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Concentration and health risk valuation of polycyclic aromatic hydrocarbons in *Tilapia zilli* in selected wetlands

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ABSTRACT: Globally, food safety is growing, and the residues of PAHs (polycyclic aromatic hydrocarbons) in fish species above the standard threshold could elicit serious public and community health concerns. This study investigated the PAHs contamination concentrations of *Tilapia zilli* in the wetlands of the Niger Delta. The study was conducted from March to August 2023, and fish samples were collected within these periods from 5 wetlands. The results obtained were as follows: pyrene; 2.46±0.11 μg/kg, chrysene; 2.54±0.12 μg/kg, BaP; 2.52±0.11 μg/kg, BaA; 2.54±0.13 μg/kg and BbF; 2.54±0.13 μg/kg. The mean results of characterising the PAHs in the fish were insignificant at $p>0.05$. The results of the health risk evaluation showed that Beneze (a) pyrene had the highest value (12.33 μg/kg), followed by Benzo (a) anthracene and Benzo (b) fluoranthene (1.51 and 1.20) of the toxicity equivalent factors. Meanwhile, the sum across the station was significantly higher than the standard limits. The CDI (chronic daily intake) results obtained in this study for each PAH (polycyclic aromatic hydrocarbon) congener were considerably high and fluctuated across the stations. However, the results for the ILCR (incremental life cancer risk) showed that the values were higher (1×10^{-2}) than the threshold set for this study (1×10^{-6} to 1×10^{-4}). The fish were thus contaminated above the acceptable limit; therefore, they are not fit for human consumption or for compounding animal feed.

1. INTRODUCTION

Fish is a major component of human and animal nutrition globally. It is highly cherished by people of varying races, economic classes, and diverse health statuses. Fish is a rich source of protein, with a protein content of between 15% and 35%, minerals, and vitamins (Adamu, 2019; Aguba, 2018). The carbohydrate, low-fat, and high-density lipoprotein (cholesterol) content is highly digestible (Ogagaoghene, 2022; Okonkwo, 2021).

Fish contain eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential fats that are polyunsaturated and not produced by the human body (Betrand, 2012; Odali, 2018). Eating fish once a week reduces the risks of cardiovascular diseases in humans and improves blood vessel elasticity, thus maintaining stable blood pressure (Jone, 2018; Ogwu et al., 2022; Tedwin, 2018). Fish consumption during pregnancy reduces the risk of premature delivery (Haruna, 2018; Johnson, 2018; Samuel, 2018). It is an essential component of animal feed formulation as a protein source, providing 60–70 per cent crude protein, 10–20 percent mineral, and 2–15 percent oil (Idialu, 2022; Ossai, 2020; Oyem, 2021).

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Fish guano helps improve soil nutrient status; the glue is used in gummed tape, while isinglass is used to clarify wine, vinegar, and beer (Eden, 2013; Johnpaul, 2019; Saliu, 2018). In 2012, it was noted that fish farming enterprises engaged the services of almost 500 million people globally (FAO, 2013; Programme, 2014).

World fish production stood at 184.6 million metric tons in 2022, while African fish production in the same year was 18.62 million (FAO, 2013). In Nigeria, fish production in 2022 was estimated at 1.073 million metric tons, 313 231 from cultured fish and 759,828 from captured (fisheries) (of Statistics, 2023; Ruwani, 2023).

Interestingly, food safety is growing; however, the residues of PAHs (polycyclic aromatic hydrocarbons) in fish species above the standard threshold have been noted to cause serious public and community health concerns (Tongo et al., 2017). Fish production from fisheries in Nigeria is carried out predominantly in the Niger Delta, which doubles as the oil and gas belt of Nigeria (Ozah, 2022). Sources of polycyclic aromatic hydrocarbon include wood, coal, gasoline, and crude oil (Atshana and Atshana, 2012; Gordon, 2019; Fidel, 2020) (Atshana & Atshana, 2012; Fidel, 2020).

The Niger Delta region of Nigeria has experienced about 822 oil spillages between 2020-2023 resulting in spewing 28,003 barrels of crude into the wetlands, marine ecosystem, and terrestrial environment alike (NESREA, 2022; NOSDRA, 2018; Society, 2022).

The presence of crude oil in the wetland ecosystems could result in the bioavailability of PAHs in the environment, resulting in bioaccumulation and biomagnification in the aquatic organisms (Anani & Olomukoro, 2019; Ogwu et al., 2022).

