Faculty of Natural and Applied Sciences Journal of Basic and Environmental Research
Print ISSN: 3026-8184 e-ISSN 3043-6338
www.fnasjournals.com
Volume 2; Issue 2; March 2025; Page No. 94-100.

Analysis of Fish Samples for Heavy Metals and Total Hydrocarbon Content from Okrika And Bonny Rivers in Rivers State, Nigeria

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Abstract
This study examines the concentrations of heavy metals (cadmium [Cd], chromium [Cr], lead (Pb), arsenic (As), and nickel (Ni) and total hydrocarbon content (THC) in three fish species (Illish, Mullet, and Tilapia) from Okrika and Bonny Rivers in Rivers State, Nigeria. Fresh fish samples were collected and analyzed to assess their contamination levels. Heavy metals were determined using Atomic Absorption Spectrophotometry (AAS), while THC was analyzed using standard extraction techniques and Gas Chromatography-Mass Spectrometry (GC-MS). The results revealed that the concentrations of heavy metals in fish species exceeded permissible limits set by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) in several instances. Notably, Mullet Fish from Okrika River exhibited significantly elevated levels of Cd (2.0±0.36 mg/kg), Cr (2.02±0.12 mg/kg), Pb (10.25±1.03 mg/kg), and As (2.5±0.37 mg/kg). Similarly, fish from Bonny River also displayed high levels of contaminants, albeit generally lower than those from Okrika River. The THC levels, while below the Department of Petroleum Resources (DPR) threshold of 50 mg/kg, reflected ongoing hydrocarbon pollution, with Mullet Fish from Okrika River recording 3.08±0.06 mg/kg. Comparative analysis with existing literature highlights the severe pollution levels in these rivers, likely driven by industrial discharges, urban runoff, and oil-related activities. Species-specific trends indicate higher contaminant bioaccumulation in Mullet Fish, attributed to their benthic feeding habits. The study underscores significant environmental and public health concerns, given the reliance of local communities on these rivers for fish consumption. Urgent interventions, including stricter pollution control, sustainable waste management, and regular monitoring, are recommended to mitigate the risks posed by heavy metal and hydrocarbon contamination in aquatic ecosystems.

Keywords: Fish, Heavy Metal, Total Hydrocarbon, Okrika and Bonny, Rivers State

Introduction
Water pollution is a global environmental issue caused by the introduction of harmful substances into water bodies, which degrade water quality and disrupt aquatic ecosystems. This pollution occurs in various liquid-containing areas, including oceans, rivers, lakes, streams, and groundwater (Verma & Dwivedi, 2013). Pollutants such as toxic substances, pathogens, and organic and inorganic materials accumulate in these ecosystems, interfering with natural processes. For instance, eutrophication an oxygen-deprived state caused by excessive algae growth—can result from nutrient enrichment by pollutants (Izadiyar & Yargholi, 2010). The natural water cycle plays a role in transporting pollutants from land to aquatic environments, where they interact with soil, sediments, and living organisms. While organic pollutants may degrade through microbial action, inorganic pollutants are less likely to break down and can persist in the environment, posing long-term risks. Toxic substances such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and nickel (Ni) are particularly hazardous, even in trace amounts. These metals, along with others like mercury (Hg) and pesticides, can bioaccumulate, leading to harmful effects on both aquatic organisms and humans who consume contaminated water or fish.

