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Impact of Urban Runoff on the Benthic and Pelagic Fish Fauna in Ikpoba River: Heavy Metal Levels and Gill Pathology

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ABSTRACT: This study presents an assessment of the influence of urban runoff on fish fauna in the Ikpoba River, Nigeria, focusing on heavy metal contamination and gill pathology in benthic (*Clarias gariepinus*) and pelagic (*Oreochromis niloticus*) fish species. Samples of water, sediment, and fish gills were collected from June to August 2023. Heavy metals (Ni, Co, Cr, Pb, and Cd) were analyzed via Atomic Absorption Spectrometry (AAS), and gill pathology was evaluated through histopathological examination. Results indicated elevated concentrations of Cr and Ni in water samples, surpassing World Health Organization (WHO) standards, with Ni concentrations also elevated in sediments. However, heavy metal concentrations in fish gills remained below United States Environmental Protection Agency (US-EPA) thresholds for aquatic life, suggesting low contamination levels. Histopathological analysis revealed mild alterations in the gills, including blunted tips and prominent lacunae, attributed to metal presence. The study underscores the ecological and human health risks associated with heavy metal contamination in the Ikpoba River due to urban runoff. Findings stress the necessity of regular water quality monitoring to protect the river ecosystem. This research contributes to understanding the impact of urbanization on aquatic environments and provides insights for effective environmental management.

Keywords: Urban runoff, Heavy metals, Gill pathology, Fish fauna, Ikpoba River

Introduction

The Ikpoba River in Nigeria's Edo State sustains a thriving fishery industry but faces challenges from urban runoff, introducing pollutants, including heavy metals (Nabizadeh *et al.*, 2005; Ojeah and Oriakhi, 2022). These heavy metals, such as Cd, Pb, Ni, and Cr, pose serious risks to both aquatic organisms and human health (Farombi *et al.*, 2007).

Understanding the impact of urban runoff on the Ikpoba River's fish fauna is crucial for evaluating ecological and human health risks associated with heavy metal contamination. This study aims to provide insights into the threats posed by urbanization by assessing heavy metal levels and gill pathology (Ohiagu *et al.*, 2020; Olayinka-Olagunju, 2022). Aquatic ecosystems are crucial repositories for various forms of pollution from human activities like urbanization, industrialization, and agriculture (Mdegela *et al.*, 2009; Adeyemo *et al.*, 2020; Loto *et al.*, 2023). Urban runoff introduces a plethora of pollutants into water bodies, including pesticides, fertilizers, and sewage (Wolfand *et al.*, 2019).

In Edo State, Nigeria, the Ikpoba River receives effluents from multiple industries, raising concerns about heavy metal pollution. Heavy metals, characterized by their density exceeding 5 g cm-3 and atomic mass exceeding 20, pose substantial risks to both human health and the environment (Jadaa and Mohammed, 2023). Cd is particularly hazardous to fish and humans, sourced from activities like fossil fuel combustion and industrial processes, leading to detrimental effects (Järup, 2003; Borgmann *et al.*, 2005). Similarly, Pb is toxic, prevalent due to various human activities like mining and industrial processes (Tangahu *et al.*, 2011).

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Studies in Nigeria have shown elevated heavy metal concentrations in fish tissues, raising health concerns for consumers (Olowu *et al.*, 2010; Akan *et al.*, 2012; Izah and Angaye, 2016). Fish, sensitive indicators of environmental pollution, accumulate heavy metals, leading to physiological changes (Eagles-Smith *et al.*, 2016; Amuneke *et al.*, 2020). Gill histopathology serves as a crucial indicator of heavy metal contamination in fish, showing morphological alterations (El-Agri *et al.*, 2022; Musa and Sabiu, 2022). This study aims to bridge a critical gap by examining the impact of urban runoff on benthic and pelagic fish fauna in the Ikpoba River.

By assessing heavy metal levels and gill pathology, this research seeks to provide a comprehensive understanding of the ecological and human health risks associated with urbanization. Understanding these risks is essential for ecosystem health evaluation and the formulation of effective environmental management strategies (Ashraf *et al.*, 2012). Thus, this study contributes significantly to assessing biodiversity threats and developing interventions to safeguard both ecosystems and human health.

Materials and methods

Study area: The study was conducted in the city of Benin, nestled in the southern region of Nigeria within Edo State. Positioned at latitudes 6°11′ and 6°29′ N, and longitudes 5°33′ and 5°47′ E, Benin City enjoys a picturesque setting with an average elevation of 77.8 m above sea level, situated in the lush and diverse humid tropical rainforest belt of Nigeria (Victor and Ogbeibu, 1985; Chukwuka and Ogbeide, 2021). One of the prominent natural features in Benin City is the Ikpoba River, which meanders through the Benin-Owena basin, traversing the Egor and Ikpoba-Okha local government areas. This river serves as a vital lifeline for the local communities, supporting various activities that are integral to the livelihoods of the residents. These activities include fishing, waste disposal practices, recreational boating, and the discharge of wastewater, particularly originating from commercial establishments (Chukwuka and Ogbeide, 2021).