The circulation and stability of PAHs in the aquatic ecosystem can be influenced by the chemical nature of their aromatic rings and their physical and chemical structure (Olayinka et al., 2019). The range of PAHs in the muscles of species can provide a good history of their source emissions (Olayinka et al., 2019). Some studies using different health risk indices have shown that the concentration of PAHs in different environmental matrixes may have serious health consequences if humans ingest aquatic biota sourced from PAH-polluted water bodies (c et al., 2023; Liu et al., 2023; Olayinka et al., 2019).

Prolonged human exposure to PAHs can result in health complications such as cancer, gastrointestinal disorders, skin irritation, mutations, and intragenic damage (Otte et al., 2013; Oyo-Ita et al., 2016; Paoli et al., 2015). However, there has been a lack of information linked to health concerns in the consumption of PAHs found in benthopelagic and demersal fish species in selected wetlands of the Niger Deltas.

The PAHs investigated were pyrene, chrysene, benzo (a)pyrene (BaP), Benzo (a)anthracene (BaA), and benz(b) fluoranthene (BbF). The selected PAH congeners were chosen because of their high presence and bioavailability in the ecosystem of this region from previous studies.

The study was guided by research questions such as:

1. What are the concentrations of pyrene, chrysene, BaP, BaA, and BbF in benthopelagic and demersal fish species tissues in the Niger Delta wetlands?

2. Are the concentrations of PAHs in the fish species within the maximum permissible concentration (MPC) stipulated by EU 1255/2020 2μg/kg for fish?

3. Are the fish fit for human consumption and the formulation of animal feeds?

4. can the fish scale Code Alimentarius conditions for produce exports

1.1. Hypothesis guided the study as follows:

H_0: There is no significant difference between the PAHs in the fish species tissues and the MPC for PAHs for fish established by EU (European Union) 1255/2020.

Therefore, the focus of this study is to investigate the polycyclic aromatic hydrocarbons (PAHs) concentration in benthopelagic and demersal fish species- *Tilapia zilli* in the wetlands of the Niger Delta oil-producing hub of Nigeria. No work had been done on the health risks of PAH (polycyclic aromatic hydrocarbon) concentrations in *Tilapia Zilli* in this region. This stands as a research gap for this study. However, works on assessing PAH concentration in aquatic bodies are ubiquitous.

2. MATERIALS AND METHODS

2.1. Area of the Study

The Niger Delta comprises the nine states of Ondo, Edo, Rivers, Delta, Imo, Cross River, Akwa-Ibom, and Bayelsa, which are located in the Delta of the Niger River. Sometimes called the oil river because it's hitherto Nigeria's palm oil belt, the Niger Delta is the crude oil hub of Nigeria. Situated at latitude 5°.33223'E and longitude 6°.4692'N (Fig. 1), the Niger Delta sits at the Gulf of Guinea on the Atlantic Ocean. It covers 70,000 km^2 (27,000 sq miles), thus making up 7.5 percent of Nigeria's land mass. The Niger Delta plays host to a diversity of oil and gas companies and three petroleum refineries and petrochemicals in Port Harcourt in River State and Warri in Delta State.

2.2. Ethical considerations

The fish were obtained from the wild. They were not threatened or endangered, and no law prohibited cropping in the wetlands. Therefore, no approval or permit was obtained before the fish sample collection.

2.3. Sampling

The fish samples were collected from 5 villages with wetlands, randomly selected from 5 oil-producing communities in 5 selected oil-producing states. The states were Akwa-Ibom, Edo, Imo, Bayelsa, and Delta states, and the communities and villages where samples were collected were Akwa-Ibom state; the community randomly selected was the Eket oil-producing community; and the villages sampled were Rivers in Afala-

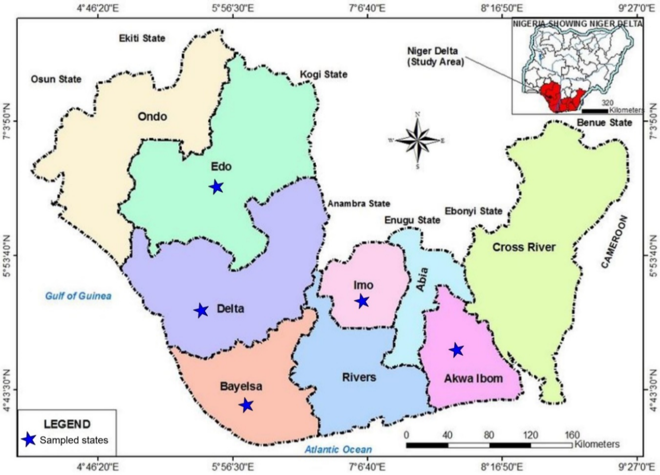

Figure 1. Map of Niger Delta showing sampled states.

