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Research Article

Histopathological Effects of Metal Exposure on Freshwater Mussels (*Anodonta marginata*) from Challawa River, Nigeria

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ABSTRACT

The widespread contamination of aquatic bodies by heavy metals has engrossed worldwide attention due to their persistence and accumulative nature. The present study determined the bioaccumulation of some heavy metals and their histological impacts on freshwater mussels. Metals assessed were Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), and Nickel (Ni) in a bivalve mussel (*Anodonta marginata*) with their levels in sediment and water of Challawa River, Kano, Nigeria. Metals were assessed using an atomic absorption spectrophotometer. Gill and digestive gland tissues were dissected and fixed, sections were analysed for histopathology. Water samples analysed had mean concentrations of Cd (0.088mg/L), Cr (0.457mg/L), Pb (0.127mg/L) and Ni (0.101 mg/L) above the World Health Organization's permissible limits of 0.003, 0.05, 0.01 and 0.07 mg/L respectively. The trend of heavy metals in water levels was Site B > Site C > Site A > Site D; similar to the trend recorded in mussels. Pearson correlation revealed positive interactions in levels of metals in water, sediment and mussels. Moderate to severe histopathological changes were recorded in the form of disruption of epithelium, necrosis, and degeneration of tissues. This was attributed to metal pollution, through several mechanisms such as oxidative stress, inflammation, changes in cellular functions and homeostasis, etc. Findings highlight the need for effective waste management practices and enforcement of environmental regulations, particularly at the industrial areas where effluents are being discharged into the water.

Keywords: Mussels; Heavy metal; Histopathology; Bioindicators; River Challawa

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INTRODUCTION

In most developed and developing countries, rapid industrialization and man's constant quest for comfort as well as changes in taste and fashion have resulted in various forms of advancement in science and technology. Industrialization is considered the bedrock of development strategies due to its significant contribution to economic growth and human welfare, but it carries with it, various devastating ecological and human disasters that have implicated industries as major contributors to environmental degradation and pollution problems of different magnitudes (Idris *et al.,* 2013). In most developing countries, water resources remain the main reservoirs of industrial, domestic and agricultural wastes. Hence, streams, and coastal environments, including banks of the rivers suffer from pollutants due to increased industrialization arising from urbanization (Wuana & Okieimen, 2011).

Heavy metal contamination has been widely reported in water samples (Ali *et al*., 2019), soil (Alsbou *et al*., 2018), sediments and fishes (Fernandez-M *et al*., 2018) and lakes (Rajeshkumar *et al.,* 2018). Anthropogenic activities like smelting, mining, agricultural and industrial processes have been the source of heavy metal contamination of various environmental matrices (Rajput *et al*., 2020). Heavy metals are known to produce free radicals which are highly reactive chemical species that can damage cells and contribute to oxidative stress, aging caused by DNA damage, cardiovascular disorders, fatigue and rare autoimmune disorder, arthritis, calcification,

chronic arterial rupture and other degenerative problems (Jan *et al.,* 2015).

Histopathological changes in animal tissues are powerful indicators of environmental stressors. Besides that, it can give the net result of adverse biochemical and physiological changes in an organism as it allows the identification of specific target organs, cells and organelles infected *in vivo* (Padrilah *et al.,* 2018, Reddy *et al.,* 2018; Abubakar *et al.,* 2014). According to Reddy (2012), histopathology is often the easiest method of assessing both short- and long-term toxic effects in field assessments.

Studies have shown that exposure to polluted water can cause histopathological changes in freshwater mussels, including inflammation, necrosis, and degeneration of tissues (Li *et al.,* 2015). For example, a study by Liu *et al.* (2018) found that freshwater mussels exposed to microplastics showed significant histopathological changes, including inflammation and necrosis of the gill and digestive tissues. Similarly, a study by Zhu *et al.* (2020) found that freshwater mussels exposed to agricultural pesticides showed histopathological changes, including degeneration of the reproductive tissues and inflammation of the digestive tissues. Another study by Khan *et al.* (2018) recorded significant histopathological alterations in a freshwater mussel in form of vacuolation, degeneration of cilia and epithelial damage from Kabul River as a result of heavy metal pollution. Alonso *et al.* (2019) also reported severe histopathologies including severe disorder of germinal cells in mussels exposed to heavy metals.

Bivalves are suspension feeders or deposit feeders, or even utilize both feeding methods. They usually feed on microscopic algae, bacteria, and detritus via a filter-feeding process. As bivalves filter large quantities of seawater, their tissues absorb some of the contaminants present in water and food particles. They accumulate trace metals from the surrounding aquatic medium across the cellular membrane (dissolved source) and from food particles (dietary source) (El-Din Saleh and El-Adham, 2018). Their wide distribution, sessile nature, ease of sampling, resistance to a wide range of contaminants make them a group of candidate species for biomonitoring programs across the globe. It has been reported that bivalves accumulate trace metals in their tissues at levels up to 100–100,000 times higher than the concentrations observed in the water in which they live (Farrington *et al*., 2016). Therefore, several chemical contaminants, including trace metals, present at undetectable levels in water can be detected in bivalves. Different species of clams, mussels, and oysters have widespread distribution across the continents, and many of those species have been successfully used for monitoring the concentrations of contaminants in the environment (Farrington *et al*., 2016).

