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# ASSESSING THE IMPACT OF URBAN RUNOFF ON FISH SPECIES IN IKPOBA RIVER: HISTOPATHOLOGICAL ANALYSIS OF METAL ACCUMULATION IN MUSCLE TISSUE

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## ABSTRACT
*This study examined the impact of urban runoff on the Ikpoba River in Benin City, Nigeria, focusing on heavy metal contamination in water, sediments, and fish species, and the histopathological changes in fish muscle tissues. The findings revealed significant levels of Pb, Cr, Ni, Co, and Cd in water, sediments, and fish tissues, with temporal fluctuations across sampling sites. Statistical analysis showed significant variations in heavy metal concentrations: Co (1.23 ± 0.34 mg/L) and Ni (0.97 ± 0.28 mg/L) in water, Pb (56.8 ± 10.2 mg/kg) in sediment, and Cr (4.32 ± 1.02 mg/kg), Ni (3.78 ± 0.85 mg/kg), and Pb (5.94 ± 1.48 mg/kg) in fish tissues ($P < 0.05$). The accumulation of these metals in Clarias gariepinus and Tilapia zilli muscle tissues raises health concerns for local communities relying on the river for food and livelihood. Histopathological analysis showed progressive muscle tissue degeneration, indicating adverse effects from pollutants. These results highlight the need for comprehensive monitoring and management to address urban runoff pollution in the Ikpoba River region. The study provides crucial data to inform policymakers, environmental managers, and local communities, supporting evidence-based actions to mitigate pollution and protect the river's ecological integrity and dependent populations.*

**KEYWORDS**: *Urban runoff, Heavy metals, Ikpoba River, Bioaccumulation, Histopathology*

## INTRODUCTION
Water is essential for human survival, fulfilling various roles including sustenance, maintaining hydration, and nutrient transportation, and is a subject of scientific research (Cunningham and Moore, 2019; Ma, 2019; Magdalena *et al.*, 2023). However, human activities have led to significant water pollution and chemical contamination, adversely affecting aquatic ecosystems and human health (Liu *et al.*, 2023). Urban runoff, a significant source of pollution, poses a severe threat to river systems because of its role in transporting various contaminants (Zanoletti and Bontempi, 2023). Studies have highlighted that urban runoff, comprising stormwater and snowmelt, carries pollutants such as heavy metals, toxic chemicals, microplastics, and pathogens, contributing to the degradation of surface water bodies (Tasseron *et al.*, 2023; Xu *et al.*, 2019; Tang, 2022; Wang *et al.*, 2024). This study focuses on the Ikpoba River in Benin City, Nigeria, a fourth-order stream with significant ecological and economic

importance that faces severe environmental challenges such as pollution, erosion, and sedimentation (Tawari-Fufeyin and Ekaye, 2007; Okonofua *et al.*, 2019; Egun and Oboh, 2022).

Urban runoff carries a myriad of pollutants, including sediments, nutrients, and organic matter, into rivers (Wen *et al.*, 2023; Zanoletti and Bontempi, 2023). Pollutants from various sources, including industrial effluents, contribute to the degradation of aquatic habitats (Ogbeide and Okoduwa 2024). The impact on benthic and pelagic fish fauna is profound, potentially altering their diversity and abundance (Hsieh *et al.*, 2023; Haseeb *et al.*, 2022). One major concern is the presence of heavy metals, which pose significant risks to both aquatic life and human health through bioaccumulation and biomagnification in the food web (Sharma *et al.*, 2023; Soliman *et al.*, 2022).

Despite the known effects of urban runoff on water quality and aquatic life, there is a notable gap in our understanding of the specific levels of metal contamination in the fish populations of the Ikpoba River, and the associated human health risks (Agashua *et al.*, 2023). Previous research has highlighted the general impacts of urban runoff; however, detailed studies focusing on the Ikpoba River and the potential health implications for local communities consuming contaminated fish are lacking (Obasohan *et al.*, 2007; Enuneku and Ineh, 2019).