Ukwa, Afaha-Atai, Ita-Idung, Atai Ndom, and Ebana. In Edo State, the communities sampled were rivers in Ikpoba/Okha, and the villages where samples were collected were rivers in Ajoku, Akpe Camp, Avbiama, Avbosa, and Ogheghe. In Imo state, the community was made up of rivers in the Oguta oil-producing community. Samples were collected from the Agwa, Nebukwu, Nkwebi, Ezi-Oru, and Egwe rivers. In contrast, in Bayelsa state, the oil community selected for sample collection was Ogbia, and the villages sampled were rivers in Abobir, Akalomani, Emaduke, Emaga, and Epebe. In Delta State, the oil-producing community selected for sample collection is Uzere, and the villages where samples were collected were rivers in Ezede, Ekregbesi, Abale, Iboro, and Uweye.

The study lasted six months, from March to August 2023, and fish sample collection was done with the assistance of local artisanal fishermen. The species sampled was *Tilapia zilli* because it is ubiquitous in the Niger Delta (Imoobe et al., 2024; Ogwu et al., 2022). The species used for the study were sampled in the wetlands in each village, bulked and composited, and stored in ice-cooled holding flasks, with which they were taken to a freezer for storage before analysis. The total bulked samples collected from each state oil-producing community were 25, and the total samples used for the study were 125.

2.4. Analysis

The samples of *Tilapia zilli* were removed from the freezer units, and the ice was allowed to thaw at room temperature. The scales of the species were removed with stainless steel scalpels and diced with stainless steel laboratory knives. They were further dehydrated by placing them in Agilent door oven model 4500 at about 105 °C for 12 hours. The dehydrated samples were blended and pulverised with high-performance laboratory Shimadzu model 2340 wrapped in aluminium foil and thoroughly labelled.

The analytical standard adopted was European Union Regulation 1255/2020, as described in Oliva et al. (2012), Nguyen et al. (2014), and Orisakwe et al. (2015). 6g of pulverised fish samples were weighed into a flask, and 45 ml of dichloromethane was introduced. A recovery study of 20μl of 20-part per million (ppm) surrogate standard sonicated for 15 minutes using an ultra-sonicator was made. The supernatant was decanted into the new sample bottle.

The processes were repeated three times for each of the bulked samples representative. Anhydrous sodium sulfate was added to the extracts to expel the remaining moisture, and this was kept at room temperature to allow the dichloroethane to expel. These effluents containing the PAHs were used to determine PAHs using Agilent high-performance liquid chromatography fitted with fluorescence detector (HPLC FLD) model 8900 containing gel permeation with purification set at 200 m×25 mm. The mobile phase was ethylene acetate cyclohexane 1:1v/v set at a column flow rate of 6ml/minute.

2.5. Quality assurance

The absorbent used for the cleanup was pre-extracted using methanol and DCM (Dichloro Methane) for over 48 hours before usage (Oyo-Ita et al., 2016; Ozaki et al., 2015). The solvents used were at the highest standard analytical grades and were further redistilled. Blank samples were analysed along with field samples. The recovery rate was 80% to 105% in the blanks and 80% to 120% of the research samples. The standard reporting limit was calculated using the lowest concentration of the calibration curve, which was divided by the actual sample weight.

2.6. Data estimation

To find out if there were any significant differences in the samples obtained, an analysis of variance (ANOVA) and the calculation of the mean and standard error (SE) were performed using the Statistical Package for Social Scientists (SPSS) 29 and Microsoft Excel 2019, Windows 10 pro program.

The applications Microsoft Excel 2019 and Windows 10 Pro were utilised to determine the potential health risks of the samples.