The current study aims to investigate the histopathological changes in freshwater mussels as a result of heavy metal bioaccumulation in River Challawa Kano, Nigeria.

MATERIALS AND METHODS

Study Area

Challawa River is located in Yandanko village in the Challawa Industrial Estate (11° 45′ 42N, longitude 8° 46′ 17E) in Kumbotso Local Government Area of Kano state. (Uzairu *et al*, 2014). Kano is located in the northern part of Nigeria covering an area extending between latitudes 12° 40′ and 10° 30′ and longitudes 7° 40′ and 9° 40′ (Uzairu *et al*, 2014). Four (4) sampling sites were selected for this research. Each sampling site comprised of three sampling points. The stations chosen were based on the different activities in the areas.

Site A: This site is near the point at which the Kano State Water Works draws its raw water to the treatment plants for purification. At this station, few human activities such as sand dredging and fishing take place.

Site B and C: These sites receive raw effluent from Challawa Industrial Area and is discharged into the river. They comprise mainly food, textile, agro-allied, plastics and tannery industries.

Site D: This site is at Tamburawa along Zaria Road close to the Tamburawa Water Treatment Plant where the river forms a confluence with River Kano. Activities such as sand dredging, farming, and fishing take place in the area.

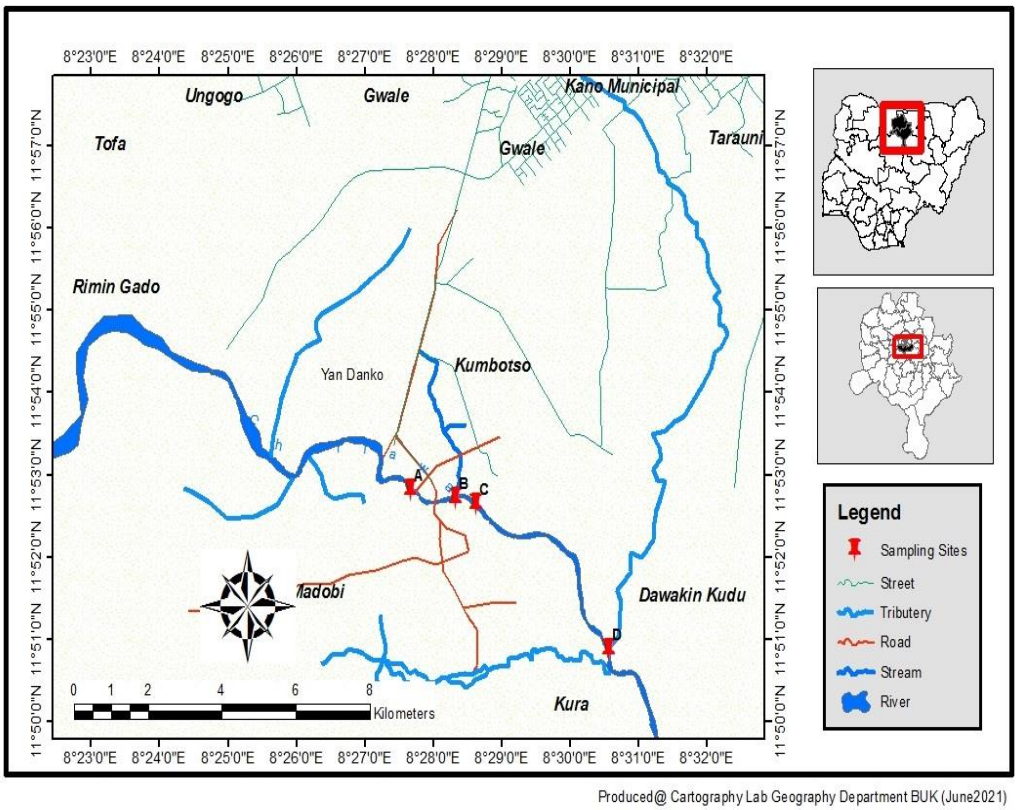

Figure 1: Map of River Challawa showing the Study Sites

Sample Collection

All the samples for this research work were collected once, every month across 12 months in triplicates. All reagents used were of analytical grade.

Water Sampling

One hundred and forty-four (144) water samples were collected from the various points in 1L sterile polyethene plastic bottles by dipping the bottles about 20 cm below the water surface and filling them to the brim. Samples were stored in an ice box, transported to the laboratory and refrigerated at about 4^{o}C prior to analysis as adopted by Indabawa (2012).

Sediment Sampling

One hundred and forty-four (144) sediment samples were collected with the aid of a manual Grab sampler at each point and stored in labeled polyethene bags. Samples were stored in an ice box, transported to the laboratory and refrigerated at about 4^{o}C prior to analysis according to International Atomic Emission Agency procedures for the collection of sediment samples (2003).

Mussel Sampling and Preparation

Species of *Anodonta marginata* were collected and identified using taxonomic guides by Lydeard *et al.* (2004). Mussels were collected at each sampling point using a modified Ekman grab sampler and were immediately transferred to the laboratory. Seventy-two (72) *Anodonta marginata* individuals were collected monthly from the different sampling points, taking care not to hurt the animals. Mussels were placed in a container with water and sediment from the sampling stations, placed in a larger box containing icepacks and then transported to the laboratory for sample preparation (maximum transit time: 2 hours) as adopted by Food Standards Agency, UK (2020). Shells of mussels were opened by carefully severing the adductor muscles followed by removing the muscle mass. Tissues were pooled in threes and completely dried at 60°C for 24 h, ground into a fine powder and stored in the desiccator prior to digestion for heavy metals analysis.