Although some metals (e.g., zinc, copper, and iron) are essential micronutrients in small quantities, they become toxic at higher concentrations. Similarly, agricultural nutrients like phosphates, nitrates, and potassium are beneficial when used appropriately but harmful when excessive amounts enter water systems (Verma & Dwivedi, 2013). Heavy metals are naturally occurring elements characterized by their high atomic weight and density, at least five times greater than that of water. While these metals have valuable applications in industries, agriculture, medicine, and technology, their widespread distribution in the environment has raised significant concerns due to their toxicity and persistence. The toxic effects of heavy metals are influenced by several factors, including the dose, route of exposure, chemical form, and the biological characteristics of the exposed individual, such as age, gender, genetics, and nutritional status (Alloway, 2013). In turn, hydrocarbons are a class of organic compounds primarily composed of carbon and hydrogen, and they exist naturally in the environment, predominantly in crude oil and natural gas. In aquatic ecosystems, hydrocarbons, particularly Total Hydrocarbons (THC), are significant pollutants due to their release from industrial discharges, oil spills, agricultural runoff, and other anthropogenic activities. This study focuses on the relationship between THC and heavy metal contamination in fish species, emphasising how these pollutants interact with aquatic environments and affect the health of aquatic organisms, including those vital for human consumption. Total Hydrocarbons (THC) serve as a comprehensive measure of all hydrocarbon compounds in an environmental sample, including aliphatic and aromatic hydrocarbons. They are often used as an indicator of pollution levels, particularly in water and sediment, because their presence reflects the intensity of contamination and the likelihood of associated ecological harm. THC contamination in aquatic systems is a pressing concern because hydrocarbons can bioaccumulate in fish tissues, adversely impacting fish health, growth, and reproduction. The bioaccumulation of THC in fish species poses a dual threat to aquatic ecosystems and human populations, as fish are an essential dietary source of protein and micronutrients in many communities, including Nigeria's riverine areas. This study addresses these concerns by examining the concentrations of heavy metals and total hydrocarbon content in fish species from these rivers, contributing to the broader effort to minimise environmental and public health risks in Rivers State, Nigeria. This study is therefore aimed at analyze fish samples for heavy metals and total hydrocarbon content (THC) from Okrika and Bonny Rivers, with a focus on understanding the extent of contamination.

Material and Methods

The study was conducted in the Okrika and Bonny Rivers in Port Harcourt, Rivers State, Nigeria, two significant aquatic environments. The Okrika River is situated in the southern part of Rivers State, within the Niger Delta region of Nigeria. This river traverses areas of significant human and industrial activity. The selected sampling location at 4°49'01.3"N 7°05'03.0"E lies near a sand fill site, a prominent economic activity in this area close to the Akpajo area. Sand filling and dredging operations are extensive along the Okrika River, providing construction materials for urban development and land reclamation projects. There are also adjacent industrial activities, including small-scale manufacturing and petroleum-related operations, as companies like Eleme Petrochemicals can be found within a 50-mile radius of the sample location. The Bonny River is a critical waterway in Rivers State, serving as a hub for industrial and maritime activities. The selected sampling location at 4°45'28.0"N 7°01'31.4"E lies within an area heavily influenced by oil and gas operations, which are the backbone of the local economy. The river supports several fabrication yards and logistical operations. While the river serves as a critical resource for local livelihoods, particularly through fishing, these economic activities contribute to environmental stressors, including industrial discharges, and maritime activities.

The two rivers of focus are critical for local subsistence activities, including fishing, and are subject to industrial and anthropogenic pollution. The fish species studied—Mullet (*Mugilidae*), Ilish (*Hilsa kelee*), and Tilapia (*Oreochromis niloticus*)—were chosen due to their ecological and economic importance. Fish samples (Mullet (*Mugilidae*), Ilish (*Hilsa kelee*), and Tilapia (*Oreochromis niloticus*)) were bought directly from the fishermen who collected them. A total of twelve fish samples were collected. The samples were wrapped in sterile aluminum foil and transported in ice-packed coolers to the laboratory to prevent microbial degradation. The fish were cleaned, identified, dissected, and oven-dried at 105ºC. For subsequent analysis, each tissue type was homogenized using a blender.

Sample treatment

The fish samples were cleaned to remove any external contaminants, such as debris or dirt, and identified to confirm their species. The fish were then dissected to separate the tissues of interest. The fleshy tissue was oven-dried at a temperature of 105°C until constant weight was achieved, ensuring the removal of all moisture content to prevent interference in subsequent analyses. After drying, the tissues were homogenized using a laboratory-grade blender to produce fine, uniform particle sizes suitable for further preparation. The twelve fish samples were reduced to six composite samples to obtain representative samples, reflecting the three species from each river. The samples collected from a single river were dried, ground, and thoroughly mixed for each fish species to ensure homogeneity. The coning and quartering method was then applied to reduce these mixtures to single representative samples. This technique involved carefully piling the ground mixture into a cone, flattening it into a uniform circular layer, and dividing it into four equal parts. Two opposite quarters were retained, while the other two were discarded.