2.7. Exposure assessment

The PAH15 health risk evaluation was based on the PAH levels found in Nigerian benthopelagic and demersal fish species. The primary example was used to demonstrate the connection between the carcinogenic risk of PAH15 and the TEFs (toxicity equivalent factors) of PAHBaP. BaP-Benz(a)pyrene (1), BaA-Benz(a)anthracene and BaF-Benzo(b) fluoranthene (0.1), Chr-Chrysene (0.01), and Pyr-Pyrene (0.001) were shown to constitute the TEF in this investigation. Using this formula (1), the BaPeq (BaP-Benz(a)pyrene equivalent) was calculated:

$$TEQBap = \sum_{i=1}^{n} Ci \times TEFi \qquad \textbf{1}$$

where the TEF congener (i) in the benthopelagic and demersal fish species and the amount of PAH in the benthopelagic and demersal fish species sample that was obtained are represented by the values of TEFi and Ci. TEQBaP-Bapeq15, the sum of all PAHs, was utilised to assess the carcinogen danger.

Using BaP as the standard equivalent level and the following equation 2 (; Dadar et al., 2017) (EPA, 1992), the CDI (chronic daily intake) of PAHs was calculated:

$$CDI\ \ (ng \times BaPeq\ per\ bw.\ d) = \sum Ci \times lRi \times ED \times \frac{ET}{Bw*AT} \quad \mathbf{2}$$

the amount of PAH15 estimated for BaPeq in ng BaPeq/g for the benthopelagic and demersal fish species is represented by the value of Ci. There were 53 exposure days (ED). The daily intake (IRi) was 20 g/day. The exposure frequency (EF) is expressed in 365 days. The European Food Safety Agency (2008) reported that the values for AT (average time), BW (body weight), and IR (ingestion rate) were 70 years (carcinogenic for 25,550 days) and 65 kg, respectively.

The following formula (3) was used to calculate the carcinogenic risk:

$$ILCR = CDI \times SF \times CF \quad \mathbf{3}$$

The conversion factor (CF) is 10^{-6}mg/mg, and the slope factor (SF) for ingesting PAH for BaP is 7.3 mg/kg/day, where the incremental life cancer risk (ILCR) is dimensionless.

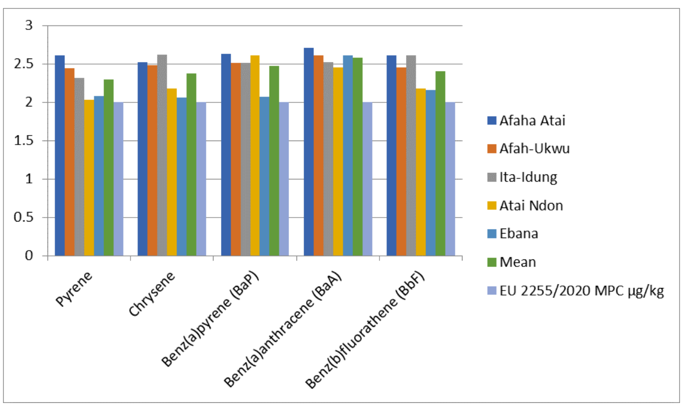

Figure 2. Results of the PAHs analysis of fish species in the wetlands of Eket oil-producing communities and EU 1255/2020 MPC in fish in μg/kg.

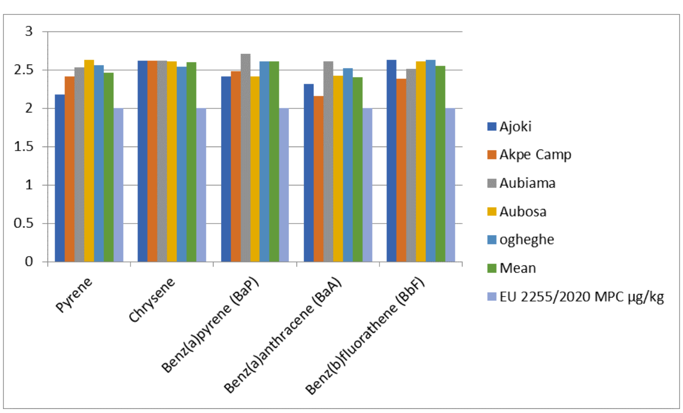

Figure 3. Results of the PAHs content in fish species in the wetlands of the Ikpoba/Okha oil-producing community and EU1255/2020 for PAHs in fish in μg/kg.