The Ikpoba River not only sustains local fishing practices but also serves as a conduit for various human activities that impact its water quality and ecosystem health (Victor and Ogbeibu, 1985; Tawari-Fufeyin and Ekaye, 2007). The coexistence of activities such as waste dumping and discharge of wastewater near the river raises concerns about potential pollution and environmental degradation risks. The dynamic interplay between human activities and the ecological integrity of the Ikpoba River underscores the need for comprehensive environmental management strategies that promote sustainable development while safeguarding the health of the river ecosystem

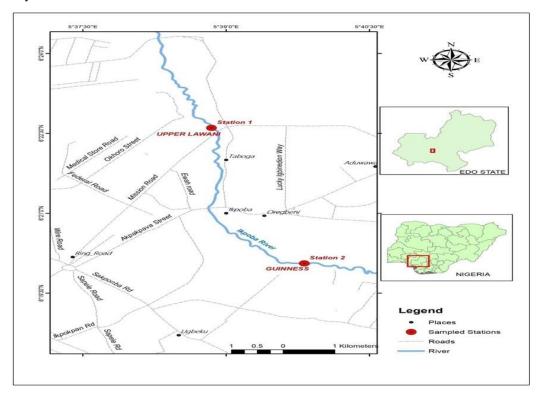


Figure 1: Map showing site locations of the study area

Sample collection and preparation: For this investigation, two sampling stations—Station 1 and Station 2 were strategically positioned based on their respective effluent characteristics. Station 1, situated at latitude $6^{\circ}22'34.39"$ N and longitude $5^{\circ}38'45.928"$ E, was considered relatively uncontaminated due to its location upstream of the effluent discharge source. In contrast, Station 2, located at latitude $6^{\circ}20'3.594"$ N and longitude $5^{\circ}39'48.906"$ E, encompasses the effluent discharge point of the prominent brewery.

Water samples were acquired from both the benthic and pelagic zones throughout the Ikpoba River during monthly collections spanning June through August 2023. Based on the method described by Onyidoh *et al.* (2017), well-cleaned 1-liter sampling bottles were employed for sample acquisition. Specifically, BOD bottles were utilized for water samples intended for biochemical oxygen demand (BOD) analyses. Upon retrieval, the filled bottles were immediately capped, labelled, and preserved on ice before transportation to the laboratory for further examination (Chukwuka and Ogbeide, 2021).

Grab sampling methods were implemented to collect sediment samples, which were subsequently wrapped in aluminium foil, appropriately labelled, and transported to the laboratory. (Onyidoh *et al.*, 2017; Chukwuka nd Ogbeide, 2021). Once in the laboratory, these sediment samples underwent drying via an oven followed by sieving utilizing a 2 mm mesh sieve. Subsequently, the dried and sieved sediment samples were kept for future analysis.

Randomly capturing fish specimens occurred using a fishing net, after which the fish were brought to the laboratory for proper identification and preservation. A total of fifty-four (54) fish were gathered, consisting of thirty from the pelagic zone and twenty-four from the benthic zone. At the laboratory, the fish were cleaned using distilled water and stored at -10° C in separate polyethylene bags treated with acid. Finally, the morphometric measurements of the fish pairs were recorded using a ruler and weighed employing a scale (Tongo *et al.*, 2022). A total of thirty fish samples of each species: fifteen *C. gariepinus* (upstream) and fifteen *O. nilotica* (downstream) were weighed, dissected, gills extracted and then transported to the laboratory in an ice-filled container where samples remained refrigerated prior to analyses.

Heavy metal analyses: Heavy metals in water and sediment samples were determined based on the methods described by Davies and Ekperusi (2021). For water samples, a volume of twenty-five milliliters (25 mL) of water sample was dispensed into a porcelain crucible, followed by the addition of 1 mL of concentrated nitric acid (HNO₃) and 3 mL of concentrated hydrochloric acid (HCl). The mixture was then subjected to heating on a steam bath for approximately 30 minutes, subsequently cooled. The resulting digest was diluted to 50 mL with distilled water and stored in plastic containers for heavy metal analysis, following a consistent protocol for all water samples.

For sediment analysis, ten grams (10 g) of ground and sieved sediment samples were weighed into a porcelain crucible. Subsequently, 25 mL of distilled water was added along with 1 mL of concentrated nitric acid (HNO₃) and 3 mL of concentrated hydrochloric acid (HCl). The sample underwent heating in a steam bath for about an hour, followed by cooling. The digested sample was then filtered and adjusted to 50 mL with distilled water. The filtrate was preserved in plastic containers for heavy metal analysis, maintaining uniform treatment procedures for all sediment samples.

Analysis of heavy metals in the water, sediment, and fish samples was done by an AAS Solar 969 Unicam Series model. Each metal Cr, Co, Cd, Ni and Pb, was determined using a specific hollow cathode lamp for its analysis. Each sample was analyzed in triplicate to ensure representative results, and the concentration of metals was calculated using a standard calibration plot method as described by (Chukwuka nd Ogbeide, 2021).

