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Comparability Assessment of Polycyclic Aromatic Hydrocarbons Tissue Load in Some Fish: Implication on Reciprocal Synergism and Risk Assessment

[1]Victor Eshu Okpashi, [2]Ogugua Victor Nwadiogbu, [3]Joshua Parkar Elijah, [4]Chibuike Samuel Ubani, [5]Obinna Aru Oje, [6]David Mbu Akpo, [7]Robert Ikechukwu Uroko and [4]Ikechukwu Noel Onwurah

[1]Environmental Toxicology and Molecular Biochemistry Unit, University of Nigeria, Nsukka, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
[3] Pharmacology Unit, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
[4]Industrial Biochemistry and Biotechnology Unit, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
[5]Department of Chemistry/Biochemistry/Molecular Biology, Federal University Ebonyi State, Nigeria
[6]Department of Environmental Education, University of Calabar, Nigeria
[7]Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture, Umudike, Nigeria

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Corresponding Author:
Victor Eshu Okpashi
Environmental Toxicology and Molecular Biochemistry Unit, University of Nigeria, Nsukka, Nigeria
Email: vic2reshu@gmail.com

Abstract: Six years after oil spill occur in Qua Ibeo river, environmental monitoring was set out to investigate residual petroleum compounds (PHCs) that are bio-accumulated by fish. Bio-concentration of residual Polycyclic Aromatic Hydrocarbons (PAHs) in fish tissues was determined in six fresh fish species. Twelve water samples were collected from Qua Ibeo river 1 KM apart. The screening was conducted using Agilent gas chromatography tandem mass spectroscopy. Results revealed 17 PAHs accumulated at variable concentrations. Bio-concentration factor and free PAHs in water was calculated by finding the ratio of PAHs concentration in fish tissue to water free PAHs concentration. The contaminant body load was extrapolated by summation of individual PAHs concentration. Results shows African Red snipper (*Lutjanus agennes*) 20.822±0.6132 with body load as the highest, Yellow tail (*Seriola lalandi*) 13.111±1.247, Atlantic Crocker (*Micropogonias undulates*) 9.8439±6.569, Tilpia (*Oreochromis niloticus*) 9.7790±12.305, Cat Fish (*Clarias gariepinus*) 7.298±4.529 and Barracuda (*Sphyraena barracuda*) 6.853±7.937 respectively. The percentage PAHs concentration in samples was also determined, for instance, African red snapper have 10.9% Indeno (1,2,3,cd) pyrene, Yellow tail 10.94% Benzo (a) pyrene, Barracuda 10.05% Indeno (1,2,3,cd) pyrene, Atlantic Croker 13.03% Indeno (1,2,3,cd) pyrene, Catfish 9.61% Indeno (1,2,3,cd) pyrene and Tilapia (*Oreochromis niloticus*) 11.84% Indeno (1,2,3,cd) pyrene respectively.

Keywords: Bio-Concentration Factor, PAHs Body Load, Risk Assessment, Public Health and Environmental Monitoring

Introduction

Many names have been used to link patients syndromes associated with sensitivity to pollutants. The difficulty in achieving scientific investigations that will agree with definition of such situation has not received a breakthrough (Rose *et al*., 2012). Contaminants body burden is organism's overall contaminant concentration, usually from air, food and water or surroundings (NRC, 1992). The organism must match with this burden. Though, there are usually expelled or put into restrictive section. This research monitored the environmental risk. The knowledge is derived from previous research works on environmental risk limits of petroleum aromatic hydrocarbons. The risk limits are calculated by concentration of substances in the organisms when they

