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EFFECT OF HEAVY METAL RUNOFF FROM IKPOBA RIVER ON THE GONAD AND ANTIOXIDANT PROPERTIES OF AFRICAN SNAIL (*Bulinus africanus*)

[*1]OGBEIDE, O. & [1]AMAYANVBO, O. C.

[1]Department of Environmental Management & Toxicology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

[*]***Corresponding Author's Email**: ozekeke.ogbeide@uniben.edu; **Tel:** 08034626868*

ABSTRACT

This study investigates the effects of urban runoff on the reproductive health and antioxidant enzyme activity in the freshwater snail *Bulinus africanus* from the Ikpoba River in Nigeria. Samples of water and sediment were collected and tested for heavy metal content, while snails were gathered for histopathology and testing of antioxidant enzyme activities. The analysis of water and sediment samples indicated no significant changes in heavy metal concentrations. However, significant variations were observed in the concentrations of heavy metals such as Cd, Co, Cr, Ni, and Pb in the muscles and gonads of the snails, The antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), exhibited notable fluctuations, with the highest increase detected in the activity of reduced glutathione (Red GSH). Histological examination of the snail gonads revealed basophilic hyperchromatic cellularity of germinal vesicles, as well as demarcated membranes with reduced cellularity of germinal vesicles and yolk granules. These findings suggest a high risk of reproductive anomalies in snails from the Ikpoba River, potentially impacting the broader ecosystem. This study underscores the need for monitoring and mitigating urban runoff to protect aquatic life and ecosystem health.

Keywords: *Antioxidant Enzymes, Bulinus africanus, Ikpoba River, Heavy Metals, Reproductive Anomalies, Urban Runoff*

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INTRODUCTION

Urbanization, a worldwide trend, has both detrimental and beneficial effects on the environment. Although it is essential for human development, it often causes environmental issues (Walsh *et al.,* 2012; Faure *et al.,* 2023). Urbanization can result in various environmental problems, such as pollution, habitat fragmentation, higher temperatures, and modifications in soil properties (Walsh *et al.,* 2012). Urban runoff, specifically, can significantly impact aquatic ecosystems. This is particularly true for species like *B. africanus*, a freshwater snail found in the Ikpoba River (Ogbeibu and Oribhabor, 2002; Ibezute *et al.,* 2016).

Urban runoff, containing diverse pollutants, can induce oxidative stress in aquatic organisms, leading to significant ecological consequences (Venditti *et al.,* 2022). Contaminants in urban stormwater runoff, including pesticides, nitrogen-containing compounds, heavy metals, and organic micropollutants, can trigger oxidative stress responses in aquatic organisms, affecting their health and survival (Gan *et al.,* 2012; Wei *et al.,* 2013). Pollutants have the potential to interfere with the antioxidant defense mechanisms of aquatic organisms, leading to physiological and biochemical imbalances that can result in diseases and have negative impacts on ecosystems (Anetor *et al.,* 2022).
This stress can result in gonadal pathology and changes in the activity of antioxidant enzymes (Nimse and Pal, 2015). It is essential to understand these effects in order to comprehend the ecological consequences of urbanization (Bulti and Abebe, 2020). While the general stress response in aquatic organisms due to pollutants has been well-documented (Winston and Di Giulio, 1991; Zhou *et al.,* 2008; Malik *et al.,* 2020; Kataoka and Kashiwada, 2021; Michan *et al.,* 2021), the specific effects on the gonadal health and antioxidant enzyme systems in *B. africanus*, are not yet fully understood.
Previous research has demonstrated that exposure to certain pollutants can lead to increased activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in other species (Aitken and Roman, 2008; Rodrigues *et al.,* 2021; Venditti *et al.,* 2022 Ibrahim *et al.,* 2023). Studies have shown that heavy metals can induce oxidative stress in freshwater snails, leading to enhanced activities of antioxidant enzymes as a defensive mechanism (Basopo and Ngabaza, 2015; Basopo and Ngabaza, 2021) However, these studies focused on different snail species and their responses to various pollutants. In contrast, the specific effects of urban runoff on the gonadal health and antioxidant enzyme activity in *B. africanus* remain largely unexplored.

Therefore, this study aims to elucidate the effects of urban runoff on the gonad pathology and antioxidant enzyme activity in *B. africanus* from the Ikpoba River. This research could provide valuable insights into the sub-lethal impacts of environmental stressors on a key species within freshwater ecosystems. Furthermore, it could contribute to the broader understanding of urban runoff's ecological implications, thereby informing conservation efforts and urban planning strategies.

METHODOLOGY

Study Area

The Ikpoba River flows in a southwesterly direction and eventually joins the Ossiomo River (Okonofua *et al.,* 2019). It originates from the Ishan Plateau and is flanked by the muddy Ikpoba slope. To assess potential contaminants, two sampling stations were chosen: Station 1 upstream and Station 2 downstream, located near a significant brewery.