Histopathological assessment of fish gills: Gill tissues were fixed using a 10% buffer solution for 24 hours, cut up into tiny fragments, and placed in a tissue cassette. Samples were then rinsed in water and processed in an Automatic Tissue Processing (ATP) machine for one hour where they remained in the processing solutions for 12 hours after which they were removed from the ATP machine. Tissue samples were embedded and by placing them in blocks of paraffin wax which provided support for the tissues. Microtomy at 3 - 5 microns was performed to section them and made to float on water in a floatation bath with a temperature of 56 °C for the removal of wrinkles and then transferred to microscopic slides. The tissues were passed through xylene to remove paraffin wax from the tissues, dehydrated using ethanol, stained using haematoxylin and eosin stains, and finally examined with the aid of a light microscope at a magnification of $\times 100$

Statistical analysis: Means and standard deviations were calculated for all the parameters related to heavy metals in the experimental fish samples. Analysis of variance (ANOVA) was employed at a 95% confidence level to compare the means for the heavy metal characteristics among different fish species from the Ikpoba River. A bioaccumulation factor was calculated for each heavy metal in fish gills and Statistical Program for Social Sciences (SPSS®), version 20.0, software was used to facilitate the analysis.

Results

Table 1 shows the monthly distribution of heavy metals in water. Across the months, upstream and downstream, Cr followed by Ni had the highest concentration, while Co had the lowest concentration of heavy metals. Pb and Cd values are below the detection limit.

Heavy Metal	June		July		August		
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	
Ni	0.04 ± 0.002	0.05 ± 0.002	0.03 ± 0.004	0.05 ± 0.002	0.03 ± 0.005	0.04 ± 0.002	
Pb	0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$0.0 {\pm} 0.0$	0.0 ± 0.0	
Cr	0.03 ± 0.003	0.05 ± 0.003	0.04 ± 0.001	0.04 ± 0.003	0.04 ± 0.004	0.05 ± 0.003	
Cd	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 + 0.0	0.0 ± 0.0	
Со	0.02 ± 0.003	0.02 ± 0.006	0.02 ± 0.003	0.03 ± 0.002	0.03 ± 0.004	0.04 ± 0.002	

Table 1: Monthly concentrations of heavy metals in water

The values are the average standard deviation in three independent analyses. All values are in mg/L. The mean difference is significant at P < 0.05

Heavy metals concentration in water: The mean concentrations of the heavy metals in water upstream and downstream across the three months are presented in Figure 2 below. Cr had the highest concentration $(0.36\pm0.005 \text{ mg/l})$ while Cohad the lowest concentration. Downstream, Cr and Ni had the highest concentration $(0.04\pm0.006 \text{ mg/l})$ and $0.04\pm0.005 \text{ mg/l}$, respectively), while Co had the lowest. Pb and Cd had no results because their heavy metal concentrations were below the detection limit.

Heavy metals concentration in sediments: According to Table 2, Ni followed by Cr had the highest concentration across the months, both upstream and downstream. Cd, on the other hand, had the lowest concentration of heavy metals. The values presented in the table are the average standard deviation from three independent analyses, and all values are given in mg/kg. Upstream, Ni had the highest mean concentration $(4.9\pm1.73 \text{ mg/l})$, while Cd had the lowest mean concentration $(0.17\pm0.04 \text{ mg/l})$. Downstream, Ni again had the highest mean concentration $(5.7\pm1.9 \text{ mg/l})$, while Cd had the lowest mean concentration $(0.2\pm0.003 \text{ mg/l})$. Overall, the results indicate that Ni and Cr consistently had higher concentrations compared to the other heavy metals in the sediments. Cd consistently had the lowest concentration.

	June		July		August		P value
Heavy	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	
metal	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	
Pb	0.362±0.019	0.474 ± 0.004	0.362±0.019	0.474 ± 0.004	5.827±0.595	5.208±0.348	P<0.05
Co	1.718±0.136	2.067±0.058	1.718±0.136	2.067±0.058	0.489±0.019	0.513±0.045	P<0.05
Cr	2.568±0.297	2.155±0.021	2.568±0.297	2.155±0.021	1.899±0.149	2.116±0.342	P>0.05
Cd	0.181±0.004	0.214±0.002	0.181±0.004	0.214±0.002	0.177±0.036	0.241±0.022	P>0.05
Ni	6.125±0.102	7.89±0.193	6.125±0.102	7.89±0.193	1.585 ± 0.061	1.447±0.117	P<0.05

Table 2: Monthly concentrations of heavy metals in sediments

The values are the average standard deviation in three independent analyses. All values are in mg/kg. The mean difference is significant at P < 0.05

Concentration of heavy metals in gills: Figure 2 shows the concentration of heavy metals in the gills of *C. gariepinus* (benthic fish) and *O. nilotica* (pelagic fish) across the months. Ni had the highest concentration across all the months while Cd had the lowest concentrations

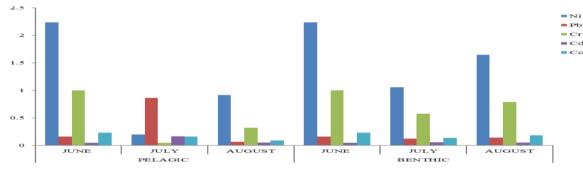


Figure 2: Concentration of heavy metals in the gills of *Oreochromis nilotica* and *Clarias gariepinus* across all the months

Figure 3 shows the mean concentration of heavy metals in the gills of *C. gariepinus* (benthic fish) and *O. nilotica* (pelagic fish). Ni had the highest concentration. The order of mean concentration in descending order was Ni> Cr> Pb>Co>Cd

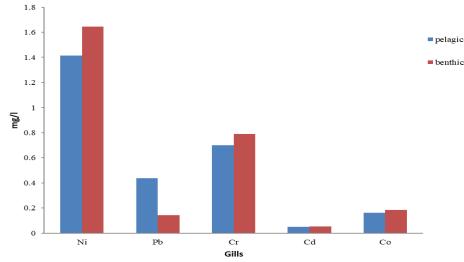


Figure 3: Mean concentration of heavy metals in the gills of C. gariepinus and O. nilotica

Histopathology of fish gills: Figure 4 shows the gills of catfish and tilapia in June. (A) showed primary lamellae (short arrow) that appear long, remarkable rakers with projected blunt tips and prominent lacuna (capillary lumen (long arrow). (B) showed long prominent rakers containing primary lamellae (short arrow), projected tips, and prominent lacuna (capillary lumen (long arrow). (C) showed unremarkable rakers with primary lamellae (short arrow) that appear long, with projected blunt tips and gill arch (long arrow).