This study aimed to assess the impact of urban runoff on aquatic ecosystems in the Ikpoba River, investigate the health and diversity of pelagic and benthic fish fauna, determine the levels of metal contamination in fish species, use advanced analytical techniques to measure metal concentrations in fish tissues, ensure accurate data collection, and provide a robust framework for assessing the risks associated with metal contamination. The findings of this study can inform policymakers and environmental managers, guide efforts to mitigate urban runoff, and protect aquatic ecosystems and public health. These results will contribute to sustainable urban planning, effective stormwater management, and conservation strategies, thereby promoting the health and well-being of local communities and ecosystems.

## METHODOLOGY

### *Study Area*

This research was conducted in Benin City, Edo State, Nigeria, located between latitudes 6º11′ and 6º29′ N and longitudes 5º33′ and 5º47′ E, with an average elevation of 77.8 meters (Victor and Ogbeibu, 1985). The Ikpoba River, a key natural feature in the area, flows through the Benin-Owena basin and the Egor and Ikpoba-Okha local government areas. It supports fishing, waste disposal, recreational boating, and commercial wastewater discharge (Chukwuka and Ogbeide, 2021). These activities impact the river's water quality and ecosystem health with waste disposal and wastewater discharge posing pollution risks (Tawari-Fufeyin and Ekaye, 2007; Victor and Ogbeibu, 1985).

