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Sustained Environmental Impact of Crude Oil Spillage in Sediment, Water and Tilapia Fish (*Tilapia Guineensis*) Specie from the Escravos River of Okpogbene and Ovuloye Communities, in the Niger Delta Region of Nigeria.

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Abstract
This study discusses the sustained environmental impact of crude oil spillage in sediment, water and Tilapia fish (*Tilapia guineesis*) specie from the Escravos river of Okpogbene and Ovuloye community. This was done to assess the level of pollution of the river by hydrocarbon. Sediment, water and fish samples were collected randomly from the study area and the control site (Ikot osukpong creek, Akwa-Ibom state). Agilent 6890N Gas Chromatography– Flame Ionization Detector (GC-FID) was used to determine the Polycyclic Aromatic Hydrocarbon (PAH) and Total Petroleum Hydrocarbon (TPH). The total concentration of PAH for sediment, water and Tilapia fish obtained for (station 1, 2 and control) were; 128.931, 226.296 and 9.814mg/kg; 75.593, 95.603 and 7.869mg/L; 75.915, 96.509 and 4.083mg/kg respectively. For TPH; 2693.456, 2706.123 and 76.274mg/kg; 825.452, 987.324 and 42.923mg/L; 310.235, 244.707 and 11.179mg/kg respectively. The concentration of PAH and TPH in Okpogbene and Ovuloye were significantly higher ($p<0.05$) than Ikot osukpong (control). PAH exceeded the WHO and EC recommended permissible limit of 10mg/l and12µg/kg for water and fish, except the controls and the United States Environmental Protection Agency (USEPA) value of 2.5mg/kg for sediment respectively. The results of TPH exceeded the Department of Petroleum Resources (DPR) permissible limit for water, sediment and fish, except the control fish sample. Diagnostic ratios applied implicated more petrogenic sources of PAHs, this indicates significant carcinogenic health risk associated with the consumption of Tilapia fish from the river. The results revealed pollution threat in the Escravos river and the need for immediate remediation.

Keywords: *Crude oil, Escravos, PAH, TPH, carcinogenic.*

1.0 Introduction

The third largest mangrove forest in the world, with divers range of plant and animal species is located in Nigeria's Niger Delta (Anejionu et al., 2015; Kuenzer et al., 2014). It is the site of oil exploration and exploitation since 1950s and has experienced over 13 million tonnes of crude oil leaks, causing significant contamination, degradation, sustained damage of the coastal environment (Sam and Zabbey, 2018), negatively impacting the country's economy and the near-shore community, decreasing government revenue and GDP. This spill's origin can be either anthropogenic or natural (Brakstad et al., 2017; Mahjoubi et al., 2018) and includes; oil pipeline vandalism, pipeline aging, oil blowouts from flow stations, accidental leaks, intentional release, cleaning of oil tankers on the high sea, roadside mechanics disposing of used oil in drains, improper handling from production sites, local transporting methods, waste from non-standard refining sites released untreated into nearby rivers and creeks (Asimiea and Omokhua, 2013), as noted by some Local (Olowu et al., 2010; Oladele and Jenyo-Oni, 2015) and international researchers (Ikem et al., 2003; Alam et al., 2012).

It is recorded that in the Niger Delta region, land, swamp, and offshore environments accounted for roughly 6%, 25%, and 69% of the total oil spilled, respectively (Obike et al., 2020), risks of oil spills are pollution from hydrocarbons (Kuenzer et al., 2014). Alam et al. (2012) and Akan et al. (2012) had also reported that untreated effluents contain a wide range of pollutants from metals to hydrocarbon to organic pollutants, which, when released into nearby water bodies, cause drastic changes in the water's chemical quality and eventually contaminate the sediment where fauna feed.

2.0 Review of Related Literature

Polycyclic aromatic hydrocarbon (PAHs) is a broad category of over 100 different chemicals, that are toxic pollutants, environmentally ubiquitous and are produced by both natural and man-made processes. Consuming these substances may cause negative health effects on aquatic organisms and humans (Fagbote and Olanipekun, 2010), various components of PAHs are suspected human carcinogens, mutagens, and endocrine disrupters (American Conference of Governmental Industrial Hygienists (ACGIH)., 2005). PAHs fractions of dissolved petroleum are bio-concentrated in and easily absorbed by the majority of finfish and shellfish due to their high lipid

solubility, dietary consumption, however has been demonstrated to be a significant pathway for human exposure (Nworu et al., 2021).

Petrogenic chemicals like PAH and TPH have been found to contaminate aquatic ecosystems i.e; sediment, water, and aquatic flora and fauna globally (Smith and Guentzel, 2010), even the water bodies of Nigeria (Enuneku and Ilegomah, 2015). High concentrations of PAHs has been reported by (Anyakora et al., 2004; Akinsanya et al., 2018).

Total petroleum hydrocarbons (TPH) is a term used to refer to a broad family of several hundred chemical compounds that originated from crude oil, typically made from hydrogen and carbon (Saranya et al, 2020). Hexane, jet fuels, diesel fuel, Stoddard solvent, mineral oils, benzene, toluene, xylenes, naphthalene, and fluorene, etc are variety of chemicals that may be present in TPH. However, TPH samples may contain only some, or a mixture of these chemicals. The widespread use of crude oil and other petroleum products for transportation, heating and industry result to the release of these petroleum products into the environment.