Sample Digestion

Water samples were digested using Nitric Acid (HNO^3). The obtained water sample (100 ml) was poured into a beaker, to which 5 ml of concentrated HNO^3 was added. The mixture was heated on a hot plate and allowed to evaporate. The volume of the solution was reduced to about 20 ml. The solution was cooled and another 5 ml of concentrated HNO^3 was added and covered with a watch glass while heating continues. One (1 ml) of HNO^3 was re-added until a clearer solution was obtained. The digests were diluted to 100 ml in a volumetric flask and transferred into a plastic container that was

acid-washed and ready for analysis (Uddin *et al.*, 2016).

Sediment samples were digested following the Nitric (HNO_3)-Perchloric ($HClO_4$) acid digestion method as adopted by Uddin *et al.* (2016).

One (1) gram of sediment was measured and added to a 250 ml capacity digestion tube and 10 ml of concentrated HNO_3 was added. The product mixture was gently heated for 30 to 45 min to ensure total oxidation of all readily oxidizable matter and allowed to cool. Following cooling, 5 ml of 70 % $HClO_4$ was added to the resultant mixture and heated gently until thick white fumes were observed. The mixture was allowed to cool and twenty (20) ml of distilled water was added to the solution and boiled again for all the fumes to be released. The solution was filtered through Whatman filter paper after cooling and transferred to a 25 ml volumetric flask followed by the addition of distilled water. The digests were kept in a labelled plastic container before analysis.

Mussel samples were digested following the Nitric (HNO^3)-Perchloric acid ($HClO_4$) digestion method as adopted by Uddin *et al.* (2016). One (1) gram of powdered mussels was weighed and added to a 250 ml capacity digestion tube and 10 ml of concentrated HNO3 was added. The product mixture was gently heated for 30 to 45 min to ensure total oxidation of all readily oxidizable matter and allowed to cool. Following cooling, 5ml of 70% $HClO_4$ was added to the resultant mixture and heated gently until thick white fumes were observed. The mixture was allowed to cool and twenty (20) ml of distilled water was added to the solution and boiled again for all the fumes to be released. The solution was filtered through Whatman filter paper after cooling and transferred to a 25 ml volumetric flask followed by the addition of distilled water. The digests were kept in labelled plastic containers prior to analysis.

Determination Of Heavy Metals

Atomic Absorption Spectrophotometer (Agilent Technologies model 200 series AA) was used to determine the levels of heavy metals in the samples digested. The spectrophotometer was set at a specific wavelength unique to each respective metal. Between two readings, distilled-deionized water aspiration was conducted. The records of the absorbance were taken from the steady galvanometer in a moment of 1, 2 min. For any sample, analysis was performed in triplicate, and the concentration of metals was calculated with the aid of a standard calibration plot (Sani *et al.,* 2016**)**.

Histological Examination

Shells of mussels were opened by carefully severing the posterior adductor muscles followed by removing the muscle mass. Histological analysis procedure was adapted from Auwioro (2010). Gills and digestive glands of mussels were each dissected and fixed with 10 % formal saline, dehydrated with ascending grade of alcohol, cleared with toluene and infiltrated with molten paraffin wax. 5 µm microtome sections were cut, mounted on microscope slides and stained with haemotoxylin and eosin. Slides were examined under a light microscope (Leica DM Model) at 100 or 400x magnification. Images were captured using a high-resolution camera (Leica ICC 50 HD) coupled to the microscope and images were analyzed using the Leica LAS EZ software (Leica Microsystems Inc, Germany).

Statistical Analysis

Two-way analysis of variance (ANOVA) was applied to determine the significant mean differences in levels of heavy metals in mussels, water and sediment between sites and seasons. Duncan's Multiple Range Test (DMRT) was used in evaluating the significant difference within the levels of independent variables (sites and seasons). Pearson Correlation (r) was used to assess the relationships between each heavy metal concentration in mussel and its corresponding concentration in water and sediment. All the data analysis was performed using R statistical software version 4.01 and the result are presented as mean and standard deviation.

$p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Heavy Metals

Levels of heavy metals (mg/L) in water from sampling sites in Challawa River

The mean level of cadmium in the water recorded was 0.088 mg/L. This value has exceeded the maximum permissible limit of 0.003mg/L for water set by the WHO (2008). This is similar to the result obtained by Akinpelu and Kuforiji (2013) in River Owo and Akan *et al.* (2013) in the Jakara waste channel. This may be due to the discharge of industrial effluents, urban and agricultural run-offs and other relevant occupational activities such as steel making, welding, electroplating etc. (Cheang *et al.,* 2022).