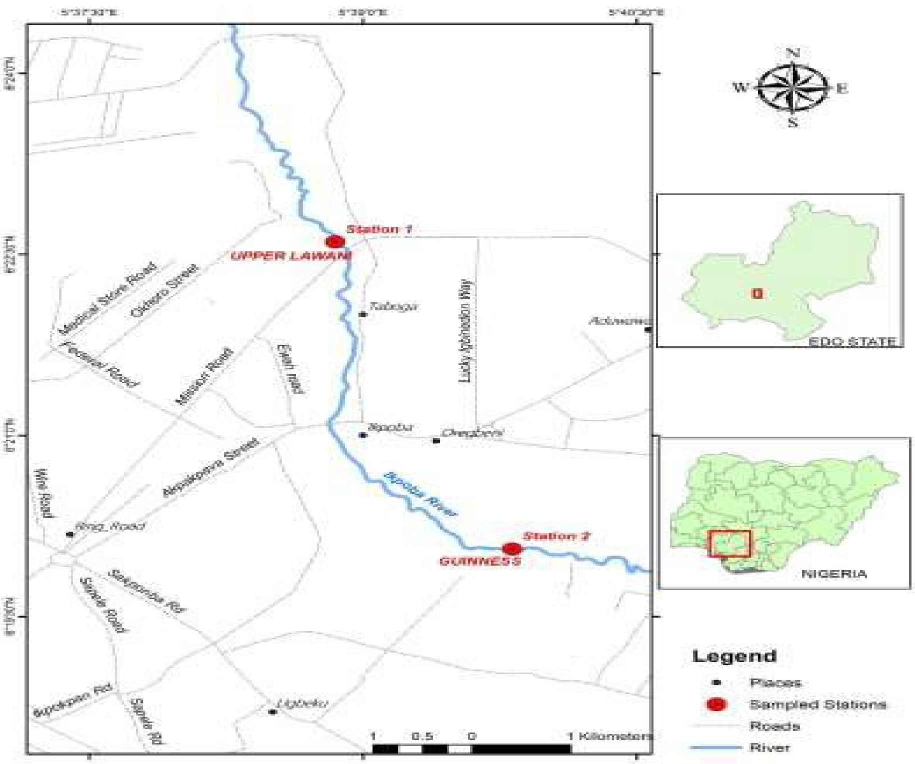

Fig. 1: Map showing the study area and sample locations

***Sampling and Analysis Procedures***

Two sites along the Ikpoba River were selected for comparative analysis. The upstream site, relatively uncontaminated and surrounded by dense vegetation, serves as the baseline reference (Schliemann et al., 2021; Arimoro, 2009). The second site, near the brewery's effluent discharge, shows significant human activity and industrial pollution (Burdon et al., 2019; Arimoro, 2009). Water samples were collected monthly between June and August 2023 from both benthic and pelagic zones, following Onyidoh *et al*. (2017). Sediment samples were collected using grab sampling, and fish were captured with nets, as outlined by Chukwuka et al. (2019). Fish were cleaned and stored in polyethylene bags at -10°C for identification and preservation. Heavy metal concentrations in water, sediment, and fish samples were determined using methods by Davies and Ekperusi (2021). Metals (Cr, Co, Cd, Ni, Pb) were analyzed with a Solar 969 Unicam Series Atomic Absorption Spectrophotometer (AAS), following Ogbeide and Okoduwa (2024). Each sample was analyzed in triplicate, with concentrations calculated using standard calibration plots.

***Extraction and Analysis of Heavy Metals***

Heavy metals in water samples were extracted following the methodologies outlined by Basanta and Ebrahim (2023) and Rana *et al*. (2021). Sediment samples were processed according to the procedures described by Lestari *et al.* (2022). Fish samples underwent processing as per the method outlined by Alhassan *et al*. (2022), followed by dilution to volume with double-distilled water in a 500 mL flask prior to analysis. Heavy metal concentrations in all samples (water, sediment, fish) were determined using an Atomic Absorption Spectrophotometer (AAS) Solar 969 Unicam Series model, utilizing the methodology detailed by Chukwuka *et al*. (2019).

***Histopathological Analysis***

The muscle tissue samples were fixed in 10% formalin to prevent decomposition (Yildirim *et al.*, 2006). The samples were then subjected to a sequential dehydration process involving graded alcohol concentrations (50, 70, 80, and 100 %), with each stage lasting 90 min (Akosman *et al.*, 2022). After dehydration, the tissues were cleared with pure xylene to eliminate residual alcohol. This was followed by a two-hour impregnation with molten paraffin wax to prepare the tissues for embedding. Once embedded, the tissues were thinly sectioned using a microtome and stained with hematoxylin and eosin (Yildirim *et al*., 2006). Finally, histological features, including pathological changes in the muscles, were examined and documented using a microscope equipped with a digital camera connected to a computer.

***Data Analysis***

The collected data were analyzed using the SPSS software version 21. Results are presented as mean ± standard deviation (S.D.) in the summary tables. One-way ANOVA followed by Duncan's Multiple Range (DMR) test was used to analyze and compare measurements across the different sampling sites.

## Results and Discussion

***Heavy Metals in Water from Ikpoba River***

The table 1 shows metal concentrations in Ikpoba River water samples across three months. In May, Cobalt (Co) measured 0.018 mg/L, Chromium (Cr) 0.038 mg/L, and Nickel (Ni) 0.043 mg/L; Lead (Pb) and Cadmium (Cd) were not detected. June saw Pb rise to 0.038 mg/L and Ni drop to 0.027 mg/L, with no Co or Cd. July recorded Cr at 0.045 mg/L and Pb at 0.033 mg/L, with no Co or Cd detected. These findings reveal fluctuations in metal levels over time in the Ikpoba River.

Table 1: Concentration of Heavy metals in water samples from Ikpoba River

| Water | May | June | July |
|---|---|---|---|
| Pb | 0 ± 0 | 0.038 ± 0.004* | 0.033 ± 0.003* |
| Co | 0.018 ± 0.002* | 0 ± 0 | 0 ± 0 |
| Cr | 0.038 ± 0.003 | 0.036 ± 0.001 | 0.045 ± 0.002* |
| Ca | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| Ni | 0.043 ± 0.001* | 0.027 ± 0.002* | 0.036 ± 0.002* |

*Indicates statistical significance ($P < 0.05$)

