenmeyer flask. The extraction was repeated two more times using additional 60 ml portions of solvent. The three solvent extracts were then combined in a 250-ml Erlenmeyer flask. A rotary evaporator apparatus was place on a hot water bath (80-90°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. The vertical position of the apparatus and the water temperature was adjusted as required to complete the concentration in 10 minutes, then the extract was dried by passing it through a glass powder funnel containing anhydrous sodium sulfate or other suitable drying agent. The extract was concentrated to less than 10 ml, by raising the temperature of the water bath, if necessary, to maintain proper distillation. The snyder column and evaporation flask was removed from the 10-ml concentrator tube. The concentrator tube containing the DCM extract was placed in an air blow down apparatus. The extract volume was adjusted to 1ml under a gentle stream of nitrogen or air. If the extract is highly colored, forms a precipitate, or stops evaporating, the final volume should be higher.(JOY, O., et al., 2024)

**2.3 For the fish (muscle) sample extraction**

A solvent mixture of acetone and methylene chloride 50:50 was prepared. Aliquot of 10g of well-mixed fish muscle sample which was measured into a solvent rinsed beaker, then 50ml of the solvent mix was added to the samples. Spike with 1ml of the surrogate mix. The sample was placed in the sonicator and sonicated for about 10 – 15 minutes at about 70oC. Anhydrous sodium Sulphate (10g) was added to the sample until a clear extract developed. Then extract solvent were poured into a round bottom flask. The process was repeated once more with an additional 50ml of solvent and the beaker was allowed to settled and decanted into the same round bottom flask. The solvent was concentrated to about 1 to 3ml. The sample was then ready for clean up using silica gel column. The columns were packed with 10g of 100-200-mesh silica gel pre-conditioned (baked) at 105oC overnight. The silica was mixed with DCM to form slurry. The analysis was carried out using gas chromatography/ Flame Ionization Detector (GC/FID), because FID has a very wide dynamic range and high sensitivity, also it has the ability detects all substance that contain carbon. (JOY, O., et al., 2024)

**2.4 Determination of risk quotient (RQ)**

**2.4.1 *Calculation of Risk Quotients***

Risk quotient (RQ) can be used when it comes to the evaluation of the potential risk of a certain pollutant or complex pollutants to aquatic ecosystem (Jiang *et al.*2014). And RQ is calculated by the method described by Caiyun *et al.* (2015).

RQ =

Where allowable concentrations of pollutants are recommended by USEPA, (2008).

**2.4.2 The human risk assessment**

The human health risk impact resulting from consumption of contaminated fish from Banegbe river and Ekpan pond were estimated using standard methods of USEPA, (2000) as described by Yusuf *et al.,* (2015) and Forsberg *et al.,* (2013).

**2.5 Determination of hazard quotient (HQ) and hazard index (HI)**

HQ =

Where RfD is the oral chronic reference dose of non-carcinogenic PAHs as shown in Table 1 and ADD is the non-carcinogenic averaged daily dose

ADD =

Where C is the concentration of non-carcinogenic PAHs,

IR is the ingestion rate of the PAHs based on the average fish consumption rate set to be 30.8g /day/ person from the annual per capita fish consumption of 11.3kg for Nigeria (FAO, 2008).

CF is the conversion factor (0.001)

BW is body weight which is set to be 70kg.

AT is the averaging time = ED×EF

where ED is the exposed duration set for 70 years life time

EF the exposed frequency set for 7 times a week for the consumption of fish.

Hazard Index (HI) which is the sum of individual hazard quotient of the non-carcinogenic PAH HI =**Σ** (HQl +HQ2 + HQ3……. HQn)

**2.6 Determination of carcinogenic or toxic equivalency of benzo (a)pyrene (TEQBaP), mutagenic equivalence of benzo (a) pyrene (MEQBaP) and lifetime cancer risk (LCR)**

The TEQBaP, MEQBaP, and LCR were calculated by the method described by Nsikak *et al.,* (2017)

(TEQBaP) = **Σn** (TEFl × Cl)

(MEQBaP) = **Σn** (MEFl × Cl)

where TEF is the toxic equivalence factor developed for individual PAHs to express the potency relative to benzo(a)pyrene as shown in Table 1 and C is the concentration of PAHs in fish.