From the results of this study, higher levels of Cadmium were observed in site B than in the other sites (0.101 mg/L). This could be because it is the point where effluent from the industrial area is being discharged into the river and hence it receives more pollutants. Similarly, sites A and D had lower mean values of Cd (both 0.08 mg/L). This is because it is upstream of the river located before the point where effluents are being discharged into the river and had less anthropogenic impact. This agrees with the findings of Sani *et al.* (2022) with levels of Cd in water ranging from 0.273 - 0.61 mg/L from

River Challawa. Drinking or use of water from River Challawa for domestic purposes thus poses a serious toxicological risk concerning cadmium intoxication according to Udiba *et al.* (2018).

Concentrations of chromium in water observed in this study have exceeded the maximum permissible limits of 0.05 mg/L set by the WHO (2008) with site B having the highest mean value (1.62 mg/L). Significant differences were observed between the study sites which could be a result of more industrial influence observed in site B. Similar results were reported by Musa and Imam (2021) from the Hadejia-Nguru wetland with values exceeding recommended limits. Chromium could gain entrance into the aquatic ecosystem through effluents discharged from dyes, leather tanneries, textiles, fertilizers etc and mussels bioaccumulate it through ingestion or by uptake through their gills. Chromium is widely used in metallurgy, electroplating, and the manufacturing of paints, pigments, preservatives, pulp and papers among others according to Kinuthia *et al.* (2020).

Copper concentration in water ranged between 0.005 and 0.012 mg/L with a mean of 0.10 mg/L which is below the maximum permissible limits of 1.0 mg/L in water. According to Padrilah *et al.* (2018), the presence of copper in water or an aquatic environment occurs through several pathways including mining activities, the discharge of industrial and agricultural waste and runoff from mineral deposits. Uzairu *et al.* (2014) opined that though widely distributed and an essential element, acute toxicity of Cu results in hypotension, coma, and death.

Levels of Pb in water ranged between 0.046 and 0.157 mg/L. The mean value of 0.127 mg/L has exceeded the WHO (2008) maximum permissible limits of 0.01 mg/L for consumption. Another study of lead concentrations in tannery effluents of River Challawa was found above the WHO (2008) limit of 0.01 mg/L with a range of 0.6250 - 0.8501mg/L. A range of 0.67-3.10 mg/L was also reported from the same study area (Bernard and Ogunleye, 2015). The concentration of Pb found in river Challawa might be a result of the anthropogenic discharge of Pb-containing wastes from industries including used items such as pipes and petrol (Akan *et al.,*2007; Sani *et al.,* 2022). Household waste suspected of containing lead is batteries, children's toys, washed paint, and plastic food or beverage packaging (Lamondo, 2021). Eshmat *et al.* (2014) also reported that the contamination of lead (Pb) in Ngemboh waters was caused by the disposal of resident waste originating from organic and non-organic materials. Budiastuti *et al.* (2016) also argued that household waste has a significant role in the presence of lead in water. A range of 0.011-0.033ppm of lead was reported in a study by Kinuthia *et al.* (2020) in wastewater from Kenya's open drainages. Lead affects the central nervous system, particularly in children and also damages the liver, kidney and immune system. At higher concentration, lead may result in metallic poisoning which can cause cancer in humans (Bakare-odunola, 2005).

Nickel in water ranged between 0.056 and 0.216 mg/L with a mean of 0.10 mg/L. This value has exceeded the WHO (2008) maximum permissible limits of 0.07 mg/L for consumption. Other values reported in the literature were 0.05mg/L, 0.68mg/L and 0.85 mg/L by Sahu *et al.* (2007), Bhatnagar *et al.* (2013) and Amanial (2015) respectively. There was no significant difference in the levels of nickel in all the sites. Kinuthia *et al.* (2020) reported values of 0.004 mg/L which was within WHO permissible limits. Shaibu and Audu (2019) reported that nickel concentrations in all the tannery effluents of River Challawa were below the WHO permissible limit of 1.0 mg/L with a range of 0.0029 - 0.0144 mg/L. The presence of nickel in tannery effluent may be attributed to chemicals used in the tanning and post-tanning processing of leather (UNIDO, 2005). At high concentrations, nickel may cause damage to DNA and cell structures (Monika *et al.,* 2011).

Table 1: Mean Concentrations of Heavy metals (mg/L) in Water across Sites and Seasons

		Cd	Cr	Cu	Ni	Pb
Sites	**A**	0.08± 0.00^{c}	0.071±0.01^{b}	0.010±0.004^{b}	0.216±0.128^{a}	0.046±0.023^{b}
	B	0.086±0.01^{b}	1.62±0.86^{a}	0.012±0.007^{a}	0.06±0.010^{b}	0.157±0.08^{a}
	C	0.101±0.01^{a}	0.07±0.02^{b}	0.011±0.005^{ab}	0.056±0.006^{b}	0.156±0.100^{a}
	D	0.08±0.00b^{c}	0.08±0.03^{b}	0.005±0.003^{c}	0.060±0.008^{b}	0.146±0.077^{a}
Seasons	**Wet**	0.086±0.01^{a}	0.589±0.96^{a}	0.010±0.006^{a}	0.094±0.080^{a}	0.128±0.09^{a}
	Dry	0.088±0.01^{a}	0.378±0.65^{a}	0.009±0.005^{a}	0.105±0.100^{a}	0.125±0.087^{a}

Means followed by superscript with different letters across the rows are significant at $p<0.05$

Levels of heavy metals (mg/kg) in sediment from sampling sites in Challawa River

Cadmium in sediment had a mean value of 0.146 mg/kg. This value is within the permissible limits of 4.9 mg/kg for sediment (WHO, 2011) and it is lower than those obtained in a study by Sani *et al.* (2022) in River Challawa and Kinuthia *et al.* (2020) in Kenya open drainage channels. The higher levels of Cd in

sediment than in water coincide with the findings of Sani *et al.* (2022). This could be due to the role of sediment which serves as the primary metals' depository where in some situations, retaining greater than 99% of the total quantity of metal in the aquatic environment (Gupta *et al.,* 2009; Sani *et al.,* 2022).