Evaluating the TPH level or concentration in a sample can serve as a common indicator of petroleum contamination at that location. TPH can enter the body through the air we breathe, food, drink, soil or by touching it, it leaves the body through urine (Nwaichi et al. 2020). The TPH compounds can impact blood, immune systems, lungs, liver, kidney, skin, eyes, cause a nerve disorder called "peripheral neuropathy," which includes numbness of the legs and feet, headache and dizziness and affects animal's developing fetus and reproduction. This is similar to the impact it has on the Ogoni people of the Niger Delta according to Yakubu (2017). Lindén and Pålsson (2013) reported high concentration of TPH in sediment from Ogoni land in Niger Delta. Yaguo et al., (2021) and Nworu et al., (2021) also, had extremely high TPH concentrations.

Crude oil exploration and exploitation pose the biggest threat to the Niger Delta and it's residents because of hydrocarbon pollution which have degraded and sustainably damaged terrestrial and marine resources, including making agricultural lands unproductive; negatively affecting water resources (marine life), biodiversity, swamps, groundwater, food security, vegetation, animals and causing human health issues due to the interaction with polluted water, biotas, soil and air in the area. These pollutants have caused severe suffering, diseases, loss of lives, loss of livelihood, hardship, rise in poverty, unemployment, underemployment, significant harm to the Niger Delta

environment, their way of life, hindered economic development and deterred foreign investment. Therefore, the aim of this study is to analyze the sustained environmental impact of PAH and TPH accumulation in sediment, water and Tilapia fish specie from the Escravos river of Okpogbene and Ovuloye community of the Niger Delta region of Nigeria. By carryout this study, inhabitants of Okpogbene/Ovuloye in Delta state, Nigeria and the world at large will be properly educated and sensitized on contact with the abiotic environment, consumption of aquatic organisms (fish) in the Escravos water body that is polluted with hydrocarbons as a result of oil spill.

3.0 Materials and Method

Study Area: This study was conducted between October and November 2023. The Escravos river in Delta State and Ikot Osukpong creek in Akwa-Ibom state (control) Nigeria served as the sample sites (Fig. 1), with a distinct dry and wet season, The Escravos is located at 5° 34' 57" N and 5° 10' 50" E. It is a distributary of the Niger River in the western Niger Delta in southern Nigeria. The river flows 57 kilometres (35 miles) in a westerly direction, passing through mangrove swamps, coastal ridges, tropical rainforest and freshwater swamps before joining the Forcados, Warri, Benin, and Ethiope rivers via a network of interconnected waterways. It ends at the Bight of Benin in the Gulf of Guinea where it empties into the Atlantic Ocean. Recreational activities, seafood, fishing and fishery products are significantly known to be provided by the estuary in the island communities and major markets in southern Nigeria. Transportation, shipping lane for major seaport and the exploration and exploitation of crude oil are the main industrial activities in the area. On the Escravos River, two sampling stations were established in the Delta State communities of Okpogbene and Ovuloye. In the Ika local government area of Akwa-Ibom State, Ikot Osukpong serves as the study's control site (Fig. 1) due to the fact that there are no oil exploitation, exploration or other marine commercial activities there.