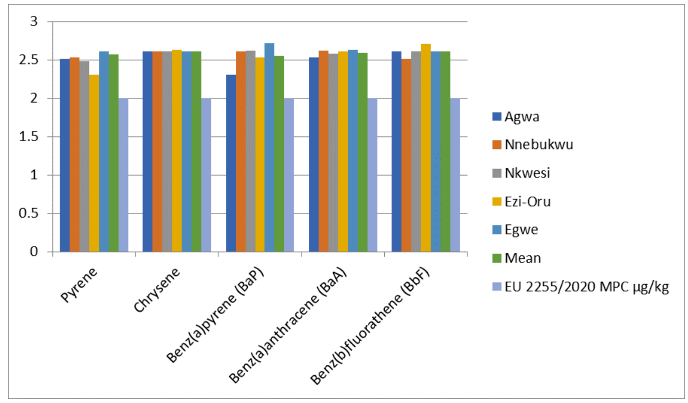

Figure 4. Results of the PAHs content in fish species in the wetlands ofOguta oil-producing community and EU 1255/2020 MPC for PAHs in fish in μg/kg.

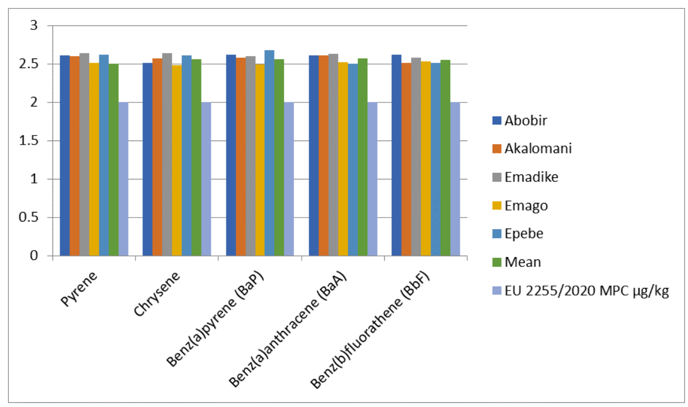

Figure 5. Results of the PAHs in fish species in the wetlands ofOgbia and EU1255/2020 MPC for PAHs in fish in μg/kg.

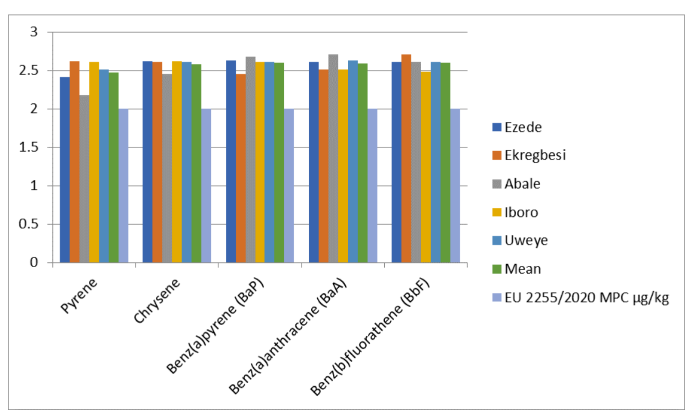

Figure 6. Results of the PAHs content of fish species inUzere wetlands and the EU 1255/2020 MPC for PAHs in fish in μg/kg

Table 1
Toxicity-equivalent factors of selected PAH congeners in fish species in the wetlands.

No of Fish sample	Designate	Beneze (a) pyrene (μg/kg)	Pyrene (μg/kg)	Chrysene (μg/kg)	Benzo (a) anthracene (μg/kg)	Benzo (b) fluoranthene (μg/kg)	
		TEQBaP	TEQBaP	TEQBaP	TEQBaP	TEQBaP	Sum
5	Afaha Atai	2.63	0.00	0.03	0.29	0.26	**3.20**
5	Afah-Ukwu	2.51	0.00	0.02	0.30	0.25	**3.08**
5	Ita-Idung	2.51	0.00	0.03	0.32	0.26	**3.11**
5	Atai Ndon	2.61	0.00	0.02	0.30	0.22	**3.15**
5	Ebana	2.07	0.00	0.02	0.31	0.22	**2.62**
	Σ	**12.33**	**0.01**	**0.12**	**1.51**	**1.20**	

*EU 1255/2020 MPC for PAHs in crawfish is 2.0 μg/kg

Table 2
Potential CDI of selected PAH congeners in fish species in the wetlands.