Sample digestion and extraction

The representative samples were then subjected to acid digestion to prepare them for heavy metal determination using Atomic Absorption Spectroscopy (AAS). For each representative sample, 2g of the homogenized fish tissue were accurately weighed using an analytical balance and transferred into a digestion flask. A mixture of concentrated nitric acid (HNO_3), perchloric acid ($HClO_4$), and sulfuric acid (H_2SO_4) in a ratio of 3:1:1 was added to the flask. This acid combination was selected for its efficacy in breaking down organic matrices and releasing bound heavy metals into solution. The digestion process was carried out on a hot plate under a fume hood to ensure safety and minimize the risk of contamination. The samples were heated gradually to near dryness, ensuring complete oxidation and dissolution of the organic matter while avoiding charring. Following digestion, the resulting solutions were allowed to cool and then diluted with deionized water to a final volume of 50 mL. The digested samples were filtered through Whatman filter paper to remove any insoluble residues, producing clear solutions suitable for AAS analysis. These filtrates were stored in clean, labeled polyethylene bottles and refrigerated at 4°C until the time of analysis to prevent any changes in metal concentrations. The heavy metal concentrations (Cd, Cr, Pb, As, and Ni) in the prepared samples were then determined using Atomic Absorption Spectroscopy (AAS). The AAS instrument was calibrated with standard solutions of known metal concentrations to ensure accuracy and reliability.

Total Hydrocarbon Content (THC) Determination

The determination of THC in fish tissue samples was carried out using the following procedure:

Each fish sample was cut into pieces and crushed in a mortar with a pestle. Ten grams of the individual fish sample was weighed using an analytical balance into a 100 mL beaker, and 60 mL of acetone and dichloromethane (1:1 v/v) was used as the extracting solvent. The beaker with the content was placed on a magnetic stirrer/heater and shaken for about 10 minutes at 70°C. The extract was decanted into a clean round-bottom flask. Then, 30 mL of fresh solvent was added, and the process was repeated. The extracts were combined, and 5 g of anhydrous sodium sulfate was added to remove water. The extract was concentrated to 3 mL with a rotary evaporator maintained at 20°C. Then, 1.5 mL of the concentrated extract was loaded on a silica gel column. The silica gel column was prepared by loading 2 g of glass wool followed by 30 g of silica gel onto a chromatographic column (2 cm internal diameter and 10 cm long). Each of the beds was conditioned with 40 mL of (95% purity) hexane to remove any organic contaminants. The 1.5 mL concentrated extract was loaded and eluted with 30 mL of (95% purity) hexane into a labeled 100 mL beaker to isolate the aliphatic hydrocarbon components in the sample.

After the hexane had almost eluted through the column but before completely letting the column dry, 30 mL of dichloromethane was added to elute the aromatic hydrocarbon contents into another labeled 100 mL beaker. Then, 2 g of anhydrous sodium sulfate was added to remove any traces of water left in the extract. The fractions were concentrated using a rotary evaporator to about 2 mL. Finally, 1 mL of the extract was transferred into a well-labeled vial, ready for gas chromatographic analysis. The samples were stored at 4°C until GC-MS analysis.

Contamination Factor

The contamination factor (CF) was employed to assess the extent of heavy metal pollution in the fish samples collected from Okirika and Bonny Rivers. This index quantifies the degree to which individual heavy metals exceed their natural background levels, providing insights into the extent of contamination in the environment.

T-Test Analysis

To statistically compare the concentrations of heavy metals and total hydrocarbon content (THC) in fish samples from the two rivers, a t-test was conducted. The t-test determines whether there is a significant difference between the mean values of two groups, in this case, the heavy metal and THC concentrations in fish samples from Okrika River and Bonny River.