might have taken in the pollutant from media. The concept of contaminants body load bear testimony to the organisms multiple sensitivity to environmental contaminants due to reciprocal synergism (NRC, 1991). A patient or an organism with multiple chemical sensitivities or synergetic contaminants effect may be identify by separating the queer offending agent and challenging after reasonable interval, under controlled conditions (Rose *et al*., 2012). Causation can be deduced by elimination of signs, removal from the hurting media and recurrence of signs with distinct contest. Petroleum hydrocarbons compounds are released into the environment media through accidents discharge of industrial effluent. Contaminants are also released into water by oil thieves and desperate crude oil vandals. Whenever pollutants such as PAHs are released directly into water column by spills or leaks, PAHs fractions will float in water and form thick surface films. Some heavier fractions of the PAHs will accumulate in the sediment, where benthic organisms may be affected. The case of oil spills in Eket has been reported, for example the Ibeno spill that occurred on May 1st, 2010, where a burst Exxon Mobil pipeline leaked over a million gallons crude oil into the coast in about seven days before the leakage was stopped and within days of the spill, thick balls of tar were calcified along the coast. After the Exxon Mobil incident, another crude oil spill occur at Ibeno, where several barrels of oil were leaked due to Vandals attack on the Shell Trans Niger pipeline (DeWitt *et al*., 1992). In 2012, over 200,000 barrels of crude oil leaked into the coastal communities of Eket. Similarly, some incidence of oil spill were reported to have occur at the Qua Iboe oil fields on August 13, 24, November 9, December 16 and 19, 2012 respectively. (Gobas *et al*., 1999) Study on PAHs shows that oil spills and gas flaring are a major route for introducing PAHs into the environment and aquatic ecosystems can be stressed. The health risk and yearly economic loss to the inhabitants due to these activities cannot be quantified. The water and sediment qualities within the study area are also influenced by sewage, indiscriminate disposal of solid waste, trading activities, abattoir (slaughter houses) and run-off from agricultural lands. PAHs are organic compounds with two or more benzene rings. Their existence results from incomplete burning of smoke materials. Therefore, PAHs are constituents and derivatives of petroleum (Atuanya and Nwogu, 2013). Spillage of oils and effluents from refineries contributory sources of PAHs pollution on aquatic ecosystem. The impact is weigh on human who live by consuming both the polluted water and contaminated fishes. The resultant effect is the hormonal alteration. Therefore, methods for estimating concentration of specific contaminants, developing accurate estimation of tissue concentrations of PAHs in aquatic organisms, monitor existing bioaccumulation in order to predict bioaccumulation due to future exposure and the use of aquatic organisms as biomarkers for precise prediction of compounds and assessment of contaminants synergetic impact and effect on aquatic organisms, gave impetus to of this investigation.

Materials and Methods

Sampling Locations

Four different locations were marked in order to collect twelve samples. The locations were established on ecological settings and anthropogenic activities. The locations were 1 km apart. Between location 1 and 4, a total of 3 km was covered.

Location 1

The sampling location was the upstream, located at Ikot Ikpe and Ikot Akpoenang at latitude 40° 55.8" and longitude 70 40.8". Anthropogenic activities in this location include fishing and boat making at the edge of the river. The water is considerably clean by human eye-sight evaluation.

Location 2

Location 2 is influenced with effluents from the meat factory along Ikot Aroku and Ikot Naidiba Village Road. Household from residential houses are also discharged into the River. The main activities are mining and loading of sand for commercial purposes. It is about 1km away from station 1 and located at latitude 40 22.9" and longitude 70 13.8".

Location 3

Location 3 was along Eket-Etinan Road, around Ebiyan and Ndon and visibly opposite Onna Local Government Area. Actions here include automobile washing, clothes and bathing. The vegetation is dominated with green bamboo strands. It is 1km apart from point 2.

Location 4

Location 4 was Ndilla, across the river are Odio and Ale Ebukuku, which derived into Ibeno local government area. The activities are mining of sand. This area is turbid due to sufficient discharge of solid wastes. It is very deep due to the sand mining activities.

Collection of River Water Sample

Water samples were collected with 12 amber bottles, each 100 mL at twelve locations on 2nd September, 2015 between 10 a.m to 3 p.m. The collected samples were extracted using normal hexane before the concentration of analytes within 30 min.

Reagents and Chemicals

All chemicals were of analytical grade and were products of British Drug House (BDH) Chemical limited, Poole England.

Collection of Fish Sample

The fish were collected randomly. Some variables influenced the site selection they include proximity to oil wells locations, gas flaring from Bonny Bright, heightened population and socio-economic activities.

Locally consumed fresh fish were used. They include: African red snipper (*Lutjanus agennes*), yellow tail (*Seriola lalandi*), atlantic crocker (*Micropogonias undulates*), tilpia (*Oreochromis niloticus*), cat fish (*Clarias gariepinus*) and barracuda (*Sphyraena barracuda*) respectively. They were collected by a resident fisherman using set nets.

Preparation, Extraction and Clean-Up Procedure of Fish Samples for Analysis

Before the extraction of fish samples, scales were removed and dissected using knife. A 15 g of the fish tissue was pounded in a clean mortar with pestle in addition to 40 g of anhydrous sodium sulphate until homogenized. Dichloromethane solvent was used for sample extraction. A 10 g of the homogenized sample was placed in 50 mL of extraction bottle and 1 mL of 60 ng/mL of 1- chloro-octadecane surrogate standard was added in the extraction bottle. The content was agitated for 5 h and allowed for 1 h to settle. The homogenates was carefully filtered through a funnel fitted with cotton wool, silica gel and sodium sulphate (Na_2SO_4) in a volumetric flask. The residue was washed and the volume made up using the extraction solvent. The sample was concentrated to 2 mL for PAHs analysis using a gas chromatography tandem mass spectroscopy.