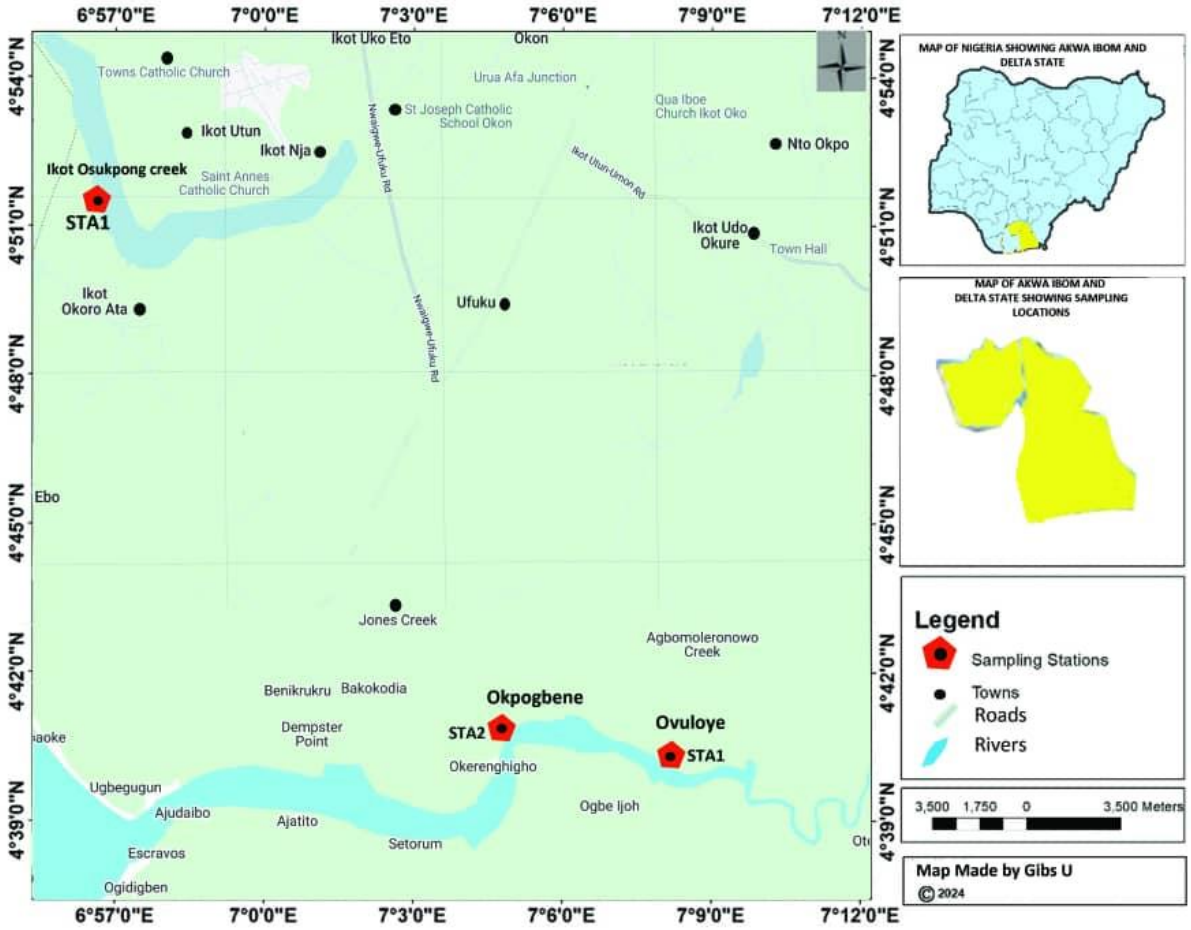


Fig. 1: A map of Delta and Akwa-Ibom State showing location of where Okpogbene, Ovuloye and Ikot Osukpong community is situated.

3.1 Sample Collection

Fresh fish samples of *Tilapia guineesis* (Tilapia), Sediment and water were collected in triplicate at each sampling station from the Escravos river. Three freshly caught fish samples each measuring 11-12.5 cm in total length (TL) were identified and obtained from local fisher men, placed in a sterile plastic container and put in a ice-filled cooler. Clean plastic bottles were used to collect 1litre of water sample in triplicates from the two rivers at a depth of 0-30 cm from the surface. A van Veen grab was used to randomly collect sediment samples in triplicate from various stations along the Escravos each weighing 500 g and ranging in depth from 10-40 cm at Warri South West Local government area in Delta State. Control samples were taken from Akwa River, which is known to have no source of petrogenic or industrial pollution in Nsit Atai Local Government Area in Akwa-Ibom State, Nigeria respectively. all in October/November 2023. The samples were then placed in a clearly labelled polythene bag and all samples transported to the laboratory for analysis.

3.2 Sample Preparation

Fish: Fresh fish samples were allowed to cool to room temperature, thoroughly cleaned in distilled water to get rid of the adhering dirt. Each fish sample's length and weigh were then measured using a metre rule and a weighing balance (G & G Elektronische waage, capacity 500 g, Model: JJ500), grounded into powder with a grinder and put in a airtight container for extraction.

Sediment: Samples of sediment were weighed with a weighing balance and placed in a container for extraction.

Sample Extraction: Thereafter, 10 g and 10ml of the fish, Sediment and water respectively was weighed and added to an Amber glass bottle each, the sample was stirred. Thereafter, supplemented with 300 µg/ml of surrogate (1-chlorooctadecane) standard. 30 ml of dichloromethane (DCM) was added as extracting solvent to the sample and the bottle was tightly corked and placed in a mechanical shaker, agitated for 5 to 6 hours at room temperature, then allowed to settle for an 1 hour and filtered through 110 mm filter paper containing 5 g of anhydrous sodium sulphate (Na_2SO_4) to remove any remaining moisture from the sample. The filtrate was left to concentrate to 1ml by evaporation overnight in a fume cupboard.

Sample Clean Up: A glass column was used for this. Glass cotton was inserted into the column as part of the preparation process.10 g silica gel and 50 ml of DCM were dissolved to create a slurry and added into the column,10 g anhydrous Na_2SO_4 and then pentane was added afterwards. After preparation of the column the concentration sample extract was combined with 20ml cyclohexane in a beaker and then transferred into prepared column. The sample extract was eluted using 30 ml pentane as solvent and eluted samples were collected in a beaker beneath the column. Additional 20 ml of pentane were added to the column to further elute the sample and 20 ml of DCM were used to rinse the column after elusion. The eluted sample was left in a fume cupboard at room temperature overnight in order to allow for evaporation.

Sample Separation and detection: The separation and detection of compounds samples were carried out using Agilent 6890N Gas Chromatography–Flame Ionization Detector (GC-FID) device. 3 µl of the concentrated sample eluded from column was injected GC vial. The blank DCM was injected into micro-syringe of GC to clean the syringe (3times) before analysis. After further rinsing of the micro-syringe with the sample also, the sample was injected into the column for

separation of compounds in the sample. The compounds were run through a flame Ionization Detector following separation and it is detected by FID. The concentration of PAH and TPH was resolved at a particular Chromatogram in mg/l.

3.3 Statistical Analysis

Data were analysed using IBM SPSS software (version 23). One-way ANOVA was used to determine the statistically significant difference. Tukey Post Hoc test was used to ascertain where the difference lies. Biota-sediment accumulation factor (BSAF) which is the concentration of contaminants accumulated from the sediment into the entire body of the fish was calculated as;

$$BSAF = \frac{C_f}{C_s}$$

Where:

C_f: Represents the concentration of contaminant in the fish

C_s: Is the concentration in the sediment sample.

The bioaccumulation factor of contaminants from water to the body (BAF f/w) of fish was calculated thus;

$$BAF = \frac{C_f}{C_w}$$

Where:

C_f: Represents the concentration of contaminants in the fish

C_w: Represents the concentration of contaminants in water.