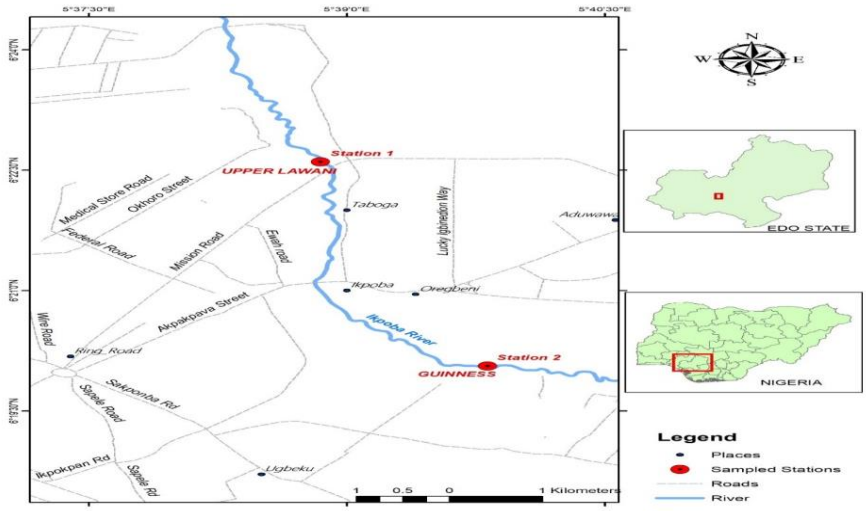

Figure 1: Map of the study area showing the sampling locations

Sample Collection

Water samples

From June to August 2023, water samples were taken from various locations along the Ikpoba River using sterile 1-liter containers, following the method described by Eric *et al.* (2017). The containers were cleaned, sealed, labeled, and transported to the laboratory on ice as per Chukwuka and Ogbeide (2021).

Sediment samples

Sediment samples were collected using a sediment grab and placed in labeled polyethylene bags that had been pre-treated with 5% nitric acid and rinsed with distilled water (Chukwuka and Ogbeide, 2021). The samples were air-dried in the laboratory for about before analysis.

Snail samples

B. africanus snails were collected, identified by the department of Animal and Environmental Biology, University of Benin (UNIBEN) experts, and cleaned. Their shells were broken to expose organs (Ibrahim *et al.,* 2023) Muscles and Reproductive organs were removed, preserved in 10% formalin, and stored at -20°C for future analysis (El-Khayat *et al.,* 2018).

Analysis

Heavy Metal Analysis

Heavy metals were detected using the methods described by Davies and Ekperusi (2021); Osioma and Iniaghe (2019). For water samples, a crucible was filled with 25 ml of the sample, 1 ml of concentrated HNO3, and 3 ml of concentrated HCl. This mixture was allowed to cool after being heated for 30 mins. The digest was then diluted with distilled water to a volume of 50 ml for storage.

For sediment samples, 10 g of ground and sieved sediment was mixed with 25 ml of water in a porcelain crucible.

Subsequently, 1 ml of conc HNO3, and 3 ml of conc HCl, was added to this mixture, then heated, cooled, and filtered. Afterwards, the digested sample was filtered and adjusted to 50 ml with distilled water. The filtrate was preserved in plastic containers for subsequent heavy metal analysis, maintaining uniform treatment procedures for all sediment samples.

Snail samples were digested with 1 ml of conc HNO3 and 3 ml of conc HCl until clear, then diluted to adjusted to 50 ml with double-distilled water. Heavy metal analysis was conducted using an AAS Solar 969 Unicam Series model. Each metal (Cr, Co, Cd, Ni, Pb) was examined using a specific hollow cathode lamp. Samples underwent three rounds of analysis, and concentrations were determined using standard calibration plots (El-Khayat *et al.*, 2018).

Histopathology Analysis

Histopathology analysis of *B. africanus* snail gonads utilized various reagents and materials, including 10% natural buffered formalin, paraffin wax, xylene, ethanol, hematoxylin, and eosin (Ünlü *et al.*, 2005; El-Khayat *et al.*, 2018), along with equipment like a microtome, cryostat, embedding station, tissue processor, staining station, microscope, incubators, and a tissue flotation bath. Muscles and Gonads were preserved in 10% formalin to prevent decomposition, dehydrated in alcohol, cleaned with xylene, and impregnated in liquid wax (Ünlü *et al.*, 2005; El-Khayat *et al.*, 2018). Impregnated samples were then segmented using a microtome and stained with Haematoxylin-Eosin (Bighiu *et al.*, 2017). After removing excess stain, slides were covered and placed in an oven at 40°C. Histological structures and any histopathological changes were observed, documented, and interpreted under a microscope, aided by a digital camera connected to a computer.