Results

Table 1 Mean concentration of heavy metals and THC in fish species from Okrika River in mg/Kg

Location	Species	Standard	Cd	Cr	Pb	As	Ni	THC
Okrika River	Illish Fish		0.06±0.03	0.04±0.05	2.99±0.2	0.11±0.04	0.09±0.04	1.15±0.05
Okrika River	Mullet Fish		2±0.36	2.02±0.12	10.25±1.03	2.5±0.37	2.02±0.37	3.08±0.06
Okrika River	Tilapia		1.22±0.13	0.92±0.19	3.17±0.45	1.27±0.14	1.24±0.14	2.03±0.05
		FAO/WHO	0.05	0.05	0.5	0.05	0.5	
		DPR		-				50

Table 2 Mean Concentration of Heavy Metals and THC in fish species from Bonny River in mg/Kg

Location	Species	Standard	Cd	Cr	Pb	As	Ni	THC
Bonny River	Illish Fish		0.01±0.05	0.04±0.01	1.56±0.25	0.06±0.06	0.06±0.02	1.03±0.08
Bonny River	Mullet Fish		0.74±0.13	1.97±0.11	6.23±048	0.79±0.14	1.99±0.13	2.15±0.09
Bonny River	Tilapia		0.86±0.22	0.42±0.18	2.61±0.28	0.91±0.23	0.88±0.23	1.33±0.10
		FAO/WHO	0.05	0.05	0.5	0.05	0.5	
		DPR		-				50

Table 3 Contamination Factor of Heavy Metals and THC of Fish Species in Okrika River

Contamination Factor							
Location	**Species**	**Cd**	**Cr**	**Pb**	**As**	**Ni**	**THC**
Okrika River	Illish Fish	1.2	0.8	5.98	2.2	0.18	0.023
Okrika River	Mullet Fish	40	40.4	20.4	50	4.04	0.0616
Okrika River	Tilapia	24.4	18.4	6.34	25.4	2.48	0.0406

Table 4 Contamination Factor of Heavy Metals and THC of Fish Species in Bonny River

Contamination Factor							
Location	**Species**	**Cd**	**Cr**	**Pb**	**As**	**Ni**	**THC**
Bonny River	Illish Fish	0.2	0.8	3.12	1.2	0.12	0.0206
Bonny River	Mullet Fish	14.8	39.4	12.46	15.8	3.98	0.043
Bonny River	Tilapia	17.2	8.4	5.22	18.2	1.76	0.0266

Table 5 T-test Analysis for Heavy Metal and THC contamination in fish species in Okrika and Bonny Rivers

Fish specie	p-value
Illish Fish	0.63
Mullet	0.42
Tilapia	0.33

Discussion

Result from the Okrika River samples shows that cadmium levels varied among the fish species. For Illish Fish (*Hilsa kelee*), the cadmium concentration was recorded at 0.06 ± 0.03 mg/kg, a relatively lower value compared to the other species analyzed. Mullet Fish (*Mugilidae*) exhibited the highest cadmium concentration at 2.0 ± 0.36 mg/kg, indicating a greater degree of exposure or bioaccumulation. For Tilapia (*Oreochromis niloticus*), the cadmium level was 1.22 ± 0.13 mg/kg, representing intermediate accumulation. The elevated levels in Mullet Fish may reflect their benthic feeding habits, as cadmium tends to accumulate in sediments and is more accessible to bottom-dwelling organisms. This work agreed with Chikodiri et al. (2022) conducted on partitioning study of heavy metals and hydrocarbons from fish samples in the Donga River. Results review that Fish such as Mullet (*Mugilidae*) and Catfish (*Clarias gariepinus*) accumulated Pb at 0.85 ± 0.14 mg/kg and Cd at 0.12 ± 0.02 mg/kg. Osuagwu and Olaifa (2018) studied the effects of oil spills on fish biodiversity in the Niger Delta. TPH concentrations in fish tissues were reported at 0.24 to 6.35 mg/kg, with Pb levels reaching 1.75 ± 0.25 mg/kg in Tilapia (*Oreochromis niloticus*) and 0.89 ± 0.13 mg/kg in Catfish (*Clarias gariepinus*).