MEF mutagenic equivalence factor proposed for individual PAHs to express the mutagenic potency relative to benzo(a)pyrene as also shown in Table 1

LCR = Average Daily Dose for carcinogenic or mutagenic (ADDt) × cancer slope factor

Where; ADDt = TEQ (carcinogenic or mutagenic) × IR × CF×ED×EF×/BW×AT

***Table 1: Proposed benzo (a) pyrene equivalent factors for carcinogenic (TEF) and Mutagenic toxicity (MEF) and toxicity value for polycyclic aromatic hydrocarbons***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| PAH compound | TEF | MEF | RfD(mg/kg-d) | CSF(1/mg/kg-d) |
|  |  |  |  |  |
| Naphthalene | 0.001 |  | 2.00×10-02 |  |
| Acenaphthylene | 0.001 |  | 2.00×10-02 |  |
| Acenaphthene | 0.001 |  | 6.00×10-02 |  |
| Florene | 0.001 |  | 4.00×10-02 |  |
| Phenanthrene | 0.001 |  |  |  |
| Anthracene | 0.01 |  | 3.00×10-02-1 |  |
| Fluoranthene | 0.001 |  | 4.00×10-02 |  |
| Pyrene | 0.001 |  | 3.00×10-02 |  |
| Benzo(a) anthracene | 0.1 | 0.082 |  | 7.30× 10-1 |
| Chrysene | 0.001 | 0.017 |  | 7.30× 10-3 |
| Benzo(b) fluoranthene | 0.1 | 0.25 |  | 7.30× 10-1 |
| Benzo(a)pyrene | 1.0 | 1.0 |  | 7.30 |
| Benzo(k)fluoranthene | 0.01 | 0.11 |  | 7.30× 10-2 |
| Indeno (1,2,3) pyrene | 0.1 | 0.31 |  | 7.30× 10-1 |
| Dibenzo(a,h) anthracene | 1.0 | 0.29 |  | 7.30 |
| Benzo(g,h,i) perylene | 0.01 |  | 4.00×10-02 |  |

From (Yusuf *et al.,*2015)

**2.7 Statistical Analysis**

Descriptive (mean and standard deviation) statistical analysis was used to present data in numerical and graphical forms.

3. results and discussion

**3.1 Results**

**Table 2: Weight of fishes for PAHs Analysis during dry and raining season (January 2016 and January 2016 ) from Banegbe river and Ekpan pond**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Point of collection | Weight (g) | Length (cm) | Weight (g) | Length (cm) |
| Up-stream | 215±0.01 | 9.6±0.00 | 271±0.01 | 14.2±0.032 |
| Middle-stream | 340±0.00 | 18±0.01 | 440±0.01 | 22.66±0.01 |
| Down-stream | 265±0.03 | 13.7±0.01 | 259±0.04 | 12.84±0.010 |
| Ekpan | 178±0.01 | 9.6±0.02 | 223±0.06 | 11.54±0.06 |

***Polycyclic aromatic hydrocarbon concentrations in Banegbe River at different locations (up-stream, middle-stream and down-stream) and reference points (Ekpan pond water).***

The result of the PAHs from different points of Banegbe River and the control water body (Ekpan pond) revealed that middle-stream and down-stream had the highest concentration of total PAHs of 4.695mg/l and 4.442 mg/l respectively, with benzo (a) anthracene having the highest values respectively, when compared to up-stream of Banegbe River. Then followed by Ekpan pond water with the value of 3.274 mg/l and all the sixteen PAHs analysed present in it, with fluoranthene having the highest value of 2.032 mg/l.

***Table 3 Polycyclic aromatic hydrocarbons concentrations in Banegbe River at different locations (up-stream, middle-stream and down-stream) and reference points (Ekpan pond water).***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Components** |  | **Banegbe River** |  | **Reference (control) site** |
|  | **Up-stream** | **Middle-stream** | **Down-stream** | **Pond water** |
| **Naphthalene** | ND | ND | ND | 0.013 |
| **Acenaphthylene** | 0.110 | 0.063 | 0.014 | 0.013 |
| **Acenaphthene** | 0.013 | 0.154 | 0.111 | 0.045 |
| **Florene** | 0.257 | 0.315 | 0.957 | 0.349 |
| **Phenanthrene** | 0.076 | 0.050 | 0.076 | 0.067 |
| **Anthracene** | 0.174 | 0.154 | 0.134 | 0.011 |
| **Fluoranthene** | - | ND | ND | 2.032 |
| **Pyrene** | 0.027 | 0.081 | 0.067 | 0.015 |
| **Benzo(a) anthracene** | 0.962 | 1.068 | 1.062 | 0.045 |
| **Chrysene** | 0.440 | 0.758 | 0.690 | 0.067 |
| **Benzo(b) fluoranthene** | 0.283 | 0.099 | 0.093 | 0.402 |
| **Benzo(a)pyrene** | ND | 0.642 | 0.107 | 0.071 |
| **Benzo(k) fluoranthracene** | ND | 0.712 | 0.605 | 0.132 |
| **Indeno (1,2,3) pyrene** | ND | 0.689 | 0.682 | 0.032 |
| **Dibenzo(a,h) anthracene** | ND | 0.190 | 0.097 | 0.020 |
| **Benzo (g,h,i) perylene** | ND | ND | ND | 0.021 |
| Total (mg/L) | **1.340** | **4.695** | **4.442** | **3.274** |
| Mean |  |  |  |  |
| WHO permissive limit for each PAHs in water | **0.00002** | | | |