Surface water rivers are increasingly contaminated with heavy metals, posing significant risks to aquatic ecosystems and human health (Ali *et al*., 2022; Afzaal *et al*., 2022). Metals like Pb, Cr, and Ni in the Ikpoba River adversely affect fish growth, development, feeding, breeding, and behaviour (Sharma *et al*., 2024; Minaopunye *et al*., 2023). These metals can also impair water quality, hydrogen ion concentrations, dissolved oxygen levels, and turbidity, negatively impacting aquatic biota such as plankton, fish, and benthos (Yunusa *et al*., 2023; Aziz *et al*., 2023). Bioaccumulation of heavy metals in food chains raises significant health concerns when contaminated aquatic organisms are consumed (Jadaa and Mohammed, 2023; Haroon *et al*., 2022).

Studies have shown that the Ikpoba River in Benin City, Nigeria, is polluted by industrial effluents containing high levels of metals like Pb, Cu, Cr, Mn, Fe, Zn, and Ni (Enuneku and Ineh, 2020; Olele *et al*., 2013; Ojeah and Oriakhi, 2022; Igboanugo *et al*., 2013). Effluents from anthropogenic and industrial activities contribute significantly to heavy metal levels in Nigeria's freshwater, often exceeding permissible limits (Abalaka, 2015). Globally, heavy metals like Fe, Pb, Cr, and Hg in surface water frequently exceed permissible limits, posing ecosystem and health risks (Kumar *et al*., 2023). In Greece, heavy metal contamination in surface waters necessitates further monitoring and management (Karaouzas *et al*., 2021). Similarly, the Upper Yamuna River Basin's metal concentrations exceed acceptable limits, posing health risks (Jaiswal *et al*., 2022). Urban rivers near industrial areas in Bangladesh show significant heavy metal toxicity, impacting human health (Proshad *et al*., 2021). Studies in other regions, such as a highly industrialized river in Vietnam (Hoang *et al*., 2020) and the Bay of Bengal coast (Islam *et al*., 2021), highlight similar concerns. Agricultural runoff in Andhra Pradesh, India, also causes heavy metal contamination, emphasizing the need for monitoring and proactive measures (Pericherla and Vara, 2024). In Nigeria, concentrations of Cd, Cr, Mn, Ni, and Pb in surface freshwater often exceed recommended levels, posing ecological and public health risks (Bawa-Allah, 2023).

***Heavy Metals in Sediment Samples from Ikpoba River***

The table shows the concentrations of various metals in the sediment samples from the Ikpoba River over three months. In May, the highest concentrations were observed for Ni (7.007 mg/kg), followed by Cr (2.362 mg/kg), Co (1.892 mg/kg), Pb (0.418 mg/kg), and Cd (0.197 mg/kg). In June, Pb levels increased significantly to 3.316 mg/kg, whereas Ni levels decreased drastically to 1.312 mg/kg. By July, Pb levels had further increased to 5.517 mg/kg, becoming the metal with the highest concentration. These findings highlight the significant temporal fluctuations in metal concentrations in sediments of the Ikpoba River.

Table 2; Concentration of Heavy metals in sediment samples from Ikpoba River

| Sediment | May | June | July |
|---|---|---|---|
| Pb | 0.418 | 3.316* | 3.316* |
| Co | 1.892* | 0.314 | 0.314 |
| Cr | 2.362 | 1.962 | 1.962 |
| Ca | 0.197 | 0.144 | 0.144 |
| Ni | 7.007* | 1.312 | 1.312 |

*Indicates statistical significance ($P < 0.05$)

Heavy metals in Ikpoba River sediments fluctuated significantly over three months, with Ni peaking in May and Pb rising highest by July. These metals can bioaccumulate in fish, posing health risks (Bhuyan *et al*., 2023; Islam *et al*., 2023;

Jumaat and Hamid, 2023). Sediment metal concentrations vary due to human activities and can affect aquatic organisms (Yang *et al*., 2023; Shorna *et al*., 2021; Lim *et al*., 2021; Sharma *et al*., 2024).