The mean chromium concentration in sediment reported was 1.1 76 mg/kg. Site B had the highest concentration of chromium in sediment (2.51 mg/kg) which differs significantly from other sites. This could be a result of the frequent discharge of effluents from tannery and textile industries from the industrial area which keeps accumulating deep beneath the river bed over time. A higher concentration of chromium in sediment than in water was observed in this study and it was attributed to the fact that sediment serves as the primary depository for metals where in some situations, retain greater than 99 % of the total quantity of metals in the aquatic environment. This agreed with the findings of Sani *et al.* (2022) in River Challawa, Kinuthia *et al.* (2020) in Kenya and Godwin and Chinenye (2016) in Bodo Creek, Niger-Delta. A higher level of Cr in sediment was reported by Kinuthia *et al.* (2020) from Kenya's open drainage (45.19 ppm) and it was attributed to the presence of waste in the study area which may pose risks and hazards to humans and ecosystems through direct contact or ingestion, food chain, contaminated drinking water, reduced food quality among others.

The concentration of Cu in sediment ranged between 0.042 and 0.122 mg/kg with a mean of 0.074 mg/kg. Ashraf *et al.* (2012) and El-Moselhy *et al.* (2014) stated that the accumulation of metals in organisms depends on several factors such as the trophic level, location, feeding behavior, size, age, duration of exposure to metals and concentration of metals. As reported by Yigit *et al.* (2017), copper is available in the natural environment and is known as an essential element for the growth and metabolism of all living organisms.

Pb in sediment was found ranging between 19.19 in site D and 22.29 mg/kg in site B which differs significantly in the two sites. The mean level of Pb in this study was 20.80 mg/kg which exceeded the safe limits of Pb in sediment. Sani *et al.* (2022) reported higher levels of Pb in sediment from the same study area ranging from 43.64 – 53.61 mg/kg. Lead content in the sediment is higher than in the water, this is because the heavy metals settle in the sediment. Cahyani (2017) found that the levels of lead in water ranged from 0.042 - 0.104 mg/L while the sediment ranged from 1.56 - 1.98 mg/kg. Environmental factors like temperature influence the concentration of lead in sediment. Happy (2012) states that a drop in water temperature will cause metals to settle into the sediments easily. Parallui (2013) also states that an increase in water temperature can reduce the absorption of heavy metals in fine particles from the pollution that settles on the bottom of the water.

Nickel in the sediment of River Challawa in this study was found to range between 0.296 and 0.335 mg/kg. This is below the range obtained by Kinuthia *et al.* (2020) which was 11.70 to 29.87 ppm who reported that sediments in wastewater channels may enrich with pollutants present in wastewater with time.

Table 2: Mean Concentrations of Heavy metals (mg/kg) in Sediment across Sites and Seasons

	Sites	Cd	Cr	Cu	Ni	Pb
Sites	**A**	0.042 ± 0.007^{a}	0.671 ± 0.13^{c}	0.046 ± 0.009^{c}	$0.316\pm0.021_{ab}$	20.15 ± 3.34^{ab}
	B	0.044 ± 0.002^{a}	2.51 ± 0.94^{a}	0.042 ± 0.007^{c}	$0.298\pm 0.019_{b}$	22.29 ± 4.77^{a}
	C	0.043 ± 0.002^{b}	0.49 ± 0.05^{c}	0.083 ± 0.033^{b}	$0.296\pm 0.017_{b}$	21.54 ± 5.97^{ab}
	D	0.052 ± 0.007^{a}	1.02 ± 0.32^{b}	0.121 ± 0.002^{a}	$0.335\pm 0.081_{a}$	19.19 ± 5.27^{b}
Seasons	**Wet**	0.045 ± 0.006^{a}	0.045 ± 0.006^{a}	0.076 ± 0.039^{a}	0.311 ± 0.04^{a}	20.61 ± 5.08^{a}
	Dry	0.046 ± 0.007^{a}	0.046 ± 0.007^{a}	0.0712 ± 0.035^{a}	0.311 ± 0.04^{a}	21.04 ± 4.99^{a}

Note: Means followed by superscript with different letters across the rows are significant at $p<0.05$

Levels of heavy metals (mg/kg) in mussels from sampling sites in Challawa River

Cadmium in mussels was recorded with a mean of 0.259 mg/kg. This coincides with the results obtained by Zhelyazkov *et al.* (2018) in mussels (0.280 mg/kg) from the Varna Bay of the black sea as a result of effluent discharge from chemical industries etc. In a previous study on heavy metal content of mussels (*M. galloprovincialis*) from Varna Bay, Stancheva *et al.* (2011) and Yuliango *et al.* (2019) reported Cd concentrations of 0.044 mg/kg (mussels) and 0.19 mg/kg (green mussels) respectively which are lower than the results from this study. Yuliango *et al.* (2019) also reported means of 1.44 mg/kg in blood mussels, 1.92 mg/kg in oysters, 0.56 mg/kg in clams and a higher value of 15.34 mg/kg in scallops from Indonesia which have all exceeded the mean found in this study. Ndiaye *et al.* (2020) reported site-specific differences in levels of Cd in tissues of mussels in the Dakar coast and was explained by the differences in the anthropogenic activities of the

sites as also observed in this study and by Sani *et al.* (2022).