***Polycyclic aromatic hydrocarbons concentrations in the Papyrocranus afer samples from Banegbe River at different locations (up-stream, middle-stream and down-stream) during dry season (January 2016) and rainy season (July 2016).***

The PAHs of fish samples from different locations of Banegbe River during dry and rainy season revealed, that fish from the middle-stream point of Banegbe River during dry season bioaccumulated the highest total PAHs level of 0.472 mg/kg with naphthalene, fluoranthene, pyrene, benzo(a) anthrancene, crysene, benzo (b) fluoranthrene and benzo (a) pyrene detected. It was then followed by down-stream of Banegbe River with total PAHs levels of 0.350mg/kg with nine PAHs detected. Fish from up-stream of Banegbe had the least concentration with anthracene and benzo (g,h,i) perylene not detected out of the sixteen PAHs during dry season.

During rainy season the concentration of PAHs in the fish at the different locations at Banegbe River were relatively lower when compared with fish harvested during the dry season at all the different locations. Fish from down-stream during rainy bioaccumulated the highest total PAHs level of 0.267mg/kg with acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, and chysene detected. It was then followed by middle-stream point of Banegbe River with total PAHs levels of 0.192mg/kg and eleven of the PAHs detected. Up-stream of river Banegbe accumulated the least concentration of 0.091mg/kg with naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, benzo (a) anthracene, and chysene detecte.

**Table 4: Polycyclic aromatic hydrocarbons concentrations in the *Papyrocranus afer* samples from Banegbe River at different locations (up-stream, middle-stream and down-stream) during dry season (January 2016) and rainy season (July 2016).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Component** | **Dry Season** |  |  | **Rainy Season** |  |  |
|  | Up-stream | Middle-stream | Down-stream | Up-stream | Middle-stream | Down-stream |
| Naphthalene | 0.002 | 0.005 | ND | 0.002 | ND | ND |
| Acenaphthalene | 0.002 | ND | 0.005 | 0.034 | 0.013 | 0.027 |
| Acenaphthene | 0.002 | ND | ND | 0.008 | 0.037 | 0.029 |
| Florene | 0.002 | ND | 0.003 | 0.003 | ND | 0.085 |
| Phenathrene | 0.002 | ND | 0.020 | 0.010 | ND | 0.019 |
| Anthracene | ND | ND | 0.008 | ND | 0.034 | 0.023 |
| Fluoranthene | 0.003 | 0.015 | 0.017 | 0.008 | 0.004 | 0.028 |
| Pyrene | 0.011 | 0.010 | 0.002 | 0.012 | 0.017 | 0.016 |
| Benzo(a) anthrancene | 0.041 | 0.028 | 0.009 | 0.003 | 0.024 | 0.014 |
| Crysene | 0.015 | 0.004 | 0.008 | 0.007 | 0.004 | 0.013 |
| Benzo(b) fluoranthrene | 0.004 | 0.406 | 0.279 | 0.002 | 0.002 | 0.017 |
| Benzo(a)pyrene | 0.003 | 0.003 | ND | ND | 0.053 | ND |
| Benzo(k)fluoranthrene | 0.002 | ND | ND | ND | 0.003 | ND |
| Indeno(1,2,3) perylene | 0.002 | ND | ND | ND | 0.005 | ND |
| Dibenzo(a,h) anthracene | 0.001 | ND | ND | ND | ND | ND |
| Benzo(g,h,i)perylene | ND | ND | ND | ND | ND | ND |
| **Total (mg/kg**) | **0.093** | **0.472** | **0.350** | **0.091** | **0.192** | **0.267** |

***Bioaccumulation factor (BAF) of PAHs in fish samples.***

The bioaccumulation factor for PAHs showed in Table 4 revealed that the BAF for PAHs in all the sampled points were below 1, in the increasing order of; Up-stream > down-stream > middle-stream > Ekpan pond.