4.0 Result and Discussion

4.1 Polycyclic aromatic hydrocarbon (PAHs) in sediment sample studied:

The analysis of sediment samples from the Delta and Akwa- Ibom State (control) rivers revealed a total of 16 PAHs (Table 1). For Delta state (Station 1), 9 PAHs; Acenaphthene, Anthracene, Fluoranthene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo(g,h,i)perylene, Benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, Dibenz(a,h) anthracene were not detected in the sediment sample, while for (station 2), 11 PAHs; Naphthalene, Acenaphthene, Anthracene, Fluoranthene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo(g,h,i)perylene,

Benzo(a)pyreneg, Indeno(1,2,3-cd)pyrene, Dibenz(a,h) anthracene were not detected.. For the control; Naphthalene, Acenaphthene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo(g,h,i)perylene, Benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, Dibenz(a,h) anthracene were not detected. Delta state and Akwa Ibom (control), had average concentrations of individual PAHs ranging from 11.568 ± 0.042mg/kg to 73.547 ± 0.024mg/kg and 1.551 ± 0.024mg/kg to 5.135 ± 0.028mg/kg respectively. The total PAH concentration for Delta (St 1 and 2) and Akwa Ibom (control) rivers is 128.931 and 226.296 and 9.814 mg/kg respectively. The total amount of carcinogenic PAHs discovered comprises of Pyrene, Chrysene and Benzo(k)Fluoranthene with mean values for Delta (St 1 and 2) and Akwa-ibom (control) as 48.512 and 130.797 and 1.551mg/kg respectively. US. DEQ (2000), categorized Benzo[k]fluoranthene as a group B2 probable human carcinogen upon lack of human and sufficient data from animal assays. Certain PAH components that pose the highest risk mostly during the period of high exposure such as; pyrene, chrysene and benzo[k]fluoranthene ATSDR., (2007), were found in the sediment samples used for this study. Nwaichi et al., (2020) also linked them to carcinogenesis. The values for the total mean concentrations of PAHs in the sediment samples at the two locations varied significantly. Numerous human activities and natural occurrences, from domestic to industrial sources, have contributed to the presence of PAHs in the environment, including the burning of fossil fuels, emissions from vehicles, incinerators, flaring gases, deposition of wildfires, pipelines leaks, gasoline leaks from engine boats, etc. (Davis et al., 2019; D'Agostino et al., 2020), seasonal variations, sediment transport processes, physicochemical properties, etc. may also have an impact on the concentration of PAHs in the environment (Ambade et al., 2023; D'Agostino et al., 2020) and primarily in Sediment, it can be attributable to the hydrophobic nature of the contaminants combined with the repository nature of the bottom sediment. In support of this study, Pang et al., (2021) conducted an assessment of ∑16PAHs in sediment of Wei River and found that the PAH levels varied between 60.50—10,241.10 ng/g, various high values were reported by (Riazet al., 2019; Asagbra et al., 2015; Kim et al., 2018). However, Lower values were also reported by (Dos-Santos et al., 2018 and Akinsanya et al., 2020a). The sediment samples in this study exceeded the USEPA guideline value of 2.5 mg/kg for Delta State and the control (non- carcinogenic), but slightly higher than the control carcinogenic PAH.

Table 1: Concentration of PAHs in Sediment

Polycyclic Aromatic Hydrocarbons (mg/kg)	Control	Delta State 1	Delta State 2
Non-Carcinogenic			
Naphthalene	-	12.250 ± 0.023	-
Acenaphthylene	3.128 ± 0.015	28.620 ± 0.012	32.506 ± 0.014
Acenaphthene	-	-	-
Fluorene	5.135 ± 0.028	23.409 ± 0.043	48.443 ± 0.024
Phenanthrene	-	16.140 ± 0.026	14.550 ± 0.023
Anthracene	-	-	-
Fluoranthene	-	-	-
Carcinogenic			
Pyrene	-	22.631 ± 0.047	57.250 ± 0.027
Benzo[a]anthracene	-	-	-
Chrysene	1.551 ± 0.024^{a}	14.313 ± 0.020^{b}	73.547 ± 0.024^{e}
Benzo[b]fluoranthene	-	-	-
Benzo[k]fluoranthene	-	11.568 ± 0.042	-
Benzo(g,h,i)perylene	-	-	-
Benzo(a)pyrene	-	-	-
Indeno(1,2,3-cd)pyrene	-	-	-
Dibenz(a,h) anthracene	-	-	-
Total PAHs	9.814^{a}	128.931^{b}	226.296^{c}
∑Carcinogenic	1.551 ± 0.024	48.512 ± 0.109	130.797 ± 0.051
LMW/HMW PAHs Ratio:	5.328	1.658	0.730

Values are presented as Mean ± SEM of triplicate determination. Mean values with different superscript alphabet in the same row are significantly different from each other at $p \leq 0.05$

4.2 Polycyclic aromatic hydrocarbon (PAHs) in water sample studied:

The analysis of water samples from the Delta and Akwa-Ibom State (control) rivers revealed a total of 16 PAHs (Table 2). For Delta state (Station 1), 9 PAHs; Acenaphthene, Phenanthrene, Pyrene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo(g,h,i)perylene, Benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, Dibenz(a,h) anthracene were not detected in the water sample, while for (station 2), 10 PAHs; Naphthalene, Fluorene, Fluoranthene, Pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo(g,h,i)perylene, Indeno(1,2,3-cd)pyrene, Dibenz(a,h)anthracene were not detected. For the control; 12 PAHs; Naphthalene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo(g,h,i) perylene, Benzo(a)pyrene, Indeno(1,2,3-cd) pyrene, Dibenz(a,h) anthracene were not detected. Delta state and Akwa-Ibom state (control), had average concentrations of individual PAHs ranging from 6.416 ± 0.015mg/L to 29.459 ± 0.017mg/L and 1.122 ± 0.011mg/L to 2.951 ± 0.018mg/L respectively. The total PAHs concentration for Delta (St 1 and 2) and Akwa-Ibom (control) rivers is 75.593 and 95.603 and 7.869 mg/L respectively. The

total amount of four carcinogenic PAHs discovered comprises of Pyrene, Benzo(a)anthracene, Chrysene and Benzo(a)Pyrene, with mean values for Delta State (St 1 and 2) and Akwa-ibom state river as 19.098 and 25.003 and 4.07 mg/L respectively, which were higher than the safe limits of 0.020 mg/L established by EPA National Recommended Water Quality Criteria for the safety of aquatic Life and human health and were above the WHO maximum permissible limit of 10 mg/l in water except for the control (WHO, 2003).