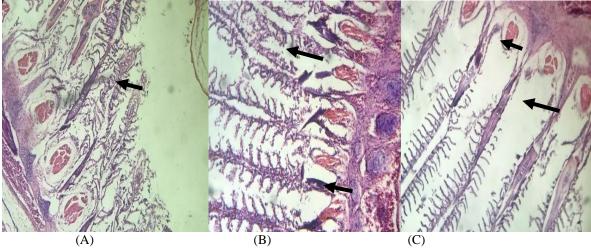


Figure 4: Fish gill histology in June

Figure 5 shows the gill features of catfish and tilapia in July. (A) showed unremarkable rakers with primary lamellae (short arrow) that appear long, with projected blunt tips and gill arch (long arrow). (B) showed unremarkable lamellae appearing short (long arrow) and visible mononuclear cells short arrow) with fatty changes (short arrow). (C) showed unremarkable blunt rakers that appear elongated (long arrow). The filament cartilage appears obliterated.

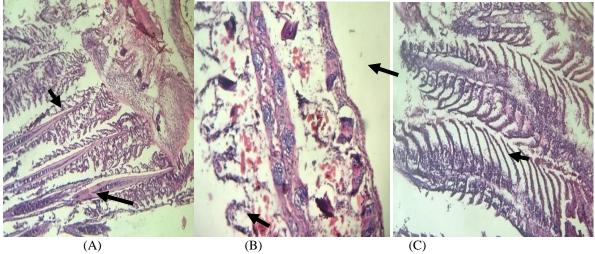


Figure 5: Fish gill histology in July

Figure 6 shows the gill features of catfish and tilapia in August. (A) showed primary lamellae (short arrow) that appear long, with unremarkable rakers with projected blunt tips and prominent lacuna capillary lumen (long arrow). (B) showed primary lamellae (short arrow) appear long, with unremarkable rakers, projected blunt tips, and prominent lacuna (capillary lumen (long arrow). (C) showed primary lamellae (short arrow) that appear short, unremarkable rakers with projected blunt tips and distorted lacuna capillary lumen (long arrow).

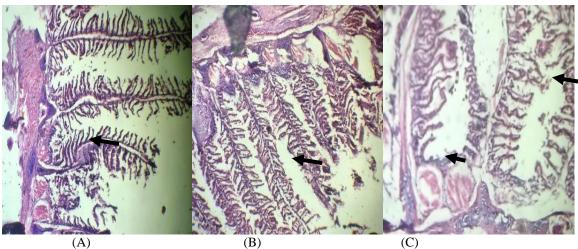


Figure 6: Fish gill histology in August

Discussion

Heavy metals are known for their adverse impacts on aquatic organisms and their potential to affect human health. This study assessed the concentrations of heavy metals in water and sediments, antioxidants, and the histopathological changes in fish samples from the Ikpoba River, Nigeria.

Heavy metals in water samples: In this study, the mean concentration of Cr in the water upstream and downstream were 0.36 ± 0.005 mg/L and 0.04 ± 0.00 mg/L, respectively. This was higher than the WHO acceptable standards and in conformity with the study carried out by (Bubu-Davies *et al.*, 2022; Ogarekpe *et al.*, 2023). This study was also in correlation with a previous study carried out by Mohiuddin *et al.* (2022) where the mean Cr concentration was much higher than the recommended value of 1 mg/kg. The high concentrations of these metals in the current study can be attributed to the influx of stormwater runoff into the river during the rainy season (Islam *et al.*, 2023; Younas *et al.*, 2023).

Interestingly, Pb and Cd were undetectable in the water samples, while Co concentrations remained within permissible limits. However, both Cr and Ni concentrations exceeded World Health Organisation (WHO, 2004) recommendations, posing potential risks to aquatic organisms and human health.