***Table 5: Bioaccumulation factor (BAF) of PAHs in Papyrocranus afer samples during dry season***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Up-stream | Middle-stream | Down-stream | Ekpan pond |
| **Conc of PAHs in fish** | 0.093 | 0.472 | 0.350 | 0.494 |
| **Conc of PAHs in water** | 1.340 | 4.695 | 4.442 | 3.274 |
| BAF | **0.069** | **0.101** | **0.079** | **0.151** |

***Estimated hazard risk (HR) of PAHs on the Papyrocranus afer from Banegbe river and Ekpan pond during dry and rainy season.***

The estimated HR for PAHs in Table 5 shows that all sampled points were greater than 1, and in the increasing order during the dry season as follow; Ekpan pond > middle-stream > down-stream > up-stream for PAHs.

***Table 6: Estimated hazard risk (HR) of PAHs on the fish from Banegbe and Ekpan pond during dry and rainy season***

|  |  |
| --- | --- |
| POINTS | PAHs (HR) |
|  | **Dry Season** |
| Middle-stream | 39.3 |
| Up-Stream | 7.75 |
| Down-Stream | 29.2 |
|  | **Rainy Season** |
| Middle-stream | 16 |
| Up-Stream | 7.58 |
| Down-Stream | 22.25 |
| Ekpan pond | 41.12 |

***Non-carcinogenic effect for the consumption of PAHs contaminated fish during the dry season.***

The non-carcinogenic effect for the consumption of PAHs contaminated fish during the dry season for all the sampled points presented in Table 6 using hazard quotient and hazard index revealed that the individual PAH hazard quotient and total hazard index of all the sampled locations were less than 1.

***Table 7 Risk assessment of PAHs based on non-carcinogenic equivalence using HQ and HI samples dry season.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Non- Carcinogenic equivalency** |  | **Banegbe River** |  | **Reference (Control site)** |
|  | **Up-stream** | **Middle-stream** | **Down-stream** | **Ekpan pond** |
| Naphthalene | 0.000052 | 0.00013 | ND | ND |
| Acenaphthylene | 0.000520 | ND | 0.00013 | ND |
| Acenaphthene | 0.000017 | ND | ND | ND |
| Florene | 0.000026 | 0.00039 | 0.000039 | 0.000091 |
| Phenanthrene | ND | ND | ND | ND |
| Anthracene | ND | ND | 0.000139 | ND |
| Fluoranthene | 0.000039 | ND | 0.000022 | ND |
| Pyrene | 0.000191 | 0.000173 | 0.000035 | 0.0000173 |
| Benzo(g,h,i) perylene | ND | ND | ND | ND |
| ΣHI | 0.000845 | 0.000693 | 0.000365 | 0.000264 |
|  |  |  |  |  |

***Non-carcinogenic effect for the consumption of PAHs contaminated fish during the rainy season.***

Result of non-carcinogenic effect for the consumption of PAHs contaminated fish during the rainy season during rainy season using hazard quotient and hazard index showed in Table 7, revealed that the individual PAH hazard quotient and the total hazard index of all the sampled locations were all less than 1, in the following order of increase: down-stream > Up-stream > middle-stream.

***Table 8: Risk assessment based on non-carcinogenic equivalence using HQ and HI samples rainy season.***

|  |  |  |  |
| --- | --- | --- | --- |
| **Non-Carcinogenic equivalency** | **Up- Stream** | **Middle-stream** | **Down- Stream** |
|  |  |  |  |
| Naphthalene | 0.000052 | ND | 0.000070 |
| Acenaphthylene | 0.00156 | ND | ND |
| Acenaphthene | 0.000069 | 0.00039 | 0.000251 |
| Florene | 0.000065 | ND | 0.001105 |
| Phenanthrene | ND | ND | ND |
| Anthracene | ND | 0.000069 | 0.000399 |
| Fluoranthene | 0.000234 | 0.000052 | 0.000364 |
| Pyrene | 0.000208 | 0.000295 | 0.000277 |
| Indeno(1,2,3)perylene | ND | 0.000065 | ND |
| ΣHI | 0.002188 | 0.000877 | 0.002466 |

***Carcinogenic toxicity (TEQ) relative to Benzo (a) pyrene (B[a]P) equivalence for the consumption of PAHs contaminated fish during the dry season.***

The result of carcinogenic toxicity (TEQ) relative to Benzo (a) pyrene (B(a)P) equivalence using lifetime cancer risk, revealed that the total TEQB[a]P value was in the following increase order of; down-stream > middle-stream > Ekapan pond >up-stream. With all the sampled locations at Banegbe above the USEPA standard of 10-6 during rainy season is presented in Table 8.