Biochemical Analysis

Determination of Catalase (CAT)

The methods of Sinha (1972); Greenwld (1985); Kadhum and Hadwan (2021) were used to determine the catalase (CAT) activity. A mixture of 0.5 ml of plasma and 5.0 ml of H_2O_2 was added, and the mixture was allowed for 30 minutes. The reaction was stopped by adding 1.5 ml of 6M H_2SO_4 and 7 ml of 0.01 M $KMnO_4$, mixed, and left for 10 minutes. Absorbance was read at 480 nm within 30-60 seconds against distilled water. An enzyme blank was run simultaneously with 1.0 ml of distilled water instead of H_2O_2. Enzyme activity was expressed as µmoles of H_2O_2 decomposed per minute per milligram of protein, calculated using the formula in equation 1.

$$Activity = \frac{\text{OD}}{\text{min}} \text{x Vx M x V x L x Y} \ldots\ldots\ldots \quad 1$$

Estimation of Superoxide Dismutase Activity (SOD) Activity

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of adrenaline autoxidation at pH 10.2, following the method by Sun *et al.* (1988) and Campos-Shimada *et al.* (2020)). Carbonate buffer (0.05 M) was prepared and adjusted to pH 10.2 using Sodium hydroxide. Plasma volume of 100 ml was mixed with 125 ml of carbonate buffer and 150 ml of adrenaline solution. Distilled water mixed with carbonate buffer served as a reference. Absorbance was read at 420 nm. Enzyme concentration was calculated as units/mg protein as indicated in in equation 2.

$$protein = \frac{\%\ inhibition}{50\ X\ Y} \ldots\ldots\ldots\ldots\ 2$$

Glutathione Peroxidase (GPx) Analysis

The activity of Glutathione peroxidase (GPx) in *B. africanus* samples was determined following the method by Flohé and Günzler (1984) and Radwan *et al.* (2010). A 0.2 ml of plasma was mixed with phosphate buffer, H_2O_2, distilled water, and pyrogallol. The reaction mixture was incubated for 30 minutes at room temperature. Absorbance was measured at 480 nm. The activity of GPx was calculated using the formula (Equation 3).

$$Activity = \frac{\text{OD/min x vt x Df}}{\text{E x Vs x Y}} \quad \ldots\ldots\ 3$$

Determination of Malondialdehyde (MDA)

Using the thiobarbituric acid assay, which was detailed by Draper and Hadley (1990); Buege and Aust (1978), malondialdehyde (MDA) levels were discovered. After adding 1.0 milliliter of the sample to a TCA-TBA-HCL (thiobarbituric acid-trichloroacetic acid-hydrochloric acid) solution, the mixture was heated in a boiling water bath for 15 minutes. After cooling, the solution was centrifuged, and absorbance was determined using the formula (Equation 4).

$$\text{MDA (mol/mg protein)} = \frac{\text{A x V x 100}}{\text{M x V x Y}} \quad \ldots\ldots\ 4$$

Data Analysis

The collected data was statistically processed using SPSS software, version 21. The processed data was represented in summary tables as Mean ± S.E. To evaluate the measurements within each sampling site, analytical methods such as One-way ANOVA and Duncan Multiple Range (DMR) test were used.

RESULTS AND DISCUSSION

Heavy Metals in water

The study conducted on the Ikpoba River, presented in Figure 2, focused on the concentrations of cadmium (Cd), nickel (Ni), cobalt (Co), lead (Pb), and chromium (Cr) in the water during June, July, and August. Cd and Pb were not detected in the water at either the upstream or downstream locations throughout the three months, indicating their absence in the river water. The study observed variations in the concentrations of Ni, Co, and Cr over the three months at both locations.

The absence of Cd and Pb is noteworthy due to their known harmful effects (Sall *et al.,* 2020; Singh *et al.,* 2022). Ni, Co, and Cr were detected, posing a potential threat to aquatic ecosystems (Sall *et al.,* 2020; Yunusa *et al.,* 2023). Although their concentrations remained relatively stable over three months, they still present risks. Human exposure, primarily through consuming contaminated fish, can lead to cardiovascular diseases, developmental abnormalities, and neurological disorders (Yunusa *et al.,* 2023; Hu *et al.,* 2024). Even at low levels, these metals are systemic toxicants, causing organ dysfunction and oncogenic effects (Chris *et al.,* 2023; Yunusa *et al.,* 2023). Heavy metals in water adversely affect aquatic life, often leading to toxicity and mortality (Sall *et al.,* 2020).

Many people are exposed to heavy metals through the consumption of contaminated fish, leading to various health issues (Chris *et al.,* 2023). Previous studies on the Ikpoba River have also reported the presence of heavy metals, emphasizing the need for continuous monitoring (Igboanugo *et al.,* 2013; Olele *et al.,* 2013; Wangboje and Ekundayo 2013; Imiuwa *et al.,* 2014; Osa-Iguehide *et al.,* 2016; Adegbite *et al.,* 2018; Adeleke *et al.,* 2023).