The values for the total mean concentrations of PAHs at the two stations varied significantly. These findings implied that, the high concentration of PAHs obtained at these sites may have resulted from a variety of industrial activities, including oil exploration and exploitation, spills, vandalization of oil pipelines, pipelines aging, oil blowouts from flow stations, unintentional leaks, intentional release, cleaning of oil tankers on the high sea, local transportation methods etc. The level of PAHs found in the water sample from Akwa-ibom (control) may have resulted from negligence during engine oil removal from the boat or seepage of diesel during transportation by engine boats. El-Naggar et al., (2021) Reported that water with a total PAHs level of more than 10 µg/L is considered to have a high level of PAHs pollutants, therefore, the control water may not be considered high PAHs Polluted water. Skin irritation and inflammation have been found to be triggered by a combination of several PAHs. Although anthracene, Benzo(a)pyrene and naphthalene can cause direct skin irritation, while anthracene and Benzo(a)pyrene have demonstrated to be skin sensitizers in both humans and animals, meaning they can cause sensitive skin reaction. This suggest that people living in and around the study area are at high risk of having cancer and other severe illness. Inhalation, ingestion and dermal contact are the primary routes of PAHs exposure to humans. In accordance with the high values in this study, Nwaichi et al. (2020), observed that the total PAHs concentration in the water samples from Abuloma and Okujagu-ama rivers were 244.03±40.84 and 6.41±1.4 mg/L, respectively. But Nasher et al. (2013), Reported a lower value (6.1-46 mg/L) for PAHs Concentration, just like (Akinsanya et al., 2020a and Ogbonna and Origbe 2021) when compared to this study.

Table 2: Concentration of PAHs in water

Polycyclic Aromatic Hydrocarbons (mg/L)	Control	Delta State 1	Delta State 2
Non-Carcinogenic			
Naphthalene	-	13.344 ± 0.018	-
Acenaphthylene	2.161 ± 0.011	8.286 ± 0.015	9.447 ± 0.012

Acenaphthene	1.645 ± 0.019	-	14.153 ± 0.008
Fluorene	-	6.416 ± 0.015	-
Phenanthrene	-	-	29.459 ± 0.017
Anthracene	-	16.784 ± 0.012	17.541 ± 0.020
Fluoranthene	-	11.665 ± 0.019	-
Carcinogenic			
Pyrene	2.951 ±0.018	-	-
Benzo[a]anthracene	-	8.576 ± 0.024	-
Chrysene	1.122 ± 0.011[a]	10.522 ± 0.015[b]	13.460 ± 0.034[c]
Benzo[b]fluoranthene	-	-	-
Benzo[k]fluoranthene	-	-	-
Benzo(g,h,i)perylene	-	-	-
Benzo(a)pyrene	-	-	11.543 ± 0.026
Indeno(1,2,3-cd)pyrene	-	-	-
Dibenz(a,h) anthracene	-	-	-
Total PAHs	7.869 ± 0.059[a]	75.593 ± 0.118[b]	95.603 ± 0.109[c]
∑Carcinogenic	4.073	19.098	25.003
LMW/HMW PAH Ratio:	0.932	2.958	2.824

Values are presented as Mean ± SEM of triplicate determination. Mean values with different superscript alphabet in the same row are significantly different from each other at $p \leq 0.05$

4.3 Polycyclic aromatic hydrocarbon (PAHs) in fish sample studied:

The analysis of fish samples from the Delta and Akwa- Ibom State (control) rivers revealed a total of 16 PAHs (Table 3). For Delta state (Station 1), 9 PAHs; Acenaphthene, Anthracene, Fluoranthene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo (g,h,i)perylene, Benzo(a)pyrene, Indeno (1,2,3-cd)pyrene, Dibenz (a,h)anthracene, were not detected in the fish sample, while for (station 2), 11 PAHs; Naphthalene, Acenaphthene, Anthracene, Fluoranthene, Benzo[a]anthracene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo (g,h,i)perylene, Benzo(a)pyrene, Indeno (1,2,3-cd)pyrene, Dibenz(a,h) anthracene, were not detected For the control; no carcinogenic PAHs were detected. Delta state and Akwa Ibom (control), had average concentrations of individual PAHs ranging from 6.587 ± 0.020 mg/kg to 27.151 ± 0.023 mg/kg and 1.590 ± 0.023 mg/kg to 2.493 ±0.015 mg/kg respectively. The total PAHs concentration for Delta (St 1 and 2) and Akwa Ibom (control) rivers is 75.915 and 96.509 and 4.083 mg/kg respectively. The total amount of carcinogenic PAHs discovered comprises of Pyrene, Chrysene and Benzo(k)Fluoranthene with mean values for Delta (St 1 and 2) as 29.074 and 40.877 mg/kg. A number of carcinogenic PAHs have also been identified in water and food samples, Zelinkova and Wenzl (2015). Fishes when contaminated with high concentration of carcinogenic PAHs, becomes toxic and unfit for human consumption. Nwaichi et al. (2020), also reported that the

presence of these substances in the environment has become a global concern, since the primary route through which humans are exposed to this contamination is through the consumption of contaminated food, water and marine products like fish. The mean values for carcinogenic PAHs are high when compared with WHO standard permissible limits of 0.001µg/g for fish (WHO, 2003).