The elevated level of Cr in both upstream and downstream water may be a result of pollution from the various industries close to the river (Gil et al., 2014; Ostoich et al., 2014). The sources of high Cr content in water in Nigeria are primarily anthropogenic, resulting from human activities such as mining, industrial processes, and waste disposal (Ishaku and Ezeigbo, 2010). Industrial wastewater is discharged into streams and rivers without treatment in many countries, particularly in developing countries like Nigeria. This leads to the contamination of water bodies and poses a threat to both human health and aquatic life (Wang and Yang, 2016; Bijekar et al., 2022; Mmonwuba et al., 2023). Similar studies conducted in the Shitalakshya river of Bangladesh (Jolly et al., 2023) and the Rupsha River in Bangladesh (Kubra et al., 2023) found that heavy metals, including Cr, were present in the sediment and water samples. The presence of Cr in high concentrations in the Spekboom River in South Africa was also attributed to contamination from anthropogenic activities (Addo-Bediako et al., 2021). Additionally, the Taipu River in China showed higher concentrations of Cr in the midstream, which was influenced by industrial activities and hydrological conditions (Yao et al., 2022). These findings suggest that the pollution from industries close to the rivers can contribute to the elevated levels of Cr in both upstream and downstream water. Exposure of aquatic life to Cr in the water column can lead to significant changes in fish physiology and metabolism. Studies by Muthulingam (2017) and Sarkar et al. (2016) have shown that fish exposed to Cr exhibit various behavioral abnormalities, including erratic swimming, mucous secretion, changes in body color, and loss of appetite (Bakshi nd Panigrahi, 2018).) This was in exact correspondence with the study carried out by Abidemi-Iromini et al. (2022) where the concentration of Cr in the flesh of C. nigrodigitatus and O. niloticus within and between locations indicated that O. niloticus from Lagos Lagoon had a higher value than recommended limits (0.013 mg/g) (USFDA., 1993); (0.020 mg/g) (FAO, 2004).

In this study, the mean concentration of Cr in water samples collected both upstream and downstream was found to be $0.36 \pm 0.005 \text{ mg/L}$ and $0.04 \pm 0.00 \text{ mg/L}$, respectively. These concentrations exceeded the acceptable standards set by the WHO and were consistent with previous studies conducted by Bubu-Davies *et al.* (2022) and Ogarekpe *et al.* (2023). Similarly, the findings of this study were in line with research by Mohiuddin *et al.* (2022), which reported elevated Cr levels surpassing the recommended threshold of 1 mg/kg. The elevated concentrations of Cr observed in the water samples were attributed to the influx of stormwater runoff into the river during the rainy season, as highlighted in studies by Islam *et al.* (2023) and Younas *et al.* (2023).

Interestingly, Pb and Cd were undetectable in the water samples, while Co levels remained within permissible limits. However, both Cr and Ni concentrations exceeded the WHO recommendations, posing potential risks to aquatic organisms and human health.

The high levels of Cr detected in both upstream and downstream water samples may be linked to pollution from nearby industries, as indicated by studies conducted by Gil *et al.* (2014) and Ostoich *et al.* (2014). The sources of elevated Cr content in Nigerian water bodies are predominantly anthropogenic, stemming from activities such as mining, industrial processes, and improper waste disposal, as highlighted by Ishaku and Ezeigbo (2010). The discharge of untreated industrial wastewater into rivers and streams, particularly in developing countries like Nigeria, contributes to water contamination, endangering both human health and aquatic life, as noted in studies by Bijekar *et al.* (2022), Mmonwuba *et al.* (2023) and Wang and Yang (2016).

Similar studies conducted in various regions, such as the Shitalakshya River (Kubra *et al.*, 2023) and the Rupsha River (Jolly *et al.*, 2023), in Bangladesh, as well as the Spekboom River in South Africa (Addo-Bediako *et al.*, 2021) and the Taipu River in China (Yao *et al.*, 2022), have also reported elevated levels of Cr attributed to industrial contamination and anthropogenic activities. The exposure of aquatic organisms to Cr in water can lead to significant physiological and metabolic changes, as evidenced by studies by (Muthulingam, 2017) and (Sarkar *et al.*, 2016), which observed behavioral abnormalities in fish exposed to Cr, including erratic swimming, mucous secretion, changes in body color, and loss of appetite.

High levels of Cr have also been reported in Ikpoba River, Edo state in a study conducted by Enuneku and Ineh (2020). They reported concentrations of Cr, Pb, Ni, and Co in water from the Ikpoba River and found that Cr had the highest concentration among the metals analyzed. The main source of metal concentration in the water was attributed to domestic and industrial waste discharges into the river. The concentrations of metals in Ikpoba River have also been reported by Oguzie and Okhagbuzo (2010) who investigated the concentrations of Cr, Pb, Ni and Co in water samples collected from different stations along the river. Results The results from this corroborate this present study as the levels of Cr, Pb, and Ni were relatively high in urban run-off effluents, which receive wastewater from various sources including industrial and domestic activities. Similarly, findings from this study are in line with reports by Wangboje and Ekundayo (2013), who reported the concentration of Cr, Pb, Ni, and Co in water from the Ikpoba River. The mean concentration of Cr ranged from 0.02 mg/l to 0.05 mg/l, while the mean concentration of Pb ranged from 0.05 mg/l to 0.08mg/l. The mean concentration of Ni ranged from 0.022 mg/l to 0.042 mg/l. These heavy metals were found to exceed the WHO maximum permissible levels for drinking water, indicating potential risks to public health.

High levels of Cr observed in this study do not agree with the findings in a study conducted by Olele *et al.* (2013). The study found that the concentration of these metals varied, with Cr having the lowest concentration

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compared to the other metals. The main source of metal concentration in the river was attributed to effluent discharges from a rubber processing factory, a brewery, and other mini-factories in the area. Similarly, the concentration of Cr, Pb, Ni, and Co in water from the Ikpoba River was analyzed in a study conducted by Osa-Iguehide *et al.* (2016). The study found that the heavy metal concentrations in the water followed the order Pb > Cu > Zn > Cd. However, Cr levels were below the detection limit of the equipment used for analysis. The main source of metal concentration in the water is attributed to the discharge of municipal waste and industrial effluent into the river.