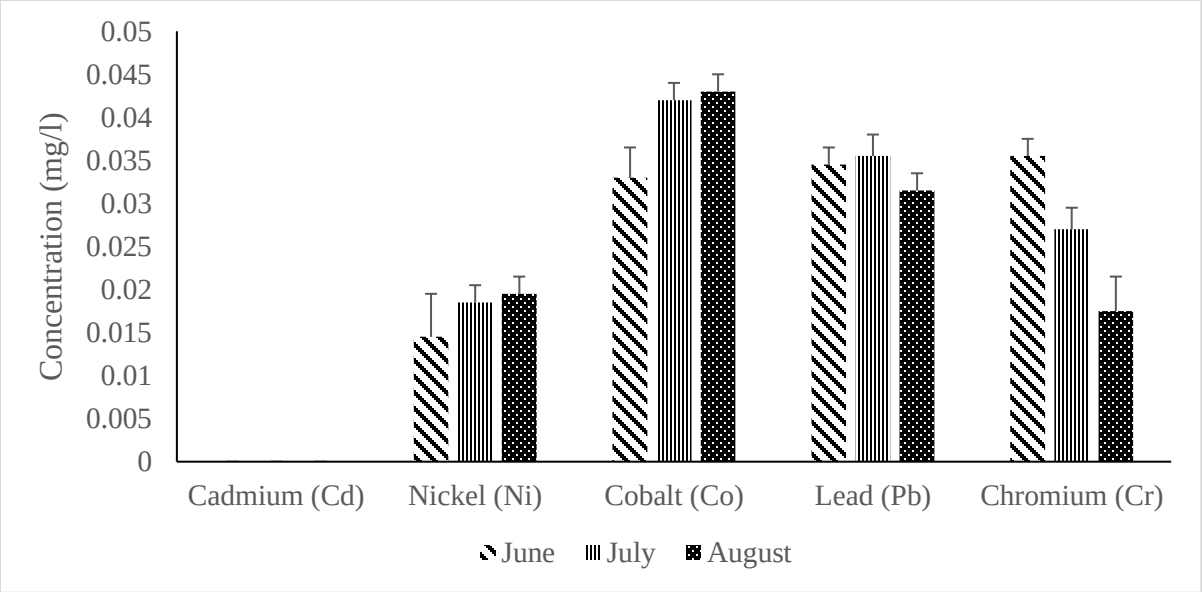

Figure 2: Heavy metal concentration in water from Ikpoba River

Heavy Metals in sediment

Figure 3 displays the concentrations of metals in the sediment of the Ikpoba River. The study spanned three months (June, July, and August) and measured concentrations at both upstream and downstream locations. Metals analyzed included cadmium (Cd), nickel (Ni), cobalt (Co), lead (Pb), and chromium (Cr). While concentrations varied over the three months at both locations, statistical analysis showed non-significant variations ($P > 0.05$).

Heavy metal contamination poses risks to ecological health, human well-being, and benthic organisms (Bhuyan *et al.,* 2023; Wojtkowska 2023; Yozukmaz and Yabanlı 2023). Previous studies have reported heavy metal presence in sediments globally (Deng *et al.,* 2009; Harikumar *et al.,* 2010; Zhu *et al.,* 2018; Singh *et al.,* 2020; Fang *et al.,* 2023; Gao *et al.,* 2023; Mosalem *et al.,* 2024). The results of this study align with research in the Ikpoba River (Enuneku and Ineh, 2020), the Olode area (Okonkwo *et al.,* 2023), and the Niger Delta region (Ehiemere *et al.,* 2022), all of which reported high Cr levels.

However, there are differences compared to previous studies. For instance, Oguzie and Okhagbuzo (2010) found Pb to be the most abundant metal in their study of the Ikpoba River, which contrasts with the higher Ni levels observed in June in this study. Additionally, Wangboje and Ekundayo (2013) reported varying levels of Cr and Pb but did not detect significant amounts of Ni or Co, which differs from the findings of this study. These differences may be due to variations in sampling locations, seasonal changes, or different analytical methods used. Despite these differences, the consistently high levels of Ni and Cr in this study indicate widespread sediment contamination, highlighting the need for comprehensive pollution management strategies to mitigate risks.