Studies show sediments often have high heavy metal levels from industrial discharge, mining, and agriculture (Lan *et al*., 2019; Tsai *et al*., 2003). In the Ikpoba River, Fe and Mn increased over time, but Cd, Pb, and Cu did not (Imiuwa *et al*., 2014). Effluents had higher metal concentrations than water (Oguzie and Okhagbuzo, 2010; Oguzie, 2006). Mean metal concentrations in sediments showed pollution in the order of Fe > Cu > Ni > Pb > Cd, with non-carcinogenic risks below thresholds (Enuneku and Ineh, 2020).

Globally, studies reviewed heavy metal trends in river sediments from 1970 to 2018, identifying metals like Cd, Cu, Ni, Pb, and Zn in various locations (Niu *et al*., 2021). Heavy metal pollution in marine sediments is a global issue, with high concentrations from anthropogenic sources in areas like southwest Mallorca Island, Spain (Romano *et al*., 2021; Robledo Ardila., 2024). Similar findings were reported in Belawan Harbor (Sulistyowati *et al*., 2023) and the Cisadane River, Indonesia, where Pb levels exceeded quality standards (Riani *et al*., 2014; Riani *et al*., 2018).

***Heavy Metals in Fish Samples from Ikpoba River***

The table presents the concentrations of various metals in fish samples from the Ikpoba River over three months. In May, *Clarias gariepinus* showed the highest concentration of Nickel (Ni) at 0.94 mg/kg, while *Tilapia zilli* had a notable Lead (Pb) concentration of 0.18 mg/kg. In June, *C. gariepinus* had a significant increase in Pb levels to 0.70 mg/kg, while all metal concentrations in *T. zilli* dropped to 0.01 mg/kg. By July, *T. zilli* showed a rise in Pb levels to 0.58 mg/kg, while metal concentrations in *C. gariepinus* decreased significantly.

Table 3; Concentration of Heavy metals in fish samples from Ikpoba River

| Month | Fish Species | Pb | Co | Cr | Cd | Ni |
|---|---|---|---|---|---|---|
| May | *C. gariepinus* | 0.06 | 0.06 | 0.50* | 0.05 | 0.94* |
| | *T. zilli* | 0.18* | 0.06 | 0.35* | 0.04 | 0.76* |
| June | *C. gariepinus* | 0.70* | 0.06 | 0.48* | 0.02 | 0.07 |
| | *T. zilli* | 0.11 | 0.01 | 0.01 | 0.01 | 0.01 |
| July | *C. gariepinus* | 0.02 | 0.00 | 0.17 | 0.00 | 0.01 |
| | *T. zilli* | 0.58* | 0.04 | 0.18 | 0.02 | 0.07 |

*Indicates statistical significance ($P < 0.05$).

Heavy metal concentrations in Ikpoba River fish fluctuated over three months, with *C. gariepinus* showing high Ni (0.94 mg/kg) in May, and *T. zilli* showing notable Pb (0.18 mg/kg). Heavy metals harm fish growth, reproduction, and respiration (Sharma *et al*., 2024; Javed and Usmani, 2019; Chen *et al*., 2023). Pb and Ni affect fish behaviour, feeding, and breeding (Areguamen *et al*., 2023; Gulati *et al*., 2022; Minaopunye *et al*., 2023). Bioaccumulation impacts fish health and physiology (Minaopunye *et al*., 2023; Chen *et al*., 2023). Differences in metal concentrations between *T. zilli* and *C. gariepinus* may be due to their biological characteristics (Saliu *et al*., 2015; Minaopunye *et al*., 2023; Martínez-Durazo *et al*., 2023). Factors like sex, age, and developmental stage influence bioaccumulation in fish (Al Mustawa *et al*., 2023; Sulato *et al*., 2022).