No post-hoc comparison was made. These results implied that the high concentration of PAHs obtained may have resulted from a variety of industrial activities and vandalisation of oil pipelines at those locations. The level of PAHs in the fish sample from Akwa-ibom (control) may have resulted from negligence during the removal of engine oil from boat or seepage of diesel during transportation by engine boats. Asagbra et al. (2015) found that the mean concentration of PAHs in fish tissue from the Warri River at Ubeji, Niger Delta, Nigeria was 1098.50 ng/g which is significantly higher than the findings from this study. Lower concentrations than this study were also reported by Harry and Ahamefule., (2023) and Ekere et al., (2019). The levels of PAHs in the fish samples analysed in this study exceeds the 12.0 µg/kg recommended regulatory limit by European Commission (2015).

Table 3: Concentration of PAHs in fish

Polycyclic Aromatic Hydrocarbons (mg/kg)	**Control**	**Delta State 1**	**Delta State 2**
Non-Carcinogenic			
Naphthalene	-	9.420 ± 0.015	-
Acenaphthylene	2.493 ± 0.015	11.333 ± 0.020	22.241 ± 0.026
Acenaphthene	-	-	-
Fluorene	-	8.540 ± 0.015	18.653 ± 0.009
Phenanthrene	-	17.548 ± 0.019	14.738 ± 0.022
Anthracene	1.590 ± 0.023	-	-
Fluoranthene	-	-	-
Carcinogenic	-	-	-
Pyrene	-	6.587 ± 0.020	27.151 ± 0.023
Benzo[a]anthracene	-	-	-
Chrysene	-	15.328 ± 0.017	13.726 ± 0.031
Benzo[b]fluoranthene	-	-	-
Benzo[k]fluoranthene	-	7.159 ± 0.022	-
Benzo(g,h,i)perylene	-	-	-
Benzo(a)pyrene	-	-	-
Indeno(1,2,3-cd)pyrene	-	-	-
Dibenz(a,h) anthracene	-	-	-
Total PAHs	4.083	75.915	96.509

∑Carcinogenic	-	29.074 ± 0.059	40.877 ± 0.054
LMW/HMW PAHs Ratio:	4.083	1.611	1.361

Values are presented as Mean ± SEM of triplicate determination. Mean values with the same superscript alphabet in the same row are significantly different from each other at $p \leq 0.05$.

4.4 Total Petroleum Hydrocarbon (TPH) in Sediment samples studied:

Total TPH in Delta state (St 1 and 2) and Akwa Ibom state River (control) were detected (Table 4) to be 7, 6 and 3 respectively. The highest average concentration is 585.677 ± 0.022 (C9) and 489.698 ± 0.033 mg/kg (C8) for Delta state (St 1 and 2) and 38.184 ± 0.018 mg/kg (C15) for Akwa Ibom state (control) respectively. The total possible TPH concentration detected during the sediment testing were; C8, C9, C11, C12, C14, C15, C17, PRISTANE, C18 and C19 for Delta St 1 and 2 and control with total values of 2,693.456 and 2,706.123 and 76.274 mg/kg respectively. The sediment sample under study shows a statistically significant difference at ($p \leq 0.05$).

These TPH levels indicates that the sediments are heavily contaminated with petroleum products and exceeds the maximum permissible limit of petroleum hydrocarbons in sediments at (30 mg/kg) set by the Federal Ministry of the Environment (FMEnv, 1991) and the acceptable limit of 50 mg/kg set by the Directorate of Petroleum Resources and are far much below the intervention limit of 5,000 mg/kg set by the DPR (DPR, 2002). The high TPH values in Delta state may have resulted from; crude oil spill via pipeline vandalism, industrial activities, accumulation of oil residues from fishing boats, municipal sewage water and surface runoff from land (Al-Saad et al., 2017a).

There are numerous ways in which pollutants enter aquatic environment and settle in the sediment, such as direct deposition due to their weight or being carried by water currents over long distances due to their stability, causing them to settle when the currents speed decreases (Liu et al., 2019). These compounds also make their way into the food chain and into humans, where they negatively impact the blood, immune system, lungs, skin and eyes, thus becoming detrimental to health and eventually return to the sediments after death, (Nwaichi et al. 2020). Therefore, The levels of TPH in sediment were consistently higher than that of water, suggesting that it is a reliable indicator of the pollution status in the river systems (Galo et al, 2022). The reason for the increase levels of TPHs in sediment after many years is due to the establishment of shore refineries (Aziz and Sabbar, 2013). Similarly, the regional variation may be related to the transfer of TPHs loads from the inshore areas to the open-shore sediments and probably related to the significant role of benthic

organisms in the bio-transport of TPH into sediment due to oil spill residues (Chouksey et al., 2004). Grain size, increases the potential for preserving TPH in the bottom structure with silty and clayey sediments. As a result, sediment analysis is a reliable indicator of the distribution of petroleum hydrocarbons (Raja et al., 2022). In agreement with this present study, Lindén and Pålsson (2013) reported high TPH value in ogoni land in the Niger Delta, Nigeria, ranging from 279 to 17 900 mg.kg-1. However, Ike-Obasi, (2023); Edori and Edori., (2021) and Uguru et al., (2022) had lower TPHs values than this study.

Table 4: TPH Concentration of sediment

TPH (ppm)	CONTROL	Delta State 1	Delta State 2
C8			489.698 ± 0.033
C9		585.677 ± 0.022	372.389 ± 0.017
C10			
C11		241.762 ± 0.051	
C12	21.542 ± 0.042[a]	413.464 ± 0.023 [a]	256.960 ± 0.017 [a]
C13			
C14		335.761 ± 0.012	
C15	38.184 ± 0.018	424.272 ± 0.012	419.515 ± 0.050
C16			
C17			368.071 ± 0.030
PRISTANE		379.840 ± 0.027	
C18	16.548 ± 0.065	312.680 ± 0.020	
Ph			
C19			431.419 ± 0.050
C20 - C40			
TOTAL	76.274 ± 0.125[a]	2693.456 ± 0.167[a]	2706.123 ± 0.197[a]

Values are presented as Mean ± SEM of triplicate determination. Mean values with the same superscript alphabet in the same row are significantly different from each other at $p \leq 0.05$.