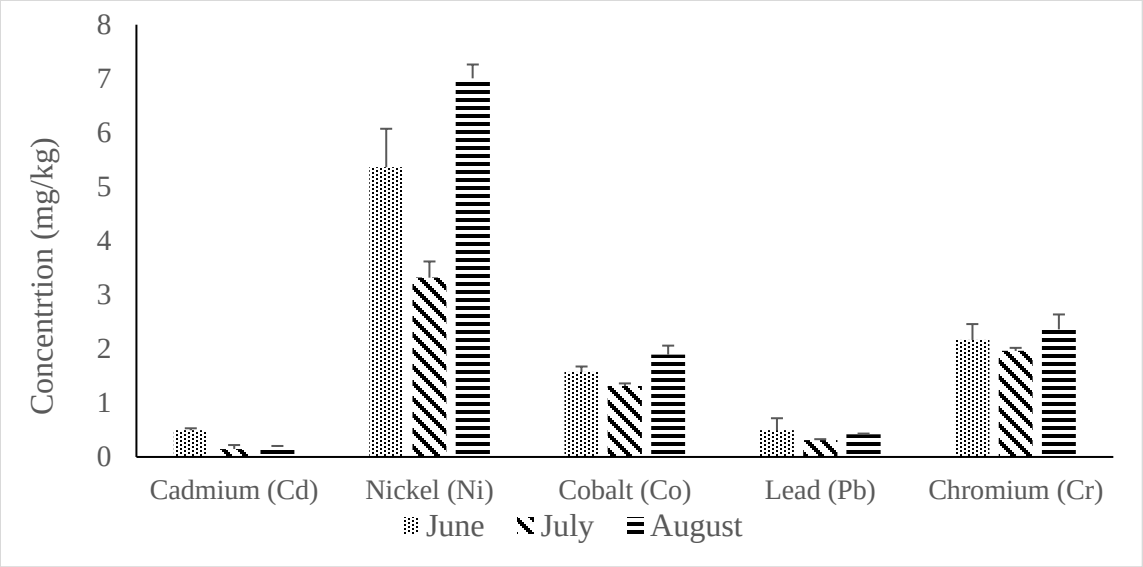

Figure 3: Heavy metal concentration in sediment from Ikpoba River

Heavy Metals in muscles and gonads of snail

The study examined heavy metal levels in the muscles and gonads of snails from the Ikpoba River over three months (June, July, and August) (Fig 4). The results showed varying levels of heavy metals in the snail muscles: Cd and Co concentrations increased, while Cr and Ni remained steady until a notable rise in August. Pb levels remained consistent until a decrease in August. Additionally, the study found a significant increase ($P < 0.05$) in heavy metal concentrations in the snail gonads from June to August. Metals included Cd, Co, Cr, and Ni, while Pb decreased slightly in August compared to June and July (Fig 5).

While the observed temporal variations suggest potential seasonal patterns, a longer study period of at least six months is necessary to draw definitive conclusions about seasonal variations in heavy metal accumulation (Burger, 2006). Urban runoff during the rainy season likely contributes to elevated heavy metal levels in the Ikpoba River, impacting aquatic organisms, including snails (Li *et al.,* 2018; Adeleke *et al.,* 2023; Iroegbulem *et al.,* 2023). Heavy metal contamination can stress aquatic life, reduce growth and reproduction, and increase mortality (Ćirić *et al.,* 2018; Jan *et al.,* 2022). It may also disrupt the aquatic food chain, risking species extinction (Ajayi and Oyewole,, 2023; Kutluyer Kocabaş *et al.,* 2023; Sharma *et al.,* 2024). Similar heavy metal accumulation has been reported in other snail species, affecting their health and behavior (Gomot, 1997; Nica *et al.,* 2012; Salih *et al.,* 2021; Ajayi and Oyewole, 2023).

These temporal variations observed in metal concentration in muscles and gonads suggest seasonal variations in heavy metal accumulation, possibly influenced by environmental changes (Tawari-Fufeyin and Egborge, 1998). Urban runoff may contribute to elevated heavy metal levels in the Ikpoba River during the rainy season, affecting aquatic organisms, including snails (Li *et al.,* 2018; Adeleke *et al.,* 2023, Iroegbulem *et al.,* 2023). Heavy metal contamination can stress aquatic life, reduce growth, reproduction, and increase mortality (Ćirić *et al.,* 2018; Jan *et al.,* 2022). Additionally, contamination may disrupt the aquatic food chain, risking species extinction (Ajayi and Oyewole, 2023; Kutluyer Kocabaş *et al.,* 2023; Sharma *et al.,* 2024). Literature reports similar heavy metal accumulation in snails, impacting their health and behavior (Gomot, 1997; Nica *et al.,* 2012; Salih *et al.,* 2021; Ajayi and Oyewole, 2023).

Previous studies on Eobania vermiculata and Helix pomatia support seasonal variations in heavy metal concentrations in snails (Tawari-Fufeyin and Egborge, 1998; Ćirić *et al.,* 2018). These findings suggest that snail species exhibit consistent responses to environmental factors, indicating that similar seasonal trends in heavy metal accumulation would likely be observed in *B. africanus* (Dhiman and Pant, 2021). This underscores the importance of snails as bioindicators for monitoring pollutants, including heavy metals.

Effluent discharge and seasonal runoffs into the Ikpoba River contribute to poor water quality and elevated heavy metal concentrations, especially near industrial sites (Enuneku and Ineh 2019; Igboanugo *et al.,* 2013). Comparing heavy metal levels in snail muscles and gonads, higher concentrations in gonads suggest potential susceptibility to reproductive organ accumulation (Duncan 2023; Kutluyer Kocabaş *et al.,* 2023). High Cu concentrations have been linked to gonad deterioration in Dosinia ponderosa (white clams), impacting reproductive health (Eraso-Ordoñez *et al.,* 2023). These findings emphasize the urgency for further research and mitigation measures to address heavy metal pollution in the Ikpoba River.