In Nigeria, heavy metals like Fe, Zn, Pb, Ni, Cr, Cu, and Cd have been found in fish from various water bodies (Aghoghovwia *et al*., 2016; Abalaka *et al*., 2020; Ekere *et al*., 2018; Ihunwo *et al*., 2020; Ezemonye *et al*., 2019). Studies on Ikpoba River fish reported heavy metals in organs and tissues, indicating environmental and health risks (Odigie *et al*., 2016; Obasohan *et al*., 2008; Oguzie, 2003; Osa-Iguehide *et al*., 2016). Globally, heavy metal contamination in fish is a widespread concern (Eneji *et al*., 2011; Ahmed *et al*., 2024; Habib *et al*., 2024; Jovanović *et al*., 2017). These studies emphasize the environmental and public health implications of bioaccumulation in aquatic ecosystems.

***Histopathology of Fish Samples from Ikpoba River***

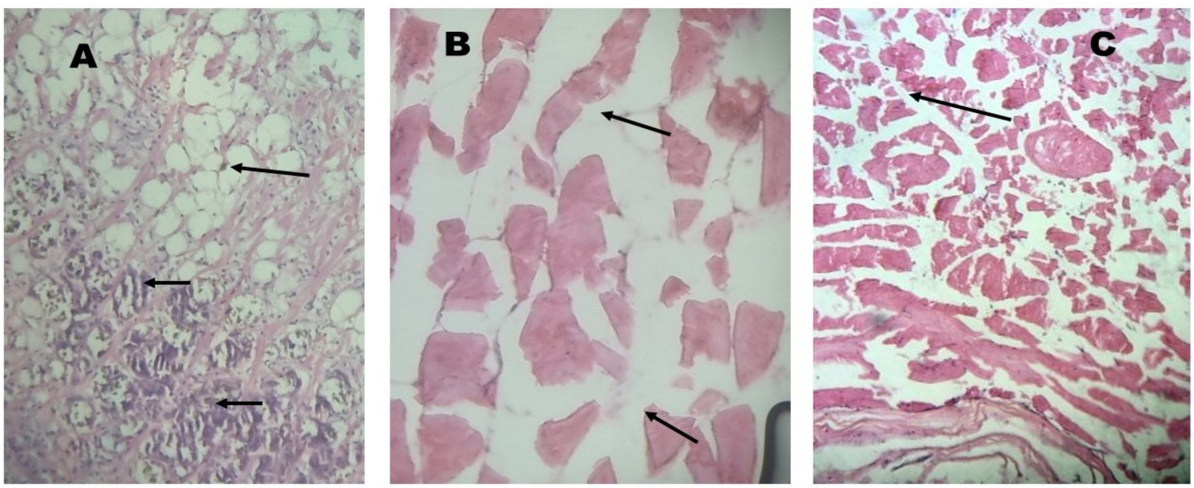


Fig. 2: Histopathology of muscle samples for *C. gariepinus* in the Ikpoba River for A) May, B) June, and C) July