Chromium in mussels was found the highest than in water and sediment with the mean value of 1.327 mg/kg. This is evident from the fact that mussels are filter-feeders and hence could bioaccumulate substances from water and sediment. Site B had the highest concentration of chromium (2.66 mg/kg) and was significantly different from sites A, C and D. Pearson correlation revealed a significant positive association in concentrations of Cr in water and mussels and Cr in sediment and mussels. This means an increase in concentration in water and mussels simultaneously brings about an increase in Cr in mussels significantly. Similar finding was observed by Sani *et al.* (2022) which states that an increase in concentration of an abiotic factor affects the concentration in a biotic factor.

Levels of copper in mussels ranged between 0.005 and 0.010 mg/kg with a mean of 0.008 mg/kg. These values are below the range of 2.4 - 4.8 mg/kg reported by Bat *et al.* (2012) in black sea, 1.3 - 1.8 mg/kg obtained by Brooks *et al.* (2012) from the Island of Gossa and 0.13 - 2.39 mg/kg by Yigit *et al.* (2017) from a fish farm in Turkey. Yuliango *et al.* (2019) also reported means of 6.94 mg/kg in blood mussels, 11.08 mg/kg in clams, 7.45 mg/kg in scallops, 10.88 mg/kg in green mussels and a higher value of 59.22 mg/kg in oysters from Indonesia which have all exceeded the mean found in this study.

Mean level of Pb found in mussels was 1.170 mg/kg. There was a significant difference in the values of Pb across all sites with site B having the highest mean value of 1.30 mg/kg. Yuliango *et al.* (2019) reported means of 5.27 mg/kg in blood mussels, 5.76 mg/kg in oysters, 2.93 mg/kg in clams, 1.27 mg/kg in green mussels and 2.00 mg/kg in scallops from Indonesia which are all above the mean found in this study. Novakov *et al.* (2021) reported lower levels of Pb than present study in mussels on the Serbian markets which ranged between 0.01-0.38 mg/kg.

Nickel in mussels was reported with a mean of 0.175 mg/kg. A study by Palermo *et al.* (2015) revealed that exposure of fishes affected antioxidant defences, increased lipid peroxidation in the liver and increased DNA damage in both blood cells and gills of fish exposed to all Ni concentrations, indicating the genotoxic potential of Ni on fish. A study by Fard *et al.* (2016) in Iran reported mean values of Ni in fish of 2.458 mg/L which has exceeded the WHO and FDA recommended values in fish. According to Brix *et al.* (2016), Ni is used in a range of industrial practices, the most important of which is the production of stainless steel. In addition to point source releases from industrial practices, several diffuse sources (natural weathering, atmospheric deposition, surface runoff) contribute to environmental Ni exposure.

Table 3: Mean Concentrations of Heavy metals (mg/kg) in Mussels across Sites and Seasons

	Sites	Cd	Cr	Cu	Ni	Pb
Sites	**A**	0.18 ± 0.02^{c}	0.92 ± 0.03^{b}	0.007 ± 0.004^{b}	0.05 ± 0.01^{c}	0.98 ± 0.18^{c}
	B	0.29 ± 0.03^{a}	2.66 ± 0.53^{a}	0.009 ± 0.005^{a}	0.06 ± 0.00^{a}	1.15 ± 0.14^{b}
	C	0.30 ± 0.05^{a}	0.86 ± 0.08^{b}	0.010 ± 0.004^{a}	0.05 ± 0.00^{b}	1.30 ± 0.27^{a}
	D	0.25 ± 0.07^{b}	0.84 ± 0.07^{b}	0.005 ± 0.002^{c}	0.06 ± 0.01^{b}	1.23 ± 0.20^{ab}
Seasons	**Wet**	0.25 ± 0.07^{a}	0.25 ± 0.07^{a}	0.007 ± 0.004^{a}	0.05 ± 0.01^{a}	1.15 ± 0.20^{a}
	Dry	0.26 ± 0.06^{a}	0.26 ± 0.06^{a}	0.008 ± 0.004^{a}	0.06 ± 0.01^{b}	1.17 ± 0.26^{a}

Means followed by superscript with different letters across the rows are significant at $p<0.05$

HISTOPATHOLOGICAL CHANGES

Histopathological alterations found in gills were disruption of the epithelium of the gill filaments and degeneration of cilia observed as shown in plates I-IV. The epithelium indicated severe pathological changes of the oedema formation and necrosis. Disaggregated cilia were observed. These alterations were observed in gill tissues of mussels from all four sites, with sites B and C recording severe damages. This could be attributed to higher levels of all the metals in these sites which receives raw industrial discharge. Khan *et al.* (2018) reported similar findings in Kabul River where histological alterations were recorded in the most polluted sites.