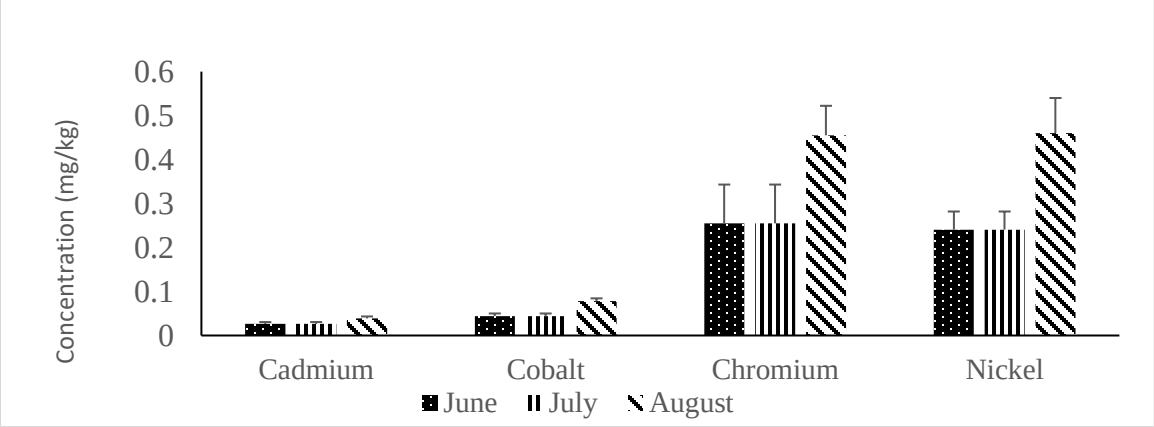

Figure 4: Heavy metal concentration in muscles of *B. africanus* from Ikpoba River

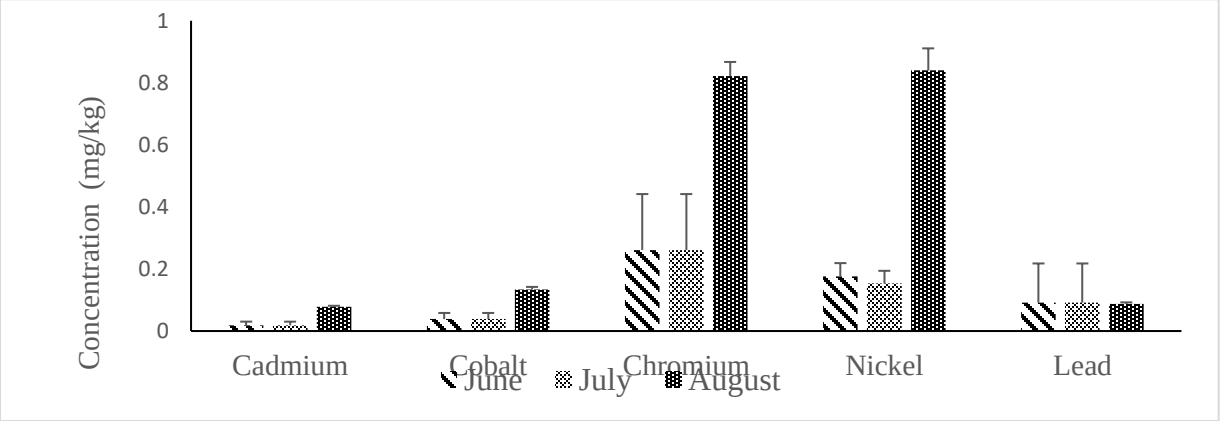

Figure 5: Heavy metal concentration in gonad of *B. africanus* from Ikpoba River

Enzyme Activity of Oxidative Stress and Antioxidants in Snails

The study compares four oxidative stress enzymes (SOD, CAT, GPx, and MDA) in snail muscles over May, June, and July (Fig 6). GPx peaks in June at 2 µg/g/protein, while SOD, CAT, and MDA show minor fluctuations, with MDA highest in May, decreasing thereafter. Figure 7 illustrates changes in oxidative stress enzymes in snail gonads

over three months. GPx surges in July, indicating increased enzyme activity, while SOD and CAT remain stable, and MDA stays consistently low.

The variations in oxidative stress enzyme concentrations in *B. africanus* muscles and gonads from the Ikpoba River (Figures 8 and 9) may result from various factors such as water quality, temperature, and food availability (Abdullahi *et al.,* 2022). The peak in GPx activity in June (muscles) and July (gonads) could indicate increased exposure to environmental pollutants, possibly due to urban runoff (Li *et al.,* 2018). These peaks might respond to heightened oxidative stress during these periods, influenced by environmental changes such as temperature fluctuations, water quality, or increased pollutant exposure (De Kock and Wolmarans, 2005). The stable concentrations of SOD and CAT suggest their consistent role in mitigating oxidative stress (Deska, 2020), while low MDA levels may indicate effective antioxidant defense mechanisms in snails (Yap *et al.,* 2023). Pollutants can disrupt the pro-oxidants and antioxidants balance, leading to oxidative stress and affecting snail reproductive and physiological functions (Adeyemi, 2014).