***Table 9: Risk assessment based on carcinogenic equivalence (TEQ), average daily dose, and lifetime excess cancer risk for consumption of fish samples during dry season.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Banegbe River** |  |  | **Reference (control) site** |
| **Carcinogenic equivalency (µg/kg)** | **Up-stream** | **Middle-stream** | **Down-Stream** | **Ekpan Pond** |
| Naphthalene | 0.002 | 0.005 | - | - |
| Acenaphthylene | 0.002 | - | 0.005 | - |
| Acenaphthene | 0.002 | - | - | - |
| Fluorene | 0.002 | - | 0.003 | - |
| Phenanthrene | 0.002 | - | 0.020 | - |
| Anthracene | - | - | 0.008 | - |
| Fluoranthene | 0.003 | 0.015 | 0.017 | 0.007 |
| Pyrene | 0.001 | 0.01 | 0.002 | 0.001 |
| Benzo(a) anthracene | 4.100 | 2.8 | 0.900 | 0.0114 |
| Chrysene | 0.0015 | 0.004 | 0.008 | 0.121 |
| Benzo(b) fluoranthene | 0.400 | 40.600 | 27.900 | 11.200 |
| Benzo(a)pyrene | 3.00 | 3.00 | - | 32.00 |
| Benzo(k)  Fluoranthene | 0.022 | - | - | 0.03 |
| Indeno (1,2,3) perylene | 0.20 | - | - | 0.200 |
| Dibenzo(a,h) anthracene | 1 | - | - | 102 |
| ΣBaP TEQ | 8.6356 | 43.424 | 28.863 | 41.3812 |
| ΣBaPTEQ ADD | 0.0049 | 0.0225 | 0.0150 | 0.08162 |
| LCR | 0.0328 | 0.1648 | 0.1096 | 0.5958 |
|  |  |  |  |  |

***Mutagenic toxicity (MEQ) relative to Benzo (a) pyrene (B[a]P) equivalence for the consumption of PAHs contaminated fish during the dry season.***

Mutagenic toxicity (MEQ) relative to Benzo (a) pyrene (B(a)P) equivalent using lifetime cancer risk showed in Table 9, revealed that the total MEQB[a]P values were in the increasing order of; down-stream > middle-stream >Ekpan pond >up-stream. With all the sampled locations at Banegbe above the USEPA standard of 10-6

***Table 10: Risk assessment based on mutagenic equivalence, average daily dose, and lifetime excess cancer risk for fish samples during dry season.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Banegbe River** |  |  | **Reference (control) sites** |
| **Mutagenic equivalency (µg/kg)** | **Up-stream** | **Middle-stream** | **Down-Stream** | **Ekpan Pond** |
| Benzo(a) anthracene | 3.362 | 2.296 | 0.738 | 9.348 |
| Benzo(b) fluoranthene | 1.00 | 101.5 | 69. 75 | 28 |
| Benzo(k) fluoranthene | 0.22 | ND | ND | 0.33 |
| Benzo(a)Pyrene | 3 | 3 | ND | 32 |
| Dibenzo(a,h) anthracene | 0.31 | ND | ND | 29.85 |
| Chrysene | 0.255 | 0.068 | 0.136 | 2.057 |
| Indeno (1,2,3,) perylene | 0.580 | ND | ND | 0.0062 |
| ΣBaP MEQ | 8.727 | 106.864 | 70.624 | 41.3812 |
| ΣBaP MEQ ADD | 0.004538 | 0.0556 | 0.03672 | 0.0029848 |
| LECR | 0.331277 | 0.40564 | 0.26809 | 0. 021790 |

***Carcinogenic toxicity (TEQ) and Mutagenic toxicity (MEQ) relative to Benzo (a) pyrene (B(a)P) equivalence for the consumption of PAHs contaminated fish during rainy season.***

The total TEQB[a]P and MEQB[a]P values using lifetime cancer risk were in the following increasing order of; down-stream > middle-stream >up-stream for TEQB[a]P. For MEQB[a]P, the order was middle-stream > downstream> upstream. The TEQB[a]P and MEQB[a]P of all the sampled locations at Banegbe were all above the USEPA standard of 10-6, as shown in Table 10