4.5 Total Petroleum Hydrocarbon (TPH) in water samples studied:

Numerous sources, such as the atmosphere, industrial sources, household waste, and untreated civil waste can release petroleum hydrocarbons into the aquatic environment. Total TPH in Delta state (St 1 and 2) and Akwa-ibom state River (control) were detected (Table 5) to be 5, 6 and 3 respectively. The highest average concentration is 231.516 ± 0.053 (C15) and 221.554 ± 0.035mg/L (C17) for Delta state (St 1 and 2) and 19.554 ± 0.060mg/L (C8) for Akwa-ibom state (control) respectively. The total possible TPH concentration detected during the water testing were

C8, C10, C12, C14, C15, C16, C17, C18, C19 and C20 for Delta St 1 and 2 and control with total values of 825.452 and 987.324 and 42.923 mg/L respectively.

TPH levels in Delta state were significantly higher than the control, but no Post Hoc comparison was made, and it exceeds the DPR permissible Limit of 10mg/l (DPR, 2002) and above the EPA 418.1 limit of 0.5 mg/L in water at all stations. These TPH levels indicates that the water is contaminated with petroleum products. The high TPH value at Delta state may have resulted from washing of petroleum products from transporting vessel tanks into the river, leaked petroleum products into the river during loading and unloading and also other industrial activities, resulting in reduced oxygen levels due to less gas diffusion through the oil-covered surface and thereby affecting aquatic organisms in the affected water body (Nwaichi, et al., 2020). The highest value of TPH concentration were observed to be between C9 to C20 range, according to this study, this suggest that the water contains a significant amount of diesel range organics and a minor amount of other crude oil components. When the TPH level in an aquatic environmental is high, organisms such as fish tend to ingest and absorb it and then pass it through food chain to other organisms and human, posing health risks when ingested by humans. Therefore, in order to prevent the impending catastrophe on the aquatic environment, the government must implement stringent environmental laws. Ibezue (2013), recorded that the mean TPH concentration in the oil-polluted marine waters of Biarra and Bodo in the Gokana area of the Niger Delta were 272.68 and 48.51 mg/L respectively, these values are lower than the average TPH concentration obtained in this study. Additionally, Adewuyi and Olowu (2012); Galo et al, (2022) and Edori & Edori (2021) also reported lower values of TPH.

Table 5: TPH Concentration of water

TPH (ppm)	CONTROL	Delta State 1	Delta State 2
C8	13.496 ± 0.011		
C9			
C10		175.596 ± 0.050	211.109 ± 0.044
C11			
C12		151.251 ± 0.067	119.520 ± 0.061
C13			
C14	19.554 ± 0.060		215.469 ± 0.094
C15		231.516 ± 0.053	
C16			221.554 ± 0.035

C17			95.953 ± 0.374
PRISTANE			
C18	9.873 ± 0.053		
Ph			
C19		127.496 ± 1.312	123.719 ± 0.043
C20		139.593 ± 0.361	
C21 - C40			
TOTAL	42.923 ± 0.124	825.452 ± 1.843	987.324 ± 0.651

Values are presented as Mean ± SEM of triplicate determination.

4.5 Total Petroleum Hydrocarbon (TPH) in Fish Samples

Total TPH in Delta state (St 1 and 2) and Akwa Ibom state River (control) were detected (Table 6) to be 6, 5 and 4 respectively. The highest average concentration is 73.427 ± 0.024 (C12) and 71.756 ± 0.039 mg/kg (C8) for Delta state (St 1 and 2) and 4.475 ± 0.077 mg/kg (C18) for Akwa Ibom state (control) respectively. The total possible TPH concentration detected during the fish testing were; C8, C9, C12, C14, C15, C17, PRISTANE, C18 and C19 for Delta St 1 and 2 and control with total values of 310.235 and 244.707 and 11.179 mg/kg respectively. There is a statistically significant difference in the fish samples studied at ($p \leq 0.05$).

This TPH levels indicates that the fish sample is contaminated with petroleum products. The high value of TPH in Delta state may have resulted from petroleum spill, accumulation of oil residues from fishing boats, municipal waste water, unusual oil leaks from industrial sites, urban and suburban surface runoff, and atmospheric deposition (Al-Saad et al., 2017a, Ogbonna and Origbe, 2021). Pollutants can enter the aquatic environment, where fish and other marine organisms live (Liu et al., 2019). Due to the continuous flow of water over their gills from eating and drinking, fish are constantly exposed to pollutants and have the tendency to accumulate several folds and higher concentration of pollutants than the surrounding water, due to the movement of these substances from multiple point sources which result in coincidental mixtures in the ecosystem (Ogbonna and Origbe, 2021), thus posing serious threat to aquatic organisms such as fishes, which are one of the main sources of protein-rich food for humans. These substances also enter humans and living organisms through the food chain where they can negatively impact the blood, immune system, lungs, skin and eyes, thus becoming detrimental to health (Nwaichi et al. 2020). Yaguo et al., (2021), recorded that the mean concentration of total petroleum hydrocarbon concentration in

tilapia muscle tissue ranged between 3170.81 ± 2076.31 and 5708.51 ± 8351.49 mg/kg, while the mean concentration of tilapia gills analyzed ranged between 6,140.38±7812.05 and 16,517±18209.90 mg/kg, which were significantly higher than the concentrations from the current study. Similar concentrations were observed by Nworu et al., (2021); Olaji et al., (2014); Ayoola et al., (2017). However, lower values were observed by Galo et al., (2022), except for the control which is 11.179mg/kg. The TPH values were above the DPR permissible Limit of 20 mg/kg for Delta State, but slightly higher than the control in this present study.