Elevated levels of oxidative stress enzymes like GPx in snails such as *B. africanus* can serve as indicators of heightened pollution and ecological disturbances in aquatic ecosystems (Ajayi and Oyewole, 2023). Monitoring these enzymes in *B. africanus* and other snail species is crucial for early detection and mitigation of environmental degradation in areas like the Ikpoba River (Osuala and Onadeko 2016; Basopo *et al.,* 2022; Panda *et al.,* 2022). The physiological responses of snails to pollutants, reflected in enzyme levels, can provide valuable insights into the overall health of aquatic ecosystems, potentially extending to other aquatic organisms.

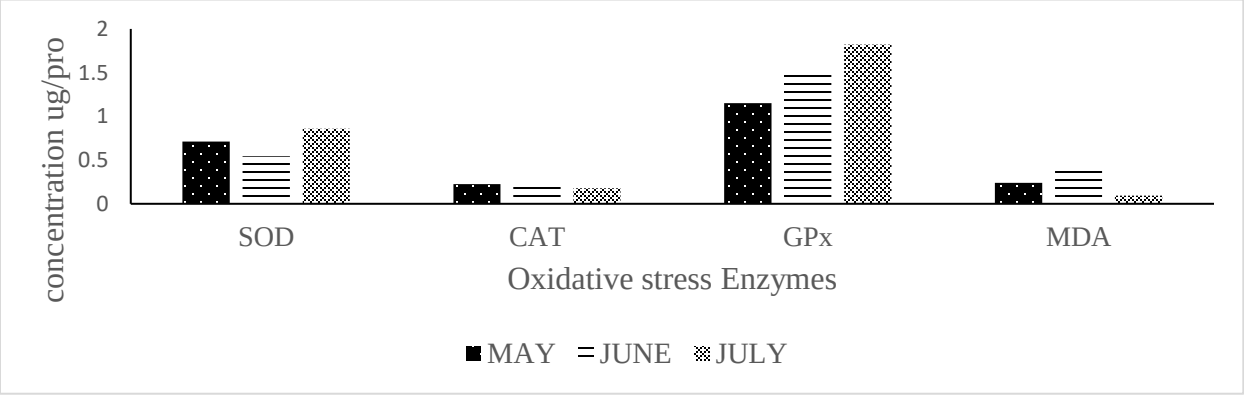

Figure 6: enzymes activity and oxidative stress in muscles of *B. africanus* from the Ikpoba River.

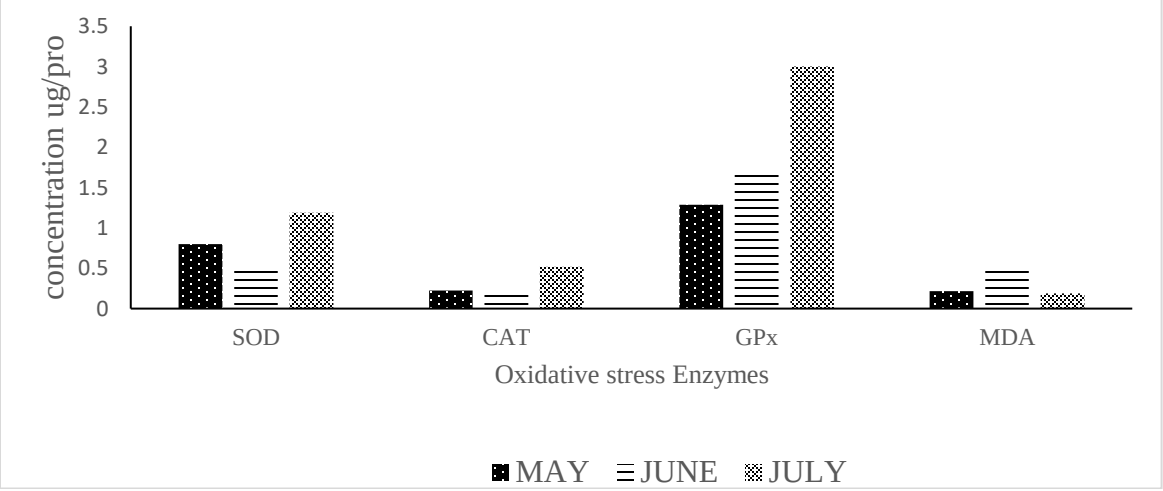

Figure 7: enzymes activity and oxidative stress in gonads of *B. africanus* from the Ikpoba River.

Histopathology assessment

The progressive degeneration in the muscle tissue of *B. africanus* from June to August, as illustrated in Figure 8, suggests a continuous pathological process induced by environmental stressors as reported by Vranković *et al*. (2020) in their study on Helix promatia. This degeneration, characterized by thickened membranes, increased fatty changes, and an increase in adipocytes, indicates the ongoing impact of pollutants on aquatic life (Li *et al.,* 2018; Ajayi and Oyewole, 2023). Snails like *B. africanus* serve as bioindicators, signaling a compromised ecosystem due to deteriorating health (Rizk *et al.,* 2014; Basopo *et al.,* 2015).