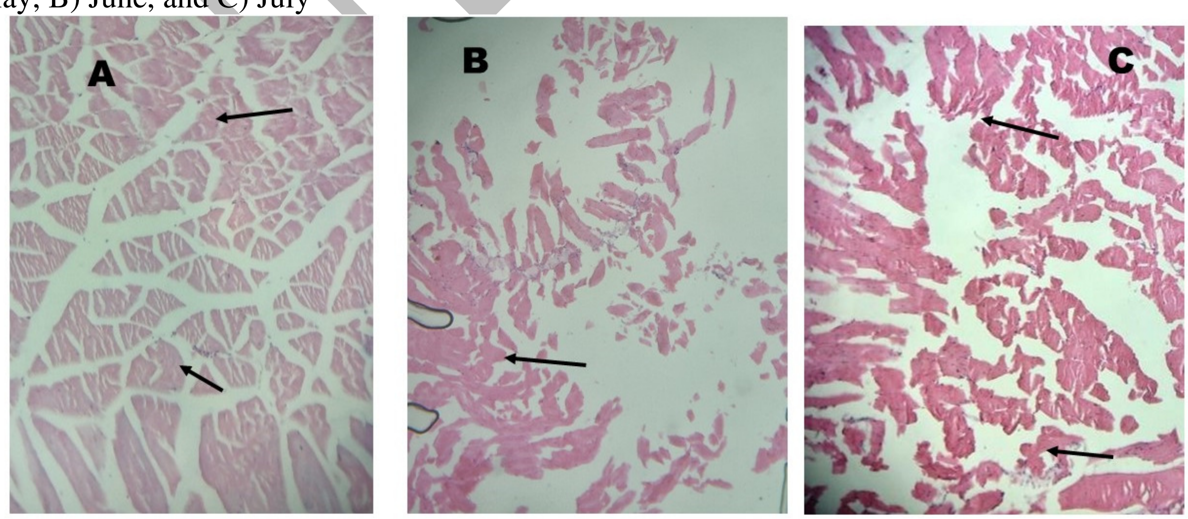


Fig. 3: Histopathology of muscle samples for *T. zili in the* Ikpoba River for A) May, B) June, and C) July

Fig. 2 (A-C) shows histopathological muscle samples of *Clarias gariepinus* from the Ikpoba River in May, June, and July. In May (Fig. 2a), muscle fibres appeared healthy and tightly packed (Nwakanma *et al*., 2020). By June (Fig. 2b), muscle fibres showed disruption and gaps, indicating damage. In July (Fig. 2c), further deterioration and loose fibres were observed, suggesting progressive degeneration. These changes align with damage due to parasitic infections and heavy metal exposure, highlighting the need for monitoring pollutants (Okunade *et al*., 2023). Similar studies in other fish species have shown muscle damage manifesting as disrupted fibres, intense staining, and gaps, affecting muscle quality and leading to issues like gaping fillets (Strateva and Penchev, 2021; Khalil *et al*., 2010; Girija and Balakrishnan, 1988; Tie *et al*., 2022; Ofstad *et al*., 2006).

Heavy metals bioaccumulate in fish, causing health problems and damaging physiological processes, development, and reproduction (Sharma *et al*., 2024; Jatav *et al*., 2023). In *C. gariepinus*, heavy metal accumulation leads to histopathological changes (Nzeve *et al*., 2014; Abalaka *et al*., 2020; El-Hak *et al*., 2022; Arojojoye *et al*., 2018; Obinna *et al*., 2021). For instance, a study in Nigeria found significant bioaccumulation linked to oxidative stress and tissue alterations (El-Hak *et al*., 2022). Heavy metal levels in *C. gariepinus* from the Niger Delta exceeded permissible limits, affecting fish health (Ehiemere *et al*., 2022).

Fig. 3 (A-C) shows T. zilli muscle samples from May, June, and July. In May (Fig. 3a), fibres were organized and less disrupted compared to June (Fig. 3b) and July (Fig. 3c), which showed degradation and gaps. This suggests progressive tissue damage likely due to heavy metal accumulation, impacting fish mobility, feeding, growth, and survival (Wu *et al*., 2023; Kaba *et al*., 2023; Lo Tamang *et al*., 2023). Studies indicate heavy metals accumulate in *T. zilli* organs, causing tissue damage and health risks to the fish and consumers (ShaAto et al., 2011; Habib *et al*., 2014; Elsharkasy *et al*., 2023).

## Conclusion

Our study on the impact of urban runoff on the Ikpoba River in Benin City, Nigeria revealed significant environmental challenges. Heavy metals, such as Pb, Cr, Ni, Co, and Cd, are prevalent in river water, sediments, and fish tissues, indicating ongoing pollution from industrial and urban sources. The accumulation of these metals in fish muscle poses health risks to local communities, with histopathological analysis confirming structural damage and degeneration. Effective stormwater management, regulation of industrial discharge, and sustainable urban development are vital for mitigating these impacts and preserving the Ikpoba River Ecosystem. Our findings can serve as a crucial resource for policymakers, environmental managers, and communities to guide evidence-based decisions to protect both rivers and their inhabitants.

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