Method of GC-MS

Gas chromatographic tandem mass spectroscopy technique was used with the following conditions. GC/MS-QP2010 Agilent Plus, ion source temperature: 200.00°C, interface temperature: 250.00°C, solvent cut time: 2.50 min, detector gain mode: MS, detector gain: 0.00 kV, threshold: 2000, column oven initial temperature: 70.0°C, injection final temperature: 250.00°C, injection Mode: Split, flow control mode: linear velocity, pressure: 116.9 kPa, total Flow: 40.8 mL min^{-1}, column flow: 1.80 mL min^{-1}, linear velocity: 49.2 cm sec^{-1}, trap and purge flow: 3.0 mL min^{-1}, Split Ratio: 20.0, high pressure injection: OFF, Carrier Gas: Helium and Splitter hold: OFF. While oven rating was as follows: Oven Temp. Program Rate Temperature (°C) Hold Time (min) Initial: 0.00 70.0 0.00 Final: 10.0 280 5.00.

Extraction of Water Samples for PAH Quantification

Liquid-liquid extraction protocol was used. A litre of sample was extracted in a 2 litre glass separating funnel fitted with a glass stopper using 30 mL hexane as extraction solvent.

The separating funnel was shaken vigorously after 3 minutes, the organic layer was allowed to visibly phase separate from the aqueous solution. The organic layer was collected into a different glass bottle. The extraction process was carried out in triplicates. Residues of water were expelled from the organic layer by passing extraction solvent through the separating funnels containing anhydrous sodium sulphate. Extracts were concentrated with rotary evaporators and water bath preset at 85°C. Concentrated extracts was transferred to a pre-weighed sample bottle and evaporated to dryness.

Interpolation of Bio Concentration Factor

The bio-concentration factor was calculated by adapting the method described by (McCarty, 1986). The Bio-Concentration Factor (BCF) is the proportion of a particular chemical in tissue to its water concentration see equation 1 below. We took notice that BCF is relevant only for accumulation from water; wherein to compare among BCFs it is important to establish that water is the only route of uptake. Contrary to The Bioaccumulation Factor (BAF) which is generally computed as the proportion between the toxicant concentrations in tissue and multiple external sources (e.g., sediment, water and diet) and is useful in determining the tendency of hydrophobic compounds to accumulate in tissue. A seldom used term, dietary accumulation, is used to determine the proportion between the concentration of a contaminant in an organism and food:

$$BCF\ with\ free\ PAHs\ in\ water = Tissue\ /\ (Water\ free) \quad (1)$$

BCF, (bio-concentration factor with free PAH in water) = [Tissue]/[Water free]

BCF is BCF predicted. For example the equation from [19]; = 0.046 K_{Ow}.

Total BCF of PAHs tissue load was calculated by summation of mean BCF of individual PAHs see Equation 2 below:

$$\sum MBCF = TBCFTL$$

Where:

MBCF = Mean bio-concentration factor
TBCFTLB = Total bio-concentration factor of tissue load

The percentage Polycyclic Aromatic Hydrocarbons (PAHs) concentration in fish sample was calculated using equation 3:

$$\begin{aligned} &percentage\ PAHs\ concentration \\ &= MBCF / TBCFTL \times 100 / 1 \end{aligned} \quad (3)$$

Where:

MBCF = Mean bio-concentration factor
TBCFTL = Total bio-concentration factor of PAHs tissue load

Statistical Analysis

Investigations were carried out in triplicate and data are presented as mean ± standard deviation using descriptive statistics. One way analysis of variance was used to compare mean difference among samples. Significance was accepted at $p<0.05$.

Results and Discussions

Many studies have shown that marine organism's bio-accumulate PAHs from the environmental media. It is not so much a query or contemplation of whether an aquatic animal will bio-accumulate PAHs, instead, what quantity of these PAHs are bio-accumulated. This and other questions are concern for assessment of human health and safety. Since human beings live by consuming this contaminated resources as food. Considering the concentration of PAHs in environmental media, controlling the bioavailability of PAHs interval which the organism enduring the contaminant and the physiology of the organism to contest with PAHs Body Load, are all tune of aquatic pollution and degeneration. Bio-concentration patterns of PAHs in marine organisms are diverse in many factors. These include, but not limited to, exposure to pollutrd media, uptake rate of contaminated feeds stock, metabolic capability, age, lipid content and feeding strategy and habit (Adams, 1987; Meador *et al*., 1995; Roesijadi *et al*., 1978; Schrap and Opperhuizen, 1990; Varanasi *et al*., 1985). These factors are considered whenever accumulations of PAHs are compared. Because biotransformation of pollutants is one of the more important processes of evaluating metabolic capacity and inclination pattern of parent compounds included in many studies of PAHs bioaccumulation to assess total PAHs uptake accurately (Bruner *et al*., 1994).