In this study, cadmium concentrations in fish species collected from the Bonny River were as follows: Mullet Fish (*Mugilidae*) recorded 0.74 ± 0.13 mg/kg, Tilapia (*Oreochromis niloticus*) showed 0.86 ± 0.22 mg/kg, and Illish Fish (*Hilsa kelee*) exhibited the lowest concentration at 0.01 ± 0.05 mg/kg. These values underscore the variability in cadmium bioaccumulation among different fish species, influenced by ecological behaviors, feeding patterns, and their interaction with the river's sediment and water column. In the Bonny River, chromium (Cr) concentrations were measured in three fish species: Mullet Fish (*Mugilidae*) showed the highest concentration at 1.97 ± 0.11 mg/kg, followed by Tilapia (*Oreochromis niloticus*) with 0.42 ± 0.18 mg/kg, and Illish Fish (*Hilsa kelee*) with the lowest at 0.04 ± 0.01 mg/kg. Inengite et al., (2010) investigated TPH and heavy metal concentrations in fish from Kolo Creek in the Niger Delta. TPH levels in fish tissues were reported between 2.43 and 9.18 mg/kg. Heavy metals included Cd (0.11 ± 0.03 mg/kg), Pb (0.89 ± 0.20 mg/kg), and Cr (0.45 ± 0.12 mg/kg), all of which exceeded WHO limits. Catfish (*Clarias gariepinus*) and Croaker (*Pseudotolithus elongatus*) were among the most affected species, exhibiting reduced growth and reproductive impairments. Also, Ndimele et al. (2018) explored the impacts of heavy metals and hydrocarbons on fish reproduction in Ogoni land. Result review that the values were above the WHO standard. Lead is a persistent and toxic heavy metal widely recognized for its detrimental effects on aquatic organisms and human health. Its presence in water bodies such as the Bonny River is often traced to industrial emissions, urban runoff, and legacy pollutants from activities like oil exploration, transportation, and waste disposal. Mullet Fish (*Mugilidae*) exhibited the highest lead concentration at 6.23 ± 0.48 mg/kg, followed by Tilapia (*Oreochromis niloticus*) with 2.61 ± 0.28 mg/kg, and Illish Fish (*Hilsa kelee*) with the lowest at 1.56 ± 0.25 mg/kg. Result from this study were compared with Avenant-Oldewage and Marx (2000) and result was significantly higher than this study. This study were above the permissible limit set by WHO. These findings indicate that lead bioaccumulation varies significantly between species, likely influenced by their feeding patterns, ecological niches, and interactions with contaminated sediments and water.

In the Okrika River samples, chromium concentrations showed notable variation across fish species. Illish Fish (*Hilsa kelee*) contained 0.04 ± 0.05 mg/kg, which is relatively low. Mullet Fish (*Mugilidae*) had a significantly higher chromium concentration at 2.02 ± 0.12 mg/kg, while Tilapia (*Oreochromis niloticus*) exhibited 0.92 ± 0.19 mg/kg. These findings suggest differing bioaccumulation capacities among the fish species, likely influenced by their feeding habits, habitat preferences, and metabolic processes. Mullet Fish, being bottom feeders, are more likely to encounter chromium-rich sediments, which act as reservoirs for heavy metals. This is in consonance with Adebisi et al. (2021). The analysis of arsenic levels in fish from the Okrika River reveals concerning concentrations, particularly in Mullet Fish (*Mugilidae*), which recorded 2.5 ± 0.37 mg/kg. Tilapia (*Oreochromis niloticus*) also showed significant accumulation at 1.27 ± 0.14 mg/kg, while Illish Fish (*Hilsa kelee*) exhibited the lowest level, 0.11 ± 0.04 mg/kg. The higher arsenic concentration in Mullet Fish could be linked to their benthic feeding habits, exposing them to arsenic-rich sediments and particulate matter. Tilapia, which inhabit both pelagic and benthic zones, may similarly encounter arsenic through sediment interactions and dietary uptake. Eneji et al. (2012) studied heavy metals in sediments and fish from the Benue River. In sediments, Zn concentrations ranged from 250 to 400 mg/kg, Cu from 45 to 80 mg/kg, and Pb from 5 to 20 mg/kg. In fish species such as Nile Perch (*Lates niloticus*), Pb concentrations were 0.62 ± 0.18 mg/kg, Cd 0.12 ± 0.04 mg/kg, and Cr 0.48 ± 0.11 mg/kg. These levels exceeded permissible limits and the result study were higher than (Eneji et al., 2012).