***Table 11: Risk assessment based on carcinogenic and mutagenic equivalence, average daily dose, and lifetime excess cancer risk for fish samples during the rainy season.***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PAH Component** | **Up-stream** | **Middle-stream** | **Down-Stream** | **Up-stream** | **Middle-stream** | **Down-Stream** |
|  | **Carcinogenic equivalence** |  |  | **Mutagenic equivalence** |  |  |
| Naphthalene | 0.002 | - | - | - | - | - |
| Acenaphthylene | 0.034 | 0.013 | 0.027 | - | - | - |
| Acenaphthene | 0.008 | 0.037 | 0.029 | - | - | - |
| Florene | 0.003 | - | 0.085 | - | - | - |
| Phenanthrene | 0.010 | - | 0.019 | - | - | - |
| Anthracene | - | 0.34 | 0.23 | - | - | - |
| Fluoranthene | 0.008 | 0.004 | 0.28 | - | - | - |
| Pyrene | 0.012 | 0.017 | 0.016 | - | - | - |
| Benzo(a) anthracene | 0.3 | 24 | 1.4 | 0.246 | 1.968 | 1.148 |
| Chrysene | 0.007 | 0.004 | 0.013 | 0.119 | 0.064 | 2.21 |
| Benzo(b) fluoranthene | 0.2 | 0.02 | 1.70 | 0.5 | 0.5 | 4.25 |
| Benzo(a)pyrene | - | 53 | - | - | 53 | - |
| Benzo(k)fluoranthene  Fluoranthene | - | 0.33 | - | - | 0.33 | - |
| Indeno(1,2,3) perylene | - | 0.5 | - | - | 1.55 | - |
| Dibenzo(a,h) anthracene | - | - | - | - | - | - |
| BaP TEQ and MTQ | 0.584 | 78.235 | 3.799 | 0.865 | 57.412 | 7.608 |
| BaPTEQ and ADD | 0.00030 | 0.04068 | 0.00197 | 0.00045 | 0.02985 | 0.00396 |
| (LCR) | 0.00222 | 0.29698 | 0.01442 | 0.00329 | 0.2179 | 0.2888 |
|  |  |  |  |  |  |  |

**3.2 Discussion**

Analysis of results

The presence of PAHs in Banegbe River water is an indication of contamination from those industries located along the river, which channelled their effluent into the river. From our results Fourteen out of sixteen priority PAHs listed by USEPA as hazardous were detected in all the different locations in Banegbe river water with most of 4 and 6 ringed PAHs, which are potential cancer-causing chemical present in them. Benzo(a) anthracene had the highest PAHs concentration in Banegbe river while benzo (b) flouranthrene was highest in fish during dry season Agbozu *et al*., (2020) detected 50 percentage of four and six-ringed PAHs compounds in the Ethiope River, which is similar to our result. The Ekpan pond water was found to contain all the sixteen PAHs which includes naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, chysene, benzo (a) pyrene, benzo (b) fluoranthene, indeno (1,2,3, cd) pyrene, benzo (k) fluoranthene, Benzo (g, h, i) perylene, dibenzo (a,h) anthracene. The last seven PAHs mentioned have been said to be probable human carcinogens. These PAHs warrant reasonable regulation and remediation. The concentration of PAHs at different points of the Banegbe River and pond water were higher when compared with recommended permissible limit of fresh water guidelines of 50 ng/L, 0.002 mg/L and 0.003 mg/L by WHO 1998, USEPA, 1996 and ANZECC, 2000, respectively. Concentrations above 50 ng/L indicate contamination by PAHs mainly through industrial point sources. PAH levels in uncontaminated groundwater are usually in the range of 0-5ng/l (Stuermer *et al.,* 1982).

Comparison with other studies

Our present study is in line with other researchers who observed the presence of PAHs in different rivers in Nigeria, which were above the WHO limit of 50ng/l. Davies and Abolude (2016) reported the presence of PAHs in the Oburun Lake Niger Delta, Nigeria with a concentration higher than this present study which was attributed to pyrolytic sources. Also, Nwineewii and Abiye (2015) detected higher PAH concentrations when compared to our results in some creeks of southeast in River state was also as a result of incomplete combustion of PAH containing materials respectively.