Table 6: TPH Concentration of fish

TPH (ppm)	CONTROL	Delta State 1	Delta State 2
C8			71.756 ± 0.039
C9		55.547 ± 0.075	58.488 ± 0.058
C10			
C11			
C12	2.385 ± 0.053[a]	73.427± 0.024[a]	46.857± 0.043[a]
C13			
C14		69.058 ± 0.027	
C15	3.148 ± 0.068[a]	24.265± 0.041[a]	29.525± 0.052[a]
C16			
C17			38.081 ± 0.043
PRISTANE	1.171 ± 0.044	49.840 ± 0.032	sss
C18	4.475 ± 0.077		
Ph			
C19		38.098 ± 0.023	
C20 - C40			
TOTAL	11.179 ± 0.242[a]	310.235± 0.222[a]	244.707± 0.235[a]

Values are presented as Mean ± SEM of triplicate determination. Mean values with the same superscript alphabet in the same row are significantly different from each other at $p \leq 0.05$

4.6 Bioaccumulation factor and Biota- Sediment accumulation factor for PAHs

The bioaccumulation and biota-sediment accumulation factor provides useful information for understanding the vertical migration of contaminants, it acts as an indicator for assessing the transport and potential risks associated with these contaminants from water and sediments to the biota in a food chain. A BAF and BSAF value >1 implies an increased accumulation of contaminants in biota, whereas a value < 1 is often considered desirable in the context of environmental protection. It suggests that a particular substance is not likely to biomagnify within

the food chain, posing fewer risks to higher trophic levels, including humans (Ekperusi, A. O., and Asiwa, D. 2024). Low BAF (<1) was reported for naphthalene (0.705 mg/kg) and phenanthrene (0.500 mg/kg) at station 2 and 3 respectively by the fish (Table S1.), the fish bioaccumulated significant amount of acenaphthylene at station 1, 2 and 3 with (1.154, 1.368 and 2.354 mg/kg), flourene at station 2 (1.331) and chrysene at station 2 and 3 (1.457 and 1.019 mg/kg) respectively. Significant BSAF of the fish shows that it accumulates phenanthrene at (1.087 and 1.013 mg/kg) at station 2 and 3 and chrysene (1.071 mg/kg) at station 2 respectively from the sediment. Results from the bioaccumulation studies suggest that (PAHs was more bioavailable in water than in Sediment. Although the Knowledge of PAHs interaction with Tilapia fish is limited.

The increased PAHs in tissues of fish resulted in increased BAFs for fish, confirming the possible uptake of PAHs from water to fish. This is contrary to the PAH uptake reported in fish in other studies (Kwok et al., 2013 and Almatari et al., 2017). PAHs are known to be metabolized by organisms through enzymatic processes. Tilapia fishes are omnivores that primarily feed on plant, algae and benthic invertebrates. Therefore, the lower BSAF in fish may have been caused by biochemical transformation and feeding habit. Uptake of PAHs by fish in nature is via a variety of pathways, including diffusion from water across the gills or through the gut from dietsor PAH-adsorbed sediments (Kelly et al., 2004). It is recognized that LMW 2-3 ring PAHs are more easily bioconcentrated from waterborne uptakes than the HMW PAHs (Cheikyula etal., 2008).

4.7 Bioaccumulation factor and Biota- Sediment accumulation factor for TPH.

Low BAF of TPH was observed in the fish for station 1, 2 and the control respectively, ranging from (C15) with 0.105- (C12) with 0.485 mg/kg. A low BSAF was also observed from station 1, 2 and control ranging from (C15) with 0.057 – (C18) with 0.270 mg/kg (Table S2). Results from the bioaccumulation studies suggest that TPH was more bioavailable in water than in sediment. The Knowledge of TPH interaction with Tilapia fish is limited. The decrease in TPH level observed in the tissues of the fish resulted in decrease BAF and BSAF of the fish, confirming the bioavailability of TPH more in water than sediment, though not likely to biomagnify within the food chain. This value is similar to the values obtained by (Victor et al., 2016, Ike-Obasi, 2023) and contrary to the values obtained by (Hu et al., 2021) this could be attributed to the feeding habit, metabolic rate, trophic transfer, many factors, such as the exposure time, the lipid content of tissues, environmental conditions, age, and sex, might affect the accumulation of PHs in fish

(Froehner et al., 2011). Tilapia fish can be an effective indicator for evaluating TPH pollution in aquatic systems.

5.0 Conclusion

The study showed that the accumulation of PAH and TPH in Tilapia fish spp, Sediment and water from the Escravos river is highly significant, indicating heavy pollution with organic matter containing petroleum products with little or no clean-up of the environment and with the presence of carcinogenic PAHs thereby making the fish toxic to health and unfit for human consumption because of Illegal petroleum crude oil refining activities.

Recommendations

1) It is therefore recommended that the consumption of tilapia fish from the Escravos river of Okpogbene and Ovuloye community and its environs should be stopped because of its impeding danger to human health.

2) Also, genuine efforts should be made to clean up polluted site by the oil companies that caused the spill, stop oil bunkery and illegal oil refining.

2) Pipeline surveillance should be a priority in the areas concerned and the water ways in general.

3) There should be sustained public and frequent enlightenment campaign on the effect of oil pollution on human and the environment.

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Data Availability

Supplementary data for this work can be found in the Supplementary material file.

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