In this study, nickel concentrations in fish from the Okrika River highlight notable levels of bioaccumulation, particularly in Mullet Fish (*Mugilidae*), which recorded 2.02 ± 0.37 mg/kg. Tilapia (*Oreochromis niloticus*) exhibited 1.24 ± 0.14 mg/kg, while Illish Fish (*Hilsa kelee*) had the lowest levels at 0.09 ± 0.04 mg/kg. The differences in nickel accumulation among these species are likely linked to their feeding behaviors and ecological niches. Mullet Fish, which are benthic feeders, are more exposed to sediment-bound nickel, where the metal tends to concentrate. Tilapia, with their omnivorous diet and benthic tendencies, also accumulate nickel but at slightly lower levels. Illish Fish, which predominantly inhabit pelagic zones, show reduced exposure to toxic elements. The results revealed notable differences in contaminant levels between the two rivers and among fish species. Mullet Fish from Okrika River exhibited the highest contamination levels for most parameters, particularly Pb (10.25 ± 1.03 mg/kg) and Cr (2.02 ± 0.12 mg/kg), far exceeding FAO/WHO permissible limits. Tilapia and Illish Fish showed relatively lower levels of heavy metals, though several parameters, especially Pb, still surpassed acceptable thresholds. THC levels were below the Department of Petroleum Resources (DPR) maximum limit of 50 mg/kg, with the highest value recorded in Mullet Fish from Okrika River (3.08 ± 0.06 mg/kg). Comparative analyses with Asuquo et al. (2004) indicated alignment with regional trends of aquatic pollution, driven by industrial discharges, urban runoff, and oil exploration activities. Edokpayi et al. (2017) results revealed elevated levels of heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), and zinc (Zn) in water and sediment samples.

Lead concentrations in water were recorded at 0.34 mg/L, exceeding the World Health Organization (WHO) limit of 0.01 mg/L for drinking water, while sediment samples showed Pb levels reaching 62.3 mg/kg. Species-specific bioaccumulation trends highlighted the influence of feeding habits and habitat preferences, with Mullet Fish, often sediment feeders, showing the highest contaminant levels. The contamination factors indicate a gradient of pollution impact across the three fish species, with Mullet Fish showing the highest levels of contamination, followed by Tilapia, and Illish Fish exhibiting the lowest levels. The high CF values for arsenic, cadmium, and chromium in Mullet Fish and Tilapia highlight the severity of pollution in the Rivers, likely driven by industrial effluents, urban runoff, and other anthropogenic activities. This pattern is consistent with their feeding habits and habitat preferences, with Mullet Fish being more exposed to sediment-associated contaminants. These findings underscore the need for immediate remediation measures to mitigate heavy metal pollution and protect aquatic ecosystems and human health in the region. The t-test analysis conducted between the heavy metal and THC concentrations in fish species from the Okrika and Bonny Rivers provides statistical insights into the variability and similarities in contamination levels across these two rivers. The p-values obtained for the Illish fish (p=0.63), Mullet fish (p=0.42), and Tilapia fish (p=0.33) offer critical implications for interpreting the findings and understanding the environmental dynamics of the two water bodies. The t-test results highlight the comparable levels of contamination in the Okirika and Bonny Rivers, emphasizing shared pollution sources and similar environmental impacts on aquatic life. While the statistical analysis does not indicate significant differences, the ecological and public health implications of heavy metal and hydrocarbon contamination remain critical. These findings call for coordinated efforts to mitigate pollution and protect the integrity of aquatic ecosystems and the health of local communities reliant on these resources.

Conclusion

This research has provided critical insights into the pollution status of Okrika and Bonny Rivers through the lens of heavy metal and hydrocarbon contamination in fish. The findings reveal extensive heavy metal pollution, particularly lead, cadmium, and chromium, which exceed international safety limits and pose significant ecological and public health threats. The higher contamination levels in Okrika River compared to Bonny River highlight localized environmental stressors likely related to industrial effluents, oil-related activities, and waste disposal practices. While THC levels remain within permissible limits, the presence of hydrocarbons in fish tissue signals ongoing contamination that, in combination with heavy metals, can amplify toxic effects. These results emphasize the urgent need for interventions to address pollution sources, protect aquatic ecosystems, and safeguard the health of communities reliant on these water bodies for food and livelihood.

Recommendations

1. The study recommends stricter environmental regulations to monitor industrial and urban pollution, public education on the health risks of consuming contaminated fish, and the establishment of treatment facilities for industrial effluents.

2. Future research should include water and sediment analysis for a comprehensive understanding of pollution dynamics. Additionally, collaboration with international agencies is encouraged to align local environmental policies with global standards and secure funding for large-scale remediation efforts.

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