Heavy metals in sediment samples: According to studies, sediments hold 99% of the heavy metals that enter aquatic systems (Li *et al.*, 2019). The results of the metals in sediment analysis of the Ikpoba River demonstrate varying concentrations of heavy metals, with a notable emphasis on Ni and Cr, exhibiting the highest levels among the analyzed metals. This observation aligns with numerous studies conducted globally, indicating a recurring trend of elevated concentrations of Ni and Cr in sediment samples from diverse aquatic environments (Zhuang *et al.*, 2018; Enuneku and Ineh, 2020; Yang *et al.*, 2023). The higher concentrations of Ni and Cr compared to other heavy metals indicate a potential source of pollution in the river.

The sources of Ni and Cr in sediment samples from the Ikpoba River can be attributed to both natural and anthropogenic sources (Genchi *et al.*, 2020; Magdy *et al.*, 2021). Anthropogenic sources of Ni and Cr in sediment are primarily associated with human activities. Industrial discharges, such as wastewater from manufacturing processes, can introduce elevated levels of Ni and Cr into the river system (Zarezadeh *et al.*, 2017; Akar *et al.*, 2021; Jin *et al.*, 2021). For example, the discharge of effluents from industries such as leather production, wood preservatives, and pigment manufacturing can contribute to the contamination of sediment with Ni and Cr (Abbas *et al.*, 2012; Nicolaus *et al.*, 2022). Other potential anthropogenic sources of Ni and Cr in sediment include urban runoff, agricultural practices, and atmospheric deposition. (León-García *et al.*, 2022; Wang *et al.*, 2024). Urban runoff can transport contaminants from roads, buildings, and other urban areas into the river, including Ni and Cr from sources such as vehicle emissions and industrial activities (Chunyuan *et al.*, 2016).

In concordance with our findings, investigations conducted in rivers such as the Saigon River (Nguyen *et al.*, 2020), Buriganga River (Ahmad *et al.*, 2010), River Gomti (Gaur *et al.*, 2005), New Calabar River (Davies nd Ekperusi, 2021), and the River Kubanni Dam (Okon *et al.*, 2022) have consistently reported heightened levels of Ni in sediment samples, affirming the prevalence of Ni contamination in riverine ecosystems. Similarly, studies conducted in the Ikpoba River (Enuneku nd Ineh, 2020), Olode area (Okonkwo *et al.*, 2023), and the Niger Delta region (Ehiemere *et al.*, 2022) have documented elevated concentrations of Cr, corroborating the findings of our investigation. However, it is important to note discrepancies in findings among certain studies. For instance, a study in the Ikpoba River (Oguzie nd Okhagbuzo, 2010) identified Pb as the predominant metal, contrasting with the prevalent Ni and Cr concentrations observed in our analysis and other related studies. Similarly, another study in the Ikpoba River (Wangboje nd Ekundayo, 2013) reported fluctuating concentrations of Cr and Pb without mentioning Ni or Co concentrations. While there may exist variances in the specific heavy metals exhibiting the highest concentrations across different studies, the recurrent identification of elevated levels of Ni and Cr in sediment samples underscores the pervasive contamination of river sediments by heavy metals. These findings emphasize the critical need for comprehensive pollution management strategies to mitigate the environmental and health risks associated with heavy metal pollution in riverine ecosystems.

Heavy metals in gills: Heavy metals can accumulate in the gills of fish, which can have detrimental effects on both the fish and human health. Studies have shown that fish species such as C. gariepinus, T. zilli, O. niloticus, A. baremose, and P. buffei have shown levels of metal accumulation in their gills (Ikwuemesi et al., 2023). The gills are a major route for heavy metal accumulation in fish, and higher concentrations of metals have been observed in the gills compared to other organs such as the liver (Choudhary et al., 2023). The accumulation of heavy metals in the gills can lead to histopathological changes and damage to the fish's respiratory system (Arojojoye et al., 2018). The gills of fish are often the first organs to be affected by heavy metals because of their direct contact with the contaminated water column (Siregar et al., 2018; Ubay et al., 2022; Shah et al., 2020). Fish rely on their gills for oxygen uptake and waste elimination, and these delicate structures possess thin membranes that readily facilitate the diffusion of heavy metals from the water into their bloodstream (Siregar et al., 2018). As a result, heavy metals quickly accumulate in gills making them the primary target for pollution. Once heavy metals breach the gill barrier, they can be distributed to other organs including the liver, kidneys, and muscle (Chima et al., 2022; Vishwakarma nd Shukla, 2023). However, the gills' continuous exposure to polluted water as the fish perspire makes them particularly vulnerable to heavy metal contamination. This may be attributed to the fact that they have the thinnest epithelium of any bodily organ, making it easy for metals to pass through as suggested by Farombi et al. (2007). This initial exposure can lead to long-term health issues for the fish and potential concerns for human consumption if the contaminated fish enter the food chain

The concentration of metals in fish gills is a crucial indicator of environmental pollution and can have significant implications for the health of aquatic organisms. Studies have emphasized the importance of

assessing heavy metal concentrations in fish gills as a reflection of the environmental status and potential risks posed by pollutants (Jabeen and Chaudhry, 2009; Elsayed *et al.*, 2011; Olgunoğlu *et al.*, 2015; Ikue *et al.*, 2019; Mustafa, 2020). In the present study, the concentration of heavy metals in the gills of *C. gariepinus* (benthic fish) and *O. nilotica* (pelagic fish) was investigated.