In this investigation, 6 different fresh fish species that are customarily consumed as food by the residents of Eket community in Akwa Ibom State Nigeria, were randomly selected for screening of PAHs levels. This help established the extent of residual PAHs accumulation in aquatic animal model. Twelve (12) water samples were collected from Qua Ibeo River at 1 km apart. The screening was carried out using Agilent gas chromatography tandem mass spectroscopy. Upon investigation, the total mean concentration of individual toxicant in water and in fish was determination and reported in triplicate. The bio-concentration factor of the fishes was extrapolated by adapting the method of McCarty (1986) see equation 1.

The Bio-Concentration Factor (BCF) is the proportion of the tissue absorption of a particular substance to its water concentration. It was kept in perspective that the BCF is relevant only to the extent of accumulation from water; to compare among BCFs, it is important to establish that water is the only route of uptake. At equilibrium, the BCF generally increases with increasing chemical hydrophobicity because of the increased fugacity or tendency of the chemical to partition into the animal's lipid rather than stay in solution (Bruner *et al*., 1994; De Mora *et al*., 2004). The results obtained as mean concentration for toxicants body residues varies from species considerably. For example, Atlantic Crocker (*Micropogonias undulates*) showed naphthalene 10.370±0.302 ppm, 2-methylnaphthalene 10.160±0.112 ppm, acenaphthylene 10.170±0.151 ppm, acenaphthene 10.403±0.431 ppm, fluorene 12.707±4.593 ppm, phenanthrene 10.360±0.355 ppm, anthracene 9.443±0.551 ppm, fluoranthene 16.416±3.787 ppm, pyrene 13.873±3.639 ppm, benzo (a) anthracene 10.240±0.832 ppm, triphenylene 10.216±0.120 ppm, benzo (e) pyrene 15.616±4.779 ppm, benzo (a) pyrene 16.200±5.232 ppm, Indeno (1, 2, 3, cd) pyrene 17.670±3.836 ppm, Benzo (g,h,i) perylene 14.126±4.564 ppm, dibenzo (a,h) anthracene 16.076±5.168 ppm and 000053-70-3-benzo(e) pyrene 13.590±5.477 ppm respectively Table 1-6.

Several researchers have noticed that PAHs concentrations in marine organisms appear to show seasonal variation, attributable to a number of factors (Atuanya and Nwogu, 2013). What customary with contaminants is that PAHs bio-accumulation in some tissues is higher, with larger proportions concentrated in the liver of vertebrates and the hepato-pancreas of invertebrates Nyarko *et al*. (2001). General, it's been observed that tissues rich in lipid preferentially bio-accumulate parent PAHs because of their strong hydrophobic nature (Umeh, 2009). Meanwhile, the total mean concentration of the individual contaminants or toxicants in water column was determined Table 1-6.

As exemplified in atlantic crocker (*Micropogonias undulates*). Results revealed 23.302±0.114 ppm for Naphthalene, 28.225±0.231 ppm 2-Methylnaphthalene, 16.564±0.220 ppm Acenaphthylene, 27.585±1.210 ppm Acenaphthene, 27.239±0.123 ppm Fluorene, 28.421±2.100 ppm Phenanthrene, 28.256±0.221 ppm Anthracene, 27.481±0.401 ppm Fluoranthene, 28.201±1.091 ppm Pyrene, 26.264±0.220 ppm Benzo (a)

anthracene, 29.077±0.329 ppm Triphenylene, 27.594±0.211 ppm Benzo (e) pyrene, 13.873±0.111 ppm Benzo (a) pyrene, 13.774±0.213 ppm Indeno (1, 2, 3, cd) pyrene, 28.383±0.210 ppm Benzo (g, h, i) perylene, 28.302±0.101 ppm Dibenzo (a,h) anthracene and 27.828±0.171 ppm for 000053-70-3-benzo(e) pyrene differently.

Accumulation may be a function of PAH hydrophobicity, but time of exposure and metabolic capacity must be considered. Several studies have shown rapid attainment of steady-state BCFs in marine organisms exposed to PAHs in water. For example, similar values (BCFs ≅ 50) were calculated for *Mytilus edulis* after 4 h of exposure to labeled naphthalene and after 4 weeks exposure to unlabeled naphthalene, indicating rapid equilibration of exposure concentrations (Widdows *et al*., 1983). The determination of BCFs in field-collected animals is uncommon, possibly because it is difficult to assure water-only exposure and accurately determine temporally variable concentrations. In this study, the bio-concentration for all the models were quantified. Results showed variable bioconcentration of toxicants in fish as shown in Table 1-6.