Nnwachukwu *et al.,* (2016) reported a total lower PAH concentration in River Niger and Benue confluence, Lokoja when compared with our present study, with only six PAH (Nap, Ph, BbF, BkF and BaP) detected. Also, Obiakor *et al*., (2014) reported a lower total concentration of PAHs in a freshwater media. Ihunwo *et al*. (2029)found up to eleven PAHs in the waters of Woji Creeks. The concentrations of PAHs in the water samples ranged between 6.029 and 29.257 ppm over a 3 –month period.

From our current result, Naphthalene and benzo (g h i) were below detectable levels at all sampled points in the Banegbe River. This was in line with the studies of Agbozu *et al.,* 2020 in the Ethiope River. And Ibigbami *et al.,* 2020 in Egbe dam southwestern Nigeria with naphthalene not detected both studies. Consequently, many common PAHs have short residence time in the water column due to volatilization, oxidation, and elimination from the system (Qiu *et al*., 2009). Their presence in water and other media connotes acute or chronic pollution and provides a link to possible human exposure and subsequent toxicity. The presence of these PAHs, especially Benzo(a) anthracene and benzo (a) pyrene at different points in the Banegbe river can pose a risk to the users of Banegbe river water as drinking and cooking water. The International Agencies for Research on Cancer (IARC) identified benzo (a) anthracene and benzo (a) pyrene as possible carcinogens to humans (IARC, 2002). The U.S Environmental Protection Agency has established drinking water criteria for benzo (a) pyrene, not to exceed 200ng/L (USEPA,1996). The Banegbe River, used as a source of drinking water for most of the population of the communities around, has 1.062mg/l and 0.107mg/l of benzo (a) anthracene and benzo (a) pyrene, respectively, that make the user of Banegbe river water at risk to cancer. The aquatic life in the river is also exposed to these contaminants, especially fishes.

PAHs in fish

Fish is an important food source for the human body. Fish provide essential fatty acids like omega 3, proteins, vitamins, and minerals. Despite their nutritive value, fish are also vulnerable to contamination by toxic industrial pollutants, such as mercury, as well as PAHs, PCBs, dioxins, flame retardants, and other lipophilic chemicals that cause potential hazard concerns to human consumers (Gado and Midany, 2003). From our studies, the concentration of PAHs was higher in water samples compared to PAHs in fish samples as shown in table (2 and 3). The PAHs in the fish sample were also in the order in which PAHs were distributed in the river water samples (middle-stream > down-stream > up-stream). This confirmed that fish are migratory and seldom settle in one place. Pollutant accumulation in fish organs (muscle) provides evidence of exposure to the contaminated aquatic environment and could be used to assess the health condition of the area from which they were collected (Aqudir and Malik, 2011).

The low concentration of PAHs in the fish samples when compared to the surrounding water environment may be due to the ability of different organisms to absorb PAHs, and this plays a major role in the potential for bioaccumulation and bioconcentration. Studies have shown that Crustaceans and fish metabolize PAH compounds more efficiently than do bivalve species such as mussels, clams, and oysters, which readily accumulate PAHs (Garrett, 2004). As such, they exhibit the highest BCFs, while fish and many crustaceans, which readily metabolize PAHs, generally obtain lower whole-body residues. And these can account for the lower residue of PAHs in the fish samples in this work when compared to the concentration in river water. PAHs are metabolized by liver mixed-function oxidases to epoxides, dihydrodiols, phenols, and quinones. The intermediate metabolites have been identified as mutagenic, carcinogenic, and teratogenic agents (Perra *et al*., 2011).

Fish samples in the Banegbe River also accumulated higher concentrations of PAHs during the dry season than the rainy season, and could be as a result of dilution from rainwater or exposure to incineration from petroleum products during the dry season. Adekunle *et al*., 2020 reported a higher PAH concentration in surface water during the dry season than wet season in River sasa in Ife North LGA of Osun State, which was attributed to a pryrolytic source. Also, Tongo *et al.,* (2018) reported a similar range of PAHs in fish from the Bonny River polluted with petroleum.

The limit for PAHs in fatty-foods and smoked foods was set to protects humans, the (EU, 2011b) consider benzo (a)pyrene as an important marker for PAH in food and EU, (2011b) proposed a total concentration of 4 PAHs (benzo (a) pyrene, benzo (a) anthracene, benzo (b) fluoranthene and chrysene) as better benchmark of the presence of PAHs in food. The Maximum Recommended Limit (MRL) for PAHs was set to be 12µg/kg for 4 PAHs and 2µg/kg for benzo (a) pyrene. Therefore, from our present results, the fish from the Banegbe River in both seasons were contaminated with PAHs.