Mussels' gills are considered to be sensitive to toxicity because of their crucial role in respiration, food absorption, and extended surface area (Mona *et al.,* 2022). Fahmy and Sayed (2017) observed gill filament dilation, epithelial lifting, necrosis, hemocyte congestion, hyperplasia, filament deformities, and cilia damage in bivalve *Coelatura aegyptiaca* exposed to metals. Similarly, epithelial damage, necrotic and ruptured cells, and deformed gill filaments were identified by Vasanthi *et al.* (2012). These pathological alterations were mostly similar to the results of this study. Histological alterations and biochemical changes of gill tissues produced by chemical stress causes disturbed metabolism, enzyme inhibition, retardation of

growth, fecundity reduction and longevity of the organism, which affects balance in the ecosystem (Sole *et al.,* 2018). Similar findings were reported by Pandey and Pathak (2016) and Balamurugan and Subramanian (2021) in freshwater mussels exposed to heavy metals and oil effluent respectively. Bhargavan (2008) reported loss of cilia, epithelial damage and elongation of gill filaments in mussels exposed to heavy metals.

Histopathological alterations found in digestive glands of mussels from the different sites were necrosis and gross changes in the epithelium of the glands as well as the stroma. The glandular epithelium was invariably detached from the stroma (plates V-VIII). This stroma adds dense accumulation of leucocytes. The integrity of the epithelium was thoroughly disrupted and a major change consisted of vertical clefts. Another important feature was noticed towards the dense accumulation of the luminal material, which also contain leucocytes, which were otherwise confined to stroma. Most of these findings were similar to those reported by Khan *et al.* (2018).

The tissues of digestive gland produced gross changes in the epithelium of the glands as well as the stroma. The glandular epithelium invariably detached from the stroma which was observed in mussels from all sampling sites. These findings were similar to those reported by Khan *et al.* (2018) from a polluted site in River Kabul. Sheir and Handy (2010) also reported disruption in the epithelial glands, tissue burden, digestive tube thickness, lumen enlargement and necrotic tissues in digestive gland of mussels exposed to heavy metals. Similar findings were also reported by Balamurugan and Subramanian (2021) in freshwater mussels exposed to oil industry effluent.

Histological alterations observed in mussel gills and digestive glands in this study were attributed to the discharge of industrial effluents into the water, since one of the key mechanisms by which heavy metals induce histopathological changes is through the generation of reactive oxygen species and the induction of oxidative stress (Rady *et al.,* 2023). ROS can damage cellular components, including DNA, proteins, and lipids, leading to cell death and tissue damage. Several researchers such as Katalay *et al.* (2016), Khan *et al.* (2018), Revel *et al* (2019) and Li *et al.* (2020) supported these findings.

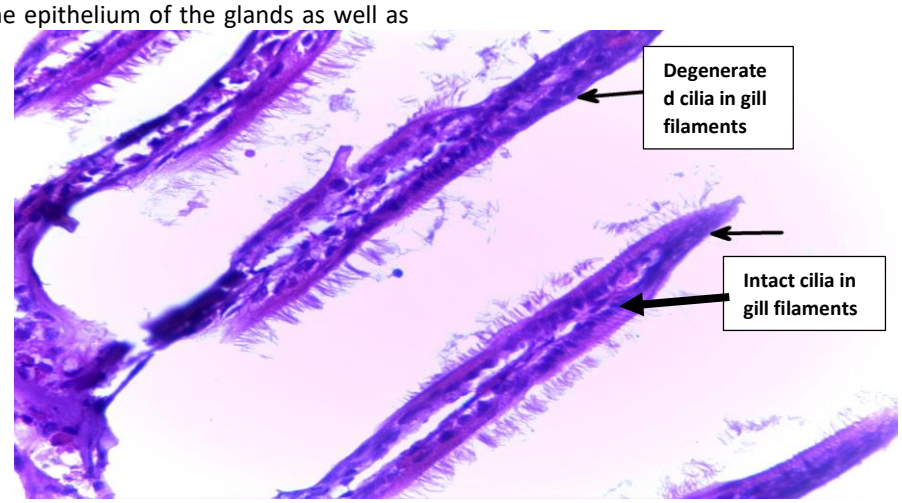

Plate I: Histological image of gills of Mussels from Site A (x400)