Ni was found to have the highest concentration among the heavy metals analyzed in the gills of both fish species across all the months. This finding is consistent with previous studies that have reported high levels of Ni accumulation in fish gills Ni accumulation in fish gills has been studied in several papers. Yousafzai and Shakoori (2008) found that the gills of *Tor putitora* from polluted waters showed higher concentrations of Ni compared to control fish. Dobryanska *et al.* (2016) investigated Coand Ni concentrations in the water, bottom deposits, and ichthyofauna of a water storage basin and observed that the biggest content of Coand Ni was found in the gills of rudd and perch. Ghosh *et al.* (2018) conducted laboratory experiments with Ni and found that the toxicity of Ni to fish is mediated primarily through the gills. Kang *et al.* (2012) used two-photon microscopy to image fish organs and found that Ni ions accumulate in fish organs in the order of kidney > heart > gill ≥ liver. Pane *et al.* (2004) studied the effects of chronic Ni exposure on rainbow trout and found that Ni accumulation was greatest in the gill, kidney, and plasma, with the plasma as the main sink for Ni.

The high concentration of Ni in fish gills suggests a potential risk to the health of these fish species and indicates the presence of Ni pollution in their respective habitats.

Studies by Aghoghovwia *et al.* (2016) and Ekeanyanwu *et al.* (2015) have observed varying trends in heavy metal accumulation in fish gills, with some metals like Ni and Cr exhibiting higher concentrations compared to others. These findings correlate with the observed effects of heavy metal exposure, as higher concentrations of metals in gills often lead to more pronounced pathological changes

Pathology of gills: Gills, being crucial respiratory and osmoregulatory organs in fish, are highly susceptible to damage from environmental pollutants, especially heavy metals (Shah *et al.*, 2020). The histopathological examination of fish gills provides valuable insights into the structural changes induced by heavy metal exposure and can help assess the overall health of fish populations. In the present study, the gills of both *C. gariepinus* and *O. nilotica* were examined for histopathological alterations.

In June, the gills of both fish species showed primary lamellae with long, remarkable rakers and prominent lacuna, indicative of healthy gill structures (Samajdar and Mandal, 2017). However, in July and August, histopathological changes observed in the gills of both fish species included unremarkable rakers, fatty changes, and obliterated filament cartilage, suggesting detrimental effects of heavy metal accumulation on the gill tissues (Abdel-Kader and Mourad, 2019). Furthermore, this study showed a high concentration of Ni followed by Cr in the gills of C. gariepinus and O. nilotica upstream and downstream across the three months except for July in the upstream, where Pb was found to be higher. This was in agreement with the findings of a study by Abidemi-Iromini et al. (2022) where Pb was found to have the highest concentration. Pb causes lamella shrinkage degeneracy of epithelium, ischemia, reduction in growth rate, branchial arterial rupture. Loss in body weight, neurological defects, renal tubular dysfunction, anemia which result in stress conditions are other defects of high concentration of Pb (Abidemi-Iromini et al., 2022). The elevated concentrations of metals, including Ni and Cr, found in the gills of C. gariepinus and O. nilotica align with previous findings of Ni pollution in aquatic environments (Ayoola, 2019). Histopathological changes in fish gills have been associated with heavy metal exposure, such as alterations in gill structure and cartilage damage (Chai et al., 2018). The observed histopathological alterations in the gills of both fish species are consistent with the detrimental effects of heavy metal accumulation on gill tissues (Salem and Ayadi, 2017).

The study also revealed an overall high concentration of Ni followed by Cr in the gills across three months, with exceptions noted for Pb in July upstream. The high concentration of Ni exceeded WHO acceptable limits for heavy metals in aquatic life, possibly due to industrial activities near the catchment area. This finding is consistent with previous studies (Atli, 2018; Wagh et al., 2023), linking Ni exposure to various effects on fish gills. Histopathological alterations observed included obliteration of filament cartilage, blunted rakers with primary lamellae, and distorted lacuna. These changes are typical responses to heavy metal exposure and have been reported in previous studies (Mabika nd Barson, 2013; Shahid et al., 2022). Notably, significant damage like necrosis and fusion of lamellae was not observed in this study, indicating lower heavy metal concentrations compared to other studies. In the study by Lace et al. (2017) necrosis in the gills and fusion of lamellae were recorded as effects of heavy metal exposure in Oreochromis niloticus in addition to damage to gill cells and shortening of lamellae, correlating the observations from this present study. Fusion of lamellae is also recorded as an effect observed in Siganus rivulatus due to heavy metal exposure by Mohammed SY et al. (2016). These effects were not detected in this study indicating that the heavy metal concentrations were not high enough to cause such significant damage. Since the fish gill is a key indication of waterborne toxins and is extremely sensitive to changes in the composition of the environment, damage to the gill epithelium is a frequent reaction seen in fish exposed to various pollutants. The amount of toxicant present and the length of exposure determine how severely the gills are harmed (Bose et al., 2013).