No. of Fish sample	Designation	Benzo (a) pyrene (μg/kg)	CDI	Pyrene (μg/kg)	CDI	Chrysene (μg/kg)	CDI	Benzo (a) anthracene (μg/kg)	CDI	Benzo (b) fluoranthene (μg/kg)	CDI
5	Afaha Atai	2.63	411.41	2.61	408.28	2.52	394.20	2.71	423.92	2.61	408.28
5	Afah-Ukwu	2.51	392.64	2.44	381.69	2.48	387.94	2.61	408.28	2.45	383.25
5	Ita-Idung	2.51	392.64	2.32	362.91	2.62	409.84	2.52	394.20	2.61	408.28
5	Atai Ndon	2.61	408.28	2.03	317.55	2.18	341.01	2.45	383.25	2.18	341.01
5	Ebana	2.07	323.81	2.08	325.37	2.06	322.24	2.61	408.28	2.16	337.89

*EU 1255/2020 MPC for PAHs in crawfish is 2.0 μg/kg

Table 3
Probable ILCR of selected PAH (Benzo (a) pyrene) in fish species in the wetlands.

No. of fish sample	Designate	Benzo (a) pyrene (μg/kg)	ILCR
5	Afaha Atai	2.63	0.02
5	Afah-Ukwu	2.51	0.02
5	Ita-Idung	2.51	0.02
5	Atai Ndon	2.61	0.02
5	Ebana	2.07	0.02

EPA (1992) standard for ILCR: *1×10^{-6} to 1×10^{-4}

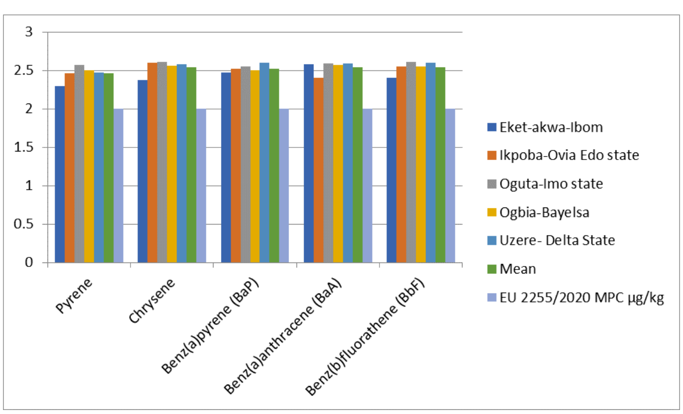

Figure 7. Results of the means comparison of the PAH content in the fish species in the wetland of oil-producing states of the Niger Delta and EU 1255/2020 MPC for fish in μg/kg.

3. RESULTS AND DISCUSSION

3.1. Variations in the concentration of PAHs in *Tilapia zilli*

Research reports on PAH contamination of aquatic organisms due to oil exploitation activities are rampant in literature in other climes (Orisakwe et al., 2015). Still, such research publications are primarily modest in the Niger Delta, and that underscores this study. The analysis of the PAH content on *Tilapia zilli* showed various contamination levels of the PAHs investigated in this study Figure 2-7.

The concentration of pyrene in the fish species was in the range of 2.30 μg/kg in the Eket oil-producing community Akwa-Ibom to 2.57 μg/kg in the Oguta oil-producing community, Imo state with a group mean concentration of 2.46 μg/kg. This concentration higher than the limit established for pyrene is similar to the reports by Papa et al. (2012) and Pergal et al. (2014). The health effects of prolonged human exposure to pyrene are skin irritation, respiratory problems, and cancer of the bladder (Ozaki et al., 2015).

Chrysene content analysis in the fish species in the wetlands of the Niger Delta oil-producing communities showed the concentration of chrysene to be between 2.37 μg/kg in Eket Akwa Ibom state to 2.58 μg/kg in Uzere Delta state with a mean content of 2.54 μg/kg. This result of a high concentration of chrysene in fish species in the oil-producing community of the Niger Delta is in agreement with the reports of Nilsen et al. (2015), Obrist et al. (2015), and Obuekwe and Semple (2013). The health implications of human exposure to chrysene include gene mutation and cardiovascular diseases (Nisha et al., 2015).

The analysis of the fish species for BaP presented varying concentrations from 2.47 in the Eket Akwa Ibom state to 2.60 μg/kg in the Uzere Delta state with a group mean of 2.54 μg/kg. Similar reports of the high content of BaP in fish species in oil-producing areas were in Obinaju et al. (2015) and Nwaichi and Ntorgbo (2016). BaP has been fingered in health complications such as abnormal neurobehavior, child asthma, cancer of the lungs, and bladder cancer (Patil et al., 2014; Pazos et al., 2010).