Table 1. Mean concentration of PAHs bio-accumulated in Atlantic Crocker (*Micropogonias undulates*)

Polycyclic aromatic hydrocarbons (PAHs)	Mean conc. of contaminant in fish (ppm)	Mean conc. of contaminant in water (ppm)	Mean Bio-Concentration factor (MBCF)	Percentage PAHs conc. (%)
Naphthalene	10.370±0.302	23.302±0.114	0.4450±2.649	4.52
2-Methylnaphthalene	10.160±0.112	28.225±0.231	0.3599±0.485	3.65
Acenaphthylene	10.170±0.151	16.564±0.220	0.6139±0.686	6.23
Acenaphthene	10.403±0.431	27.585±1.210	0.3771±0.356	3.83
Fluorene	12.707±4.593	27.239±0.123	0.4665±37.34	4.74
Phenanthrene	10.360±0.355	28.421±2.100	0.3666±0.169	3.72
Anthracene	9.443±0.551	28.256±0.221	0.3341±2.493	3.39
Fluoranthene	16.416±3.787	27.481±0.401	0.5974±9.444	6.07
Pyrene	13.873±3.639	28.201±1.091	0.4919±3.335	5
Benzo (a) anthracene	10.240±0.832	26.264±0.220	0.3899±3.782	3.96
Triphenylene	10.216±0.120	29.077±0.329	0.3513±0.365	3.57
Benzo (e) pyrene	15.616±4.779	27.594±0.211	0.5659±22.65	5.75
Benzo (a) pyrene	16.200±5.232	13.873±0.111	1.1677±47.13	11.86
Indeno (1, 2, 3, cd) pyrene	17.670±3.836	13.774±0.213	1.2829±18.01	13.03
Benzo (g, h, i) perylene	14.126±4.564	28.383±0.210	0.9769±21.73	9.92
Dibenzo (a,h) anthracene	16.076±5.168	28.302±0.101	0.5681±51.17	5.79
000053-70-3-benzo (e) pyrene	13.590±5.477	27.828±0.171	0.4884±32.03	4.96
Total BCF of PAHs tissue load			9.8439±6.569	100%

Table 2. Mean concentration of PAHs bio-accumulated in Tilpia (*Oreochromis niloticus*)

Polycyclic aromatic hydrocarbons	Mean conc. of contaminant in fish (ppm)	Mean conc. of contaminant in water (ppm)	Mean BIO Concentration Factor (BCF)	Percentage PAHs conc. (%)
Naphthalene	13.573±4.134	23.302±0.114	0.5825±36.263	5.94
2-Methylnaphthalene	13.140±5.161	28.225±0.231	0.4655±22.342	4.76
Acenaphthylene	13.767±4.794	16.564±0.22	0.8311±3.9215	8.49
Acenaphthene	13.727±4.745	27.585±1.210	0.4976±3.9215	5.08
Fluorene	18.700±0.572	27.239±0.123	0.6865±4.6504	7.02
Phenanthrene	16.590±5.088	28.421±2.100	0.5837±2.4229	5.96
Anthracene	16.616±4.958	28.256±0.221	0.5881±22.434	6
Fluoranthene	13.970±4.604	27.481±0.401	0.5083±11.481	5.19
Pyrene	13.426±5.191	28.201±1.091	0.4761±4.7580	4.84
Benzo (a) anthracene	13.280±5.076	26.264±0.220	0.5056±23.072	5.17
Triphenylene	13.840±5.018	29.079±0.329	0.4749±15.428	4.84
Benzo (e) pyrene	16.513±5.190	27.594±0.211	0.5876±24.597	6.01
Benzo (a) pyrene	16.073±5.069	13.873±0.111	1.1586±45.667	11.84
Indeno (1, 2, 3, cd) pyrene	12.586±5.105	13.774±0.213	0.9072±23.967	9.27
Benzo (g,h,i) perylene	13.473±4.876	28.382±0.210	0.4747±22.892	4.85
Dibenzo (a,h) anthracene	13.350±5.009	28.302±0.101	0.4717±49.594	4.82
000053-70-3-benzo (e) pyrene	13.263±5.245	27.828±0.171	0.4766±30.673	4.87
Total BCF of PAHs tissue load			9.7790±12.305	100%

Table 3. Mean concentration of PAHs bio-accumulated in Yellow tail (*Seriola lalandi*)