Risk Assessment

Risk quotient is used to evaluate the potential risk of certain pollutant compounds on the aquatic organism in its environment. From the present result, PAHs at all the sample points analyzed (middle-stream, downstream, upstream, and Ekpan pond fish) have the ability to cause harm to the fish. When RQ is less than one, the potential adverse effect caused by the contaminant exposure is minimal, but when greater than one, the potential adverse effect caused by the contaminant exposure may be severe (Jiang *et al.*, 2014). It has been known that pollutants like PAHs have a number of adverse effects on aquatic organisms, such as reproductive impairment and suppression of the immune system (Aguilar *et al.,* 2002), which can have long-term consequences for population viability.

The HQ was calculated based on the average daily dose and reference dose (RfD), and HI is the sum of Individual HQ for PAHs. From the result of the HQ and HI connected to the consumption PAHs contaminated fishes were found to be below unity (< 1) for all the sampled points. When HQ and HI are less than one, it implies that little or no significant potential negative influences are exerted on human health, whereas potential negative influences on human health may be assumed if HQ > 1 (Frederic and Yves, 2014). Comparing the standard unity with our result, it could be concluded that consumption of fish from all the sampled points for PAHs contamination has no effect on the 70kg adult average population of the fish consumption of 36.4g per capita per day for non-carcinogenic effect.

Carcinogenic risks for PAHs were also assessed by evaluating the carcinogenic and mutagenic potencies of individual PAHs concentration, using toxic equivalence factor (TEFs) and mutagenic equivalence factor (MEF) to express their potency relative to benzo (a) pyrene. The carcinogenic toxicity equivalent is directly associated with carcinogenicity, but the mutagenic equivalent may not be directly associated with cancer (Zeiger, 2001) and may have implications for another non-cancerous adverse health effect like pulmonary diseases, birth defects, impotency, and low intelligence quotient (Essumang *et al.,* 2013). The carcinogenic and mutagenic toxicities associated with benzo(a)pyrene were calculated using TEF and MEF values in Table 1 and used to calculate the corresponding carcinogenic toxicity equivalent daily dose, as shown in Tables 9, 10, and 11 for consumption of contaminated fish samples during the rainy and dry seasons. Carcinogenic and mutagenic risk for an adult involved in a time of 70 years for ingestion of *Papyrocranus afer* in Banegbe river were all above the USEPA unity value of 10−6 during the two seasons. This means that the consumption of fish from all sources can pose a Carcinogenic and mutagenic risk to consumers of contaminated fish, compared to the USEPA limit of 10−6 (USEPA, 2010). Akpan *et al*., 2022 reported a similar higher cancer risk of 7.10 10−7 to 7.18 10−4 when compared to USEPA limit due to ingestion of PAHs from water in adult population

From the results in Table 11, the value of LCR through ingestion of PAH-contaminated *Papyrocranus afer* indicates that residents may face a potential carcinogenic risk due to exposure to PAHs in the consumption of *Papyrocranus afer* at all sampled points during both rainy and dry seasons. This is because they were all above the USEPA unity, which the acceptable values of lower than 10−6 are considered to be negligible for carcinogenic and above are considered unacceptable (USEPA, 2010). This means that consumption of fish from these points is unsafe and may possess a carcinogenic risk in the lifetime of some of the consumers.

4. Conclusion

The study depicts the presence of PAHs in water and fish samples of the Banegbe River. The results revealed that the high molecular weight PAHs composition were more predominant than the low molecular weight PAHs in both surface waters and fish. The PAHs composition in the samples shared similar pattern and was dominated by both low molecular weight (2-3 rings PAHs) and high molecular weight PAHs (4-6 rings PAHs), which suggest pyrolytic and. petrogenic sources. This could be attributed to industrial effluent channeled into the river and run off water from the surrounding. Some of the water samples exceeded the compared international standard guidelines for groundwater and drinking water which necessitate regular and continuous monitoring of the river so as to ensure safety of the water before consumption. The study also indicates risk of cancer effect resulting from PAHs exposure through consumption of fish from the Banegbe River exceeded the acceptable thresholds in both seasons for the adult population. Therefore, the fish in the river are unsuitable for human consumption and unfit for animal feed formulation. These suggests that close attention should be given to the indiscriminate release of domestic, agricultural, and industrial micro-pollutants in the river. Also renewed call should be made to experts and policy makers to urgently develop a regulatory frame work for permissible levels of PAHs into rivers from emitting sources and the environment at large.

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