The concentrations of BaA in the fish species in the wetlands of the Niger Delta revealed the concentrations of BaA to range between 2.40 μg/kg in Ikpoba/Okha oil-producing community of Edo state to 2.59 μg/kg in Uzere in Delta state and Oguta oil producing community in Imo state with a group mean of 2.54 μg/kg. This report on elevated BaA content is similar to the reports by Pena et al. (2015). Human health complications of long-term exposure to BaA include teratogenic damage, cardiovascular complications, and cancer of the sinus (Sharma, 2014; Shen et al., 2014).

Analysis of the *Tilapia zilli* in the Niger Delta oil-producing communities for BbF contamination gave a range of contamination grades from 2.40 μg/kg in Eket Akwa Ibom to 2.60 μg/kg in Uzere Delta with a mean of 2.54 μg/kg. This result of a high concentration of BbF in fish species in the wetlands of oil production enclaves aligns with the reports of Paulik et al. (2015) and Shi et al. (2016). Exposure to BbF has been reported to cause oxidative damage, endocrine disruption, and mutation of DNA (Peters et al., 2016; Pham et al., 2015).

No significant values p>0.05 in the mean PAHs across the studied wetlands existed. The p-value was 0.47, thus rejecting $H_{0.}$

3.2. Health risk evaluation

Certain PAHs' health risk assessment results in *Tilapia zilli* revealed a high chemical concentration in the species Table 1. The TEFs were necessary because the fish species had considerable amounts of Benzo (a) pyrene, Benzo (a) anthracene, and Benzo (b) fluoranthene. If the species under investigation is taken more than the recommended daily intake, there may be a non-carcinogenic health risk. The CDI (chronic daily intake) data demonstrated a beneficial synergy between the TEF results in Table 2.

In comparison to the EPA (1992) (1×10^{-6} to 1×10^{-4}) and Liu et al. (2023) standards for exposure valuation for PAH compounds, the ILCR result (1×10^{-2}) in this study demonstrated a significant increase Table 3. As a result, eating *Tilapia zilli* people in this region carries the danger of developing cancer.

4. CONCLUSION

This study investigated the PAHs contamination status of *Tilapia zilli* in the wetlands of the Niger Delta. The results obtained were as follows: pyrene; 2.46±0.11 μg/kg, chrysene; 2.54±0.12 μg/kg, BaP; 2.52±0.11 μg/kg, BaA; 2.54±0.13 μg/kg and BbF; 2.54±0.13 μg/kg. The mean results of characterising the PAHs in the fish were insignificant at p>0.05. The results of the health risk evaluation showed that Beneze (a) pyrene had the highest value (12.33 μg/kg), followed by Benzo (a) anthracene and Benzo (b) fluoranthene (1.51 and 1.20) of the toxicity equivalent factors. Meanwhile, the sum across the station was significantly higher than the standard limits. The CDI (chronic daily intake) results obtained in this study for each PAH congener were considerably high and fluctuated across the stations. However, the results for the ILCR (incremental life cancer risk) showed that the values were higher (1×10^{-2}) than the threshold set for this study (1×10^{-6} to 1×10^{-4}).

The result of this study has further confirmed the environmental abuse occasioned by industrial activities, especially oil exploitation. The sample analysis of the fish species for PAHs contamination status has revealed that the PAHs content in the fish species in the wetlands of the Niger Delta is above critical points enunciated by EU regulation 1255/2020 for PAHs in fish. Therefore, the fish in the wetlands are unsuitable for human consumption and unfit for animal feed formulation. They cannot be exported because their contamination levels have contravened Codex Alimentarius standards for exporting the product.

Against this backdrop, the study recommended that oil companies in the Niger Delta should be mandated to adhere to the global standards spelt out for oil activities, mitigation modalities should be put in place for the impacted, and remediation of the wetlands should be commissioned to restore the wetlands to their hitherto healthy statuses for continued ecosystem services in line with sustainable economic development mantra of the United Nations.

CONFLICTS OF INTEREST

We declare no financial conflict of interest amongst us.

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AUTHOR CONTRIBUTIONS

All authors (Chukwudi Ogwu, Osikemekha Anthony Anani, Victor Ideh, Margret Awowede, Imobighe Mabel, Ogana Joy, and Ese Agbe) contributed to the research design, conceptualisation, data collection, data analysis, writing, editing, and review of this manuscript.

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