Polycyclic aromatic hydrocarbons	Mean conc. of contaminant in fish (ppm)	Mean conc. of contaminant in water (ppm)	Mean Bio-Concentration Factor (BCF)	Percentage PAHs conc. (%)
Naphthalene	18.6933±0.583	23.3017±0.114	0.8022±5.114	6.11
2-Methylnaphthalene	16.1300±5.232	28.2250±0.231	0.5714±22.64	4.35
Acenaphthylene	16.1100±5.274	16.5644±0.220	0.9726±23.97	7.41
Acenaphthene	16.5500±5.570	27.5853±1.210	0.5999±4.603	4.57
Fluorene	19.4967±0.412	27.2386±0.123	0.7068±3.349	5.39
Phenanthrene	19.3867±0.398	28.4211±2.100	0.6820±0.189	5.2
Anthracene	18.553±1.355	28.2558±0.221	0.6566±6.131	5
Fluoranthene	18.890±0.795	27.4806±0.401	0.6874±1.983	5.24
Pyrene	16.086±5.219	28.2011±1.091	0.5700±4.784	4.34
Benzo (a) anthracene	16.066±5.202	26.2642±0.220	0.6117±23.65	4.66
Triphenylene	19.066±1.050	29.0786±0.329	0.6556±3.191	5
Benzo (e) pyrene	19.100±0.078	27.5942±0.211	0.6922±0.369	5.27
Benzo (a) pyrene	19.090±0.101	13.8728±0.111	1.4344±0.909	10.94
Indeno (1, 2, 3, cd) pyrene	19.123±0.624	13.7744±0.213	1.3883±2.929	10.58
Benzo (g,h,i) perylene	19.590±0.375	28.3825±0.210	0.6902±9.277	5.26
Dibenzo (a,h) anthracene	20.203±0.937	28.3017±0.101	0.7138±9.277	5.44
000053-70-3-benzo (e) pyrene	18.796±0.571	27.8281±0.171	0.6754±3.339	5.14
Total BCF of PAHs tissue load			13.111±1.247	100%

Table 4 mean concentration of PAHs bio-accumulated in Barracuda Fish (*Sphyraena barracuda*)

Polycyclic aromatic hydrocarbons (PAHs)	Mean conc. of Contaminant in fish (ppm)	Mean conc. of Contaminant in water (ppm)	Mean Bio-Concentration Factor (BCF)	Percentage PAHs conc. (%)
Naphthalene	9.360±0.795	23.302±0.114	0.402±6.974	5.86
2-Methylnaphthalene	9.550±0.446	28.225±0.231	0.339±1.930	4.94
Acenaphthylene	9.4300±0.578	16.564±0.220	0.569±2.627	8.3
Acenaphthene	9.806±0.596	27.585±1.210	0.355±0.492	5.18
Fluorene	8.777±1.154	27.239±0.123	0.322±9.382	4.71
Phenanthrene	8.726±0.903	28.421±2.100	0.307±0.43	4.78
Anthracene	10.086±0.030	28.256±0.221	0.357±0.135	5.2
Fluoranthene	9.766±0.516	27.481±0.401	0.355±1.286	5.8
Pyrene	9.753±0.575	28.201±1.091	0.346±0.527	5.04
Benzo (a) anthracene	9.913±0.375	26.264±0.220	0.377±1.705	5.5
Triphenylene	9.826±0.422	29.077±0.329	0.337±1.283	4.91
Benzo (e) pyrene	9.960±0.338	27.594±0.211	0.361±1.602	5.27
Benzo (a) pyrene	9.473±0.677	13.873±0.111	0.683±6.099	9.97
Indeno (1, 2, 3, cd) pyrene	9.490±0.364	13.774±0.213	0.689±1.709	10.05
Benzo (g,h,i) perylene	9.800±0.616	28.383±0.210	0.345±2.933	5.03
Dibenzo (a,h) anthracene	10.093±0.565	28.302±0.101	0.357±5.594	5.21
000053-70-3-benzo (e) pyrene	9.676±0.480	27.828±0.171	0.348±2.807	5.08
Total BCF of PAHs tissue load			6.853±7.937	100%

One study have reported variable BCFs in the range of 7×10^3 to 3×10^4 in an urban area and 1×10^1 to 5×10^1 in an oil refinery area for mussels *(Mytilus edulis planulatus)* from southeast Australia Murray *et al.* (1991). Interestingly, the BCFs for benzo[b] fluoranthene, benzo [k] fluoranthene and benzo[a]pyrene were 10-44 times higher in models collected from the oil refinery sites versus those in animals collected from the urban sites. Bioconcentration factors for fish are very difficult to calculate because extensive biotransformation results in the rapid disappearance of parent PAHs from tissues. Measurement of tissue burdens of parent compounds normalized to water concentrations will severely underestimate the BCF. As described earlier, alternate measurements of PAH accumulation may be more useful in assessing exposure. In the same vain, results in Table 3 and 4 showed the similar trend. As mean bio-concentration of toxicants varies in fish tissues.