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Bioaccumulation factor (BAF): The bioaccumulation factor (BAF) values for heavy metals in the fish gills were determined to be 47.08, 0, 1.9, 0, and 8.14 for Ni, Pb, Cr, Cd, and Co, respectively, upstream, and 37.83, 0, 16.03, 0, and 4.5 for the same metals downstream. The BAF serves as a metric for assessing the concentration of heavy metals in the gills relative to their concentration in the surrounding water environment, providing insights into bioaccumulation processes (Costanza *et al.*, 2012; Raju *et al.*, 2013; Radwan *et al.*, 2022). Notably, BAF calculations derived from field-caught fish offer ecological relevance by accounting for dietary, respiratory, and dermal exposures, making them a valuable tool for screening bioaccumulation potential without significantly increasing resource requirements (Costanza *et al.*, 2012).

The Environmental Protection Agency (EPA) suggests that the BAF values obtained indicate a low potential for bioaccumulation, with Ni exhibiting a higher propensity for bioaccumulation in fish gills compared to other heavy metals both upstream and downstream, aligning with findings by Afzaal *et al.* (2022). Bioaccumulation, characterized by the gradual accumulation of chemicals in living organisms over time, occurs due to factors such as rapid absorption, poor digestion, or non-biodegradability of the chemicals. Heavy metals, being non-biodegradable, tend to bioconcentrate in various fish tissues through biosorption and metallurgical processes, particularly in the gills, which serve as the primary site for metal absorption from the aquatic environment (Makedonski *et al.*, 2017).

Several studies have investigated the bioaccumulation of heavy metals in aquatic organisms, including fish species. For example, e Silva et al. (2022) evaluated the bioaccumulation factor of toxic elements in fish species collected from a river impacted by industrial activity and found that some species showed bioaccumulation of elements such as Cu, Ni, and Zn, which can present risks to the biota and consumers. Additionally, Ikwuemesi et al. (2023) assessed the bioaccumulation of heavy metals in the gills and muscles of various fish species and found that some species showed accumulation of metals such as Zn, Cu, Fe, and Pb, with high levels of Cd, which calls for concern regarding fish consumption. Adekolurejo et al. (2023) also assessed heavy metal accumulation in fish samples and found that the concentrations of metals in water exceeded recommended limits, suggesting potential risks for human consumers ^{[I}In this study, the highest accumulation of Ni was observed in the gill tissues of both C. gariepinus and O. niloticus, while Pb and Coshowed negligible values. This finding is consistent with the field assessment conducted by Doherty et al. (2010), which investigated oxidative stress biomarkers and heavy metal levels, including Pb and zinc, as indicators of environmental pollution in selected fish species in Lagos, Nigeria. The observed metal accumulation levels are directly influenced by the prevailing concentrations of metals in the environment, which can be influenced by various physico-chemical parameters. The process of heavy metal bioaccumulation in animal tissues results from the interplay between uptake and excretion rates, where a net accumulation occurs when uptake surpasses excretion rates (Otitoloju and Olagoke, 2011). This comprehensive understanding of heavy metal bioaccumulation in fish tissues underscores the importance of continued monitoring and assessment of environmental pollutants to safeguard aquatic ecosystems and human health.

Benthic and pelagic similarities: The lack of significant differences in the results observed for benthic and pelagic fishes in this study can be attributed to several factors. Firstly, both benthic and pelagic fish species may have similar exposure pathways to heavy metals in the Ikpoba River. They could be exposed to contaminants through water intake, ingestion of contaminated food sources, or direct contact with sediments. Therefore, it is plausible that the overall contamination levels in the river affected both fish groups similarly.

Additionally, the study period and sampling locations may not have captured distinct variations in heavy metal concentrations between benthic and pelagic zones. The spatial and temporal distribution of contaminants in aquatic environments can be influenced by various factors such as water flow, sediment deposition, and pollutant sources. If the study period and sampling locations did not coincide with specific events or areas of higher contamination, it is possible that no significant differences were observed between the two fish groups.

Furthermore, the tolerance and adaptive capabilities of benthic and pelagic fish species to heavy metal exposure could be similar. Both groups may possess comparable detoxification mechanisms or physiological responses to cope with the presence of heavy metals in their gills. This similarity in response could result in comparable levels of heavy metal accumulation and gill pathology between benthic and pelagic fish species.

Conclusion

This study demonstrates that runoff into the Ikpoba River has led to a slight elevation in heavy metal concentrations within the river, resulting in increased bioaccumulation of heavy metals in the gills of *Oreochromis nilotica* and *Clarias gariepinus*. Consequently, structural alterations were observed in the gills of the collected fish samples, including obliterated filament cartilage, distorted lacuna, blunt rakers, reduced lamellae, and blunt gill arch. Fish sampled from upstream exhibited fewer disturbances compared to those from

downstream locations. Heavy metal concentrations in water samples exceeded permissible limits, although Pb and Cd were not detected. Sediments generally showed low heavy metal concentrations.

Implementing advanced treatment technologies to mitigate heavy metal discharge and other pollutants from industrial effluents is recommended. Regular monitoring of effluent quality and conducting environmental risk assessments are crucial to identify potential impacts on aquatic ecosystems and adjust operations accordingly. Toxic effects were studied at the individual species level, but future research should consider multiple biomarker-based studies across various fish species to encompass a broader spectrum of effects. Therefore, further studies and routine monitoring of the river are recommended to serve as an early warning signal of adverse environmental pollution effects.

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