Observations in Figure 9 reveal a clear progression in the development or recovery of snail gonadal tissues from June to August (Danilova *et al.,* 2022). This progression suggests a continuous developmental or recovery process influenced by environmental factors such as heavy metal exposure and reproductive periodicity variations (Pewphong *et al.,* 2020; Salinas, 2022; Eraso-Ordoñez *et al.,* 2023; Seinor and Benkendorff, 2023). Snails exhibit seasonal cycles in their reproductive tissues, reflecting fluctuations in ecosystem health and the impact of urban runoff, which can be mitigated or exacerbated by seasonal changes (Bistransin 1976; Ajayi and Oyewole 2023; Masese *et al.,* 2023). These findings underscore the significance of using snails as bioindicators to monitor environmental changes and assess ecosystem health (Pewphong *et al.,* 2020).

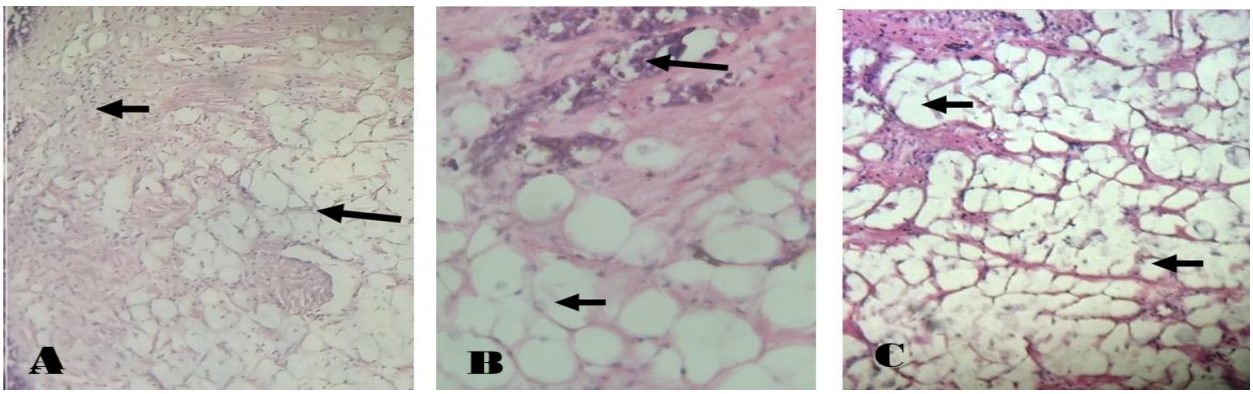

Figure 8: Histopathology of muscles of *B. africanus* from the Ikpoba River for a) June, b) July c) August.
**Muscle fiber bundles indicated by the short arrows, while the long arrows point to the surrounding adipocytes*

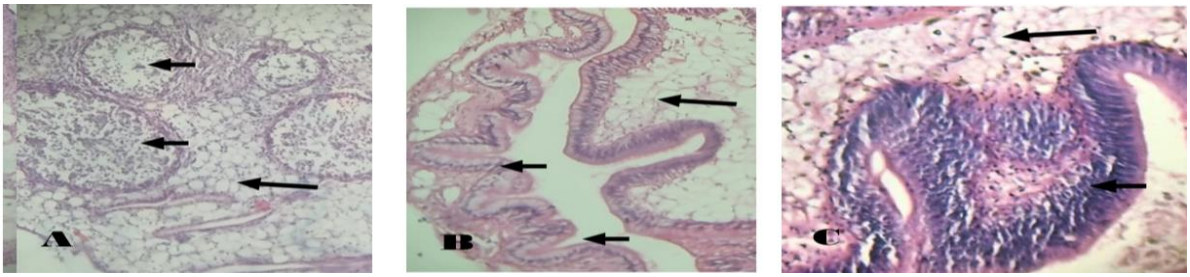

Figure 9: Histopathology of gonads of *B. africanus* from the Ikpoba River from the Ikpoba River for a) June, b) July c) August.

**The short arrows highlight areas with thickened membranes, and the long arrows mark regions with prominent fatty changes and adipocytes.*

CONCLUSION

The study on the Ikpoba River highlights the impact of urban runoff on *B. africanus* snails. While no significant changes were observed in water and sediment heavy metal content, the snails showed notable variations in heavy metal concentrations in their gonads. Antioxidant enzyme activities also varied significantly, with the greatest increase in Red GSH activity. Histological analysis revealed abnormalities in snail gonads. These findings indicate a high risk of reproductive anomalies in snails and potential heavy metal poisoning for humans or predators consuming them. Consistent monitoring and treatment measures are crucial to address pollution in the Ikpoba River, essential for *B. africanus* conservation.

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