Table 5. Mean concentration of PAHs bio-accumulated in Cat Fish (*Clarias gariepinus*)

Polycyclic aromatic hydrocarbons (PAHs)	Mean conc. of Contaminant in fish (ppm)	Mean conc. of Contaminant in water (ppm)	Mean Bio-Concentration Factor (BCF)	Percentage PAHs conc. (%)
Naphthalene	10.517±0.453	23.302±0.114	0.451±3.974	6.18
2-Methylnaphthalene	10.476±0.551	28.225±0.231	0.371±2.385	5.08
Acenaphthylene	10.170±0.500	16.564±0.220	0.613±2.272	8.4
Acenaphthene	10.097±0.023	27.585±1.210	0.366±0.019	5.01
Fluorene	10.146±0.115	27.239±0.123	0.372±0.934	5.09
Phenanthrene	10.400±0.294	28.421±2.100	0.366±0.140	5.04
Anthracene	10.373±0.458	28.256±0.221	0.367±2.072	5.03
Fluoranthene	11.646±0.803	27.481±0.401	0.423±2.002	5.79
Pyrene	10.360±0.312	28.201±1.091	0.367±0.286	5.03
Benzo (a) anthracene	10.613±0.512	26.264±0.220	0.404±2.327	5.54
Triphenylene	10.206±0.215	29.077±0.329	0.351±0.653	4.81
Benzo (e) pyrene	10.323±0.195	27.594±0.211	0.374±0.924	5.12
Benzo (a) pyrene	9.436±1.411	13.873±0.111	0.680±12.711	9.32
Indeno (1, 2, 3, cd) pyrene	9.656±1.064	13.774±0.213	0.701±4.995	9.61
Benzo (g,h,i) perylene	10.133±1.021	28.383±0.210	0.357±4.862	4.89
Dibenzo (a,h) anthracene	10.336±0.275	28.302±0.101	0.365±2.722	5
000053-70-3-benzo (e) pyrene	10.193±0.020	27.828±0.171	0.366±0.117	5.01
Total BCF of PAHs tissue load			7.298±4.529	100%

Table 6. Mean concentration of PAHs bio-accumulated in African Red snipper (*Lutjanus agennes*) fish

Polycyclic aromatic hydrocarbons (PAHs)	Mean conc. of Contaminant in fish (ppm)	Mean conc. of Contaminant in water (ppm)	Mean Bio-Concentration Factor (BCF)	Percentage PAHs conc. (%)
Naphthalene	10.517±0.453	23.302±0.114	0.451±3.974	6.18
2-Methylnaphthalene	10.476±0.551	28.225±0.231	0.371±2.385	5.08
Acenaphthylene	10.170±0.500	16.564±0.220	0.613±2.272	8.4
Acenaphthene	10.097±0.023	27.585±1.210	0.366±0.019	5.01
Fluorene	10.146±0.115	27.239±0.123	0.372±0.934	5.09
Phenanthrene	10.400±0.294	28.421±2.100	0.366±0.140	5.04
Anthracene	10.373±0.458	28.256±0.221	0.367±2.072	5.03
Fluoranthene	11.646±0.803	27.481±0.401	0.423±2.002	5.79
Pyrene	10.360±0.312	28.201±1.091	0.367±0.286	5.03
Benzo (a) anthracene	10.613±0.512	26.264±0.220	0.404±2.327	5.54
Triphenylene	10.206±0.215	29.077±0.329	0.351±0.653	4.81
Benzo (e) pyrene	10.323±0.195	27.594±0.211	0.374±0.924	5.12
Benzo (a) pyrene	9.436±1.411	13.873±0.111	0.680±12.711	9.32
Indeno (1, 2, 3, cd) pyrene	9.656±1.064	13.774±0.213	0.701±4.995	9.61
Benzo (g,h,i) perylene	10.133±1.021	28.383±0.210	0.357±4.862	4.89
Dibenzo (a,h) anthracene	10.336±0.275	28.302±0.101	0.365±2.722	5
000053-70-3-benzo (e) pyrene	10.193±0.020	27.828±0.171	0.366±0.117	5.01
Total BCF of PAHs tissue load			7.298±4.529	100%

The mean bio-concentration of Toxicants body residues for Tilpia (*Oreochromis niloticus*) was calculated, results revealed naphthalene 0.5825±36.263, 2-methylnaphthalene 0.4655±22.342, acenaphthylene 0.8311±3.9215, acenaphthene 0.4976±3.9215, Fluorene 0.6865±4.6504, phenanthrene 0.5837±2.4229, Anthracene 0.5881±22.434, fluoranthene 0.5083±11.481, pyrene 0.4761±4.7580, Benzo (a) anthracene 0.5056±23.072, triphenylene 0.4749±15.428, benzo (e) pyrene 0.5876±24.597, Benzo (a) pyrene 1.1586±45.667, Indeno (1, 2, 3, cd) pyrene 0.9072±23.967, benzo (g, h, i) perylene 0.4747±22.892, dibenzo (a,h) anthracene 0.4717±49.594 and 000053-70-3-benzo(e) pyrene 0.4766±30.673 respectively Table 2 and 3.

Correlations between PAHs in the environmental matrixes and in tissues load of contaminants, these can be useful in assessment of exposure to pollutants (Dhananjayan and Muralidharan, 2012). One study found strong gradient of PAH concentration in sediment and mussel (*Mytilus edulis and Modiolus modiolus*) tissue up to several kilometers away from a ferro-alloy smelter Boese *et al*. (1990).

Both oysters (*Crassostrea virginica*) and clams (*Rangia cuneata)* sampled in the fall contained about 2-3 times more aromatic hydrocarbons than those sampled at the same sites during the spring season (Bender *et al*., 1986), leading to the hypothesis that differences resulted

from the spawning cycle. Bio-Concentration and Bioaccumulation Factors (BCFs and BAFs) are useful ratios that can indicate steady-state exposure and expected tissue burdens are based on environmental concentrations. Table 4 present the result of toxicants body residues of yellow tail fish (*Seriola lalandi*). So far, the elucidation of BCF showed that Naphthalene 0.8022±5.114, 2-Methylnaphthalene 0.5714±22.64, Acenaphthylene 0.9726±23.97, Acenaphthene 0.5999±4.603, Fluorene 0.7068±3.349, Phenanthrene, 0.6820±0.189, Anthracene 0.6566±6.131, Fluoranthene 0.6874±1.983, Pyrene 0.5700±4.784, Benzo (a) anthracene 0.6117±23.65, Triphenylene 0.6556±3.191, Benzo (e) pyrene 0.6922±0.369, Benzo (a) pyrene 1.4344±0.909, Indeno (1, 2, 3,cd) pyrene 1.3883±2.929, Benzo (g,h,i) perylene 0.6902±9.277, Dibenzo (a,h) anthracene 0.7138±9.277 and 000053-70-3-benzo(e) pyrene 0.6754±3.339.

Some researchers have noticed a pattern of differential accumulation which differ over major PAH groups (for example, compounds containing 2 through 6 aromatic rings). Varanasi *et al*. (1985; Isaac and Labunmi, 2010), reported that 2, 3 and 5-ring PAHs were poorly taken up by amphipods (*Eohaustorius washingtonianus and Rhepoxynius abronius*) and a clam (*Macoma nasuta*) when compared to 4-ring compounds Rose *et al*. (2012). This make us extrapolates the total mean PAHs body load and percentage body load. The contaminant body load was extrapolated by summation of individual PAHs mean concentration. Following this, results shows African Red snipper (*Lutjanus agennes*) have 20.822±0.6132 body load of contaminants as the highest, followed in decreasing order by Yellow tail (*Seriola lalandi*) 13.111±1.247, Atlantic Crocker (*Micropogonias undulates*) 9.8439±6.569, Tilpia (*Oreochromis niloticus*) 9.7790±12.305, Cat Fish (*Clarias gariepinus*) 7.298±4.529 and Barracuda (*Sphyraena barracuda*) 6.853±7.937 respectively. The percentage PAHs concentration in samples was also determined, for instance, African red snapper have 10.9% Indeno (1,2,3,cd) pyrene, Yellow tail 10.94% Benzo (a) pyrene, Barracuda 10.05% Indeno (1,2,3,cd) pyrene, Atlantic Croker 13.03% Indeno (1,2,3,cd) pyrene, Catfish 9.61% Indeno (1, 2, 3, cd) pyrene and Tilapia (*Oreochromis niloticus*) 11.84% Indeno (1, 2, 3, cd) pyrene Table 5 and 6 respectively.

The amount of PAHs in a sample is useful as an indicator of residual petroleum contamination at the site and toxicants body load in sample pattern may have resulted from the volatility of the 2 and 3-ring compounds, which may be released directly without metabolism from the organism, slower uptake kinetics of the more hydrophobic PAHs and the reduced uptake of the 5 and 6-ring compounds, which are suspected of being more tightly bound to organic carbon and hence less available to organisms as exemplify in various individual toxicant. These may affect people, animals and plants over time if exposure persist.

Conclusion

The observed levels of PAHs in fish species indicates that Qua Iboe River in Eket community of Akwa Ibom state is contaminated with PAHs. Therefore, Eket community is at risk of contracting cancer and other associated hormonal diseases as a result of their persistence exposure to/and consumption of the fishes. It is believe that other fish species do bio-accumulate the toxicants and possibly, beyond permissible limit. However, periodic monitoring of the aquatic environment will give insight into the levels of PAHs in the water body. It is suggested that bio-concentration of PAHs know no boundary and that studies from each major aquatic environment are relevant. Future work on the subjects of uptake efficiency, the role of qualitative and quantitative differences in organic carbon in determining bioavailability, assumed environmental equilibrium, nutritional migration of compounds and metabolites, predictable bio-accumulation factors and the variability in toxico-kinetic parameters are functions of chemical hydrophobicity associated with environmental changes and physiological posture will enhance understanding of PAH bio-accumulation and elimination in marine organisms. These will ultimately give empirical evidence for impact and risk assessment of both aquatic population and public health and safety.

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Author's Contributions

All authors contributed equally in this investigation.

Ethics

This investigation was carried out in compliance with the Nigerian Environmental protection right.

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