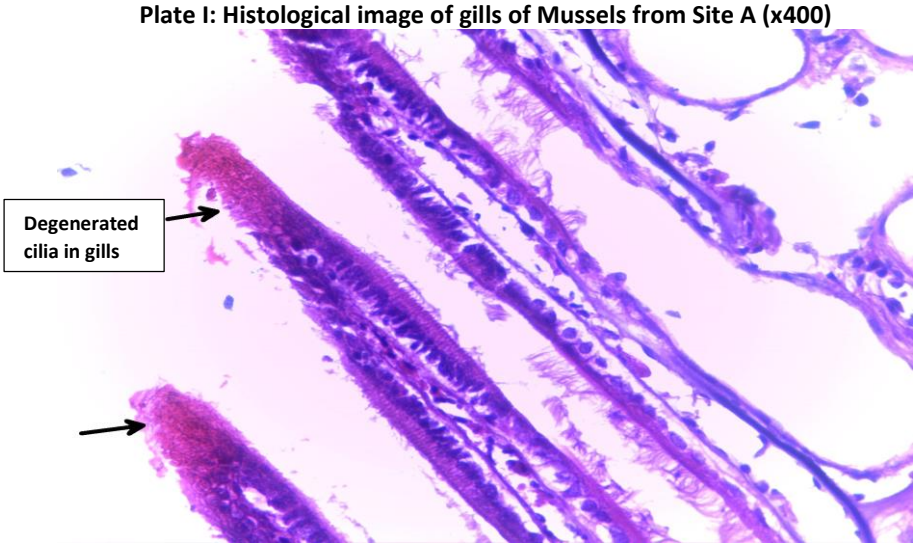

Plate II: Histological image of gills of Mussels from Site B (x400)

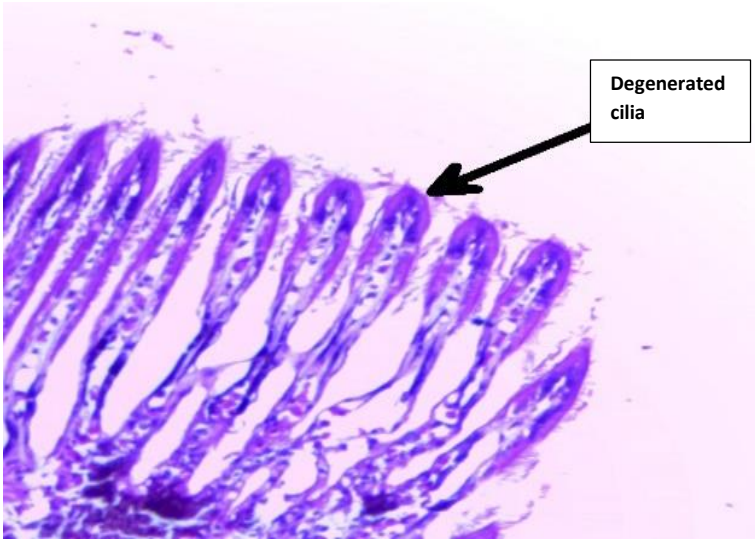

Plate III: Histological image of gills of Mussels from Site C (x400)

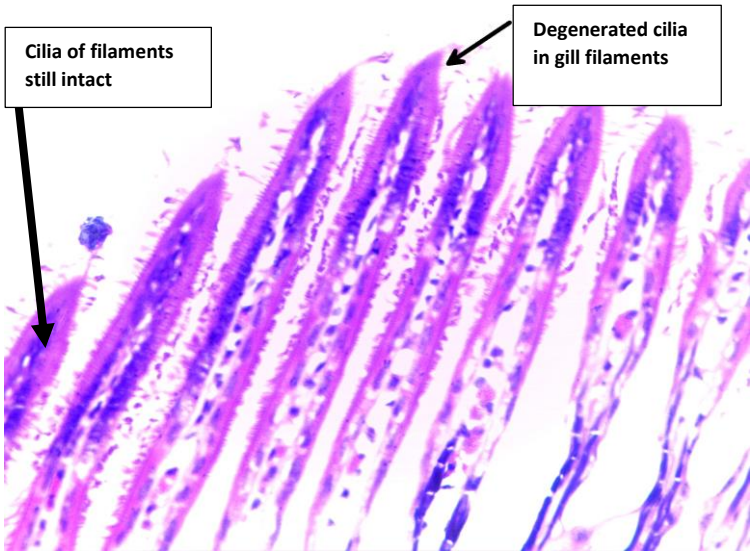

Plate IV: Histological image of gills of Mussels from Site D (x400)

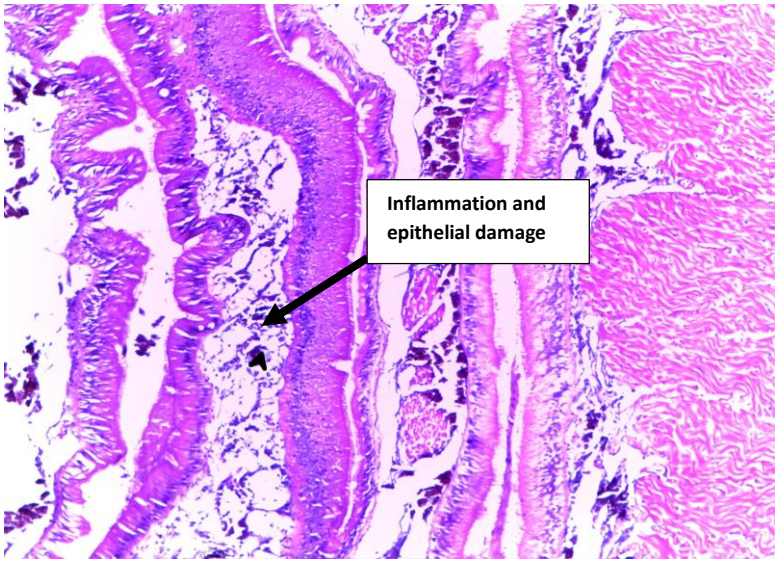

Plate V: Histological image of digestive gland of Mussels from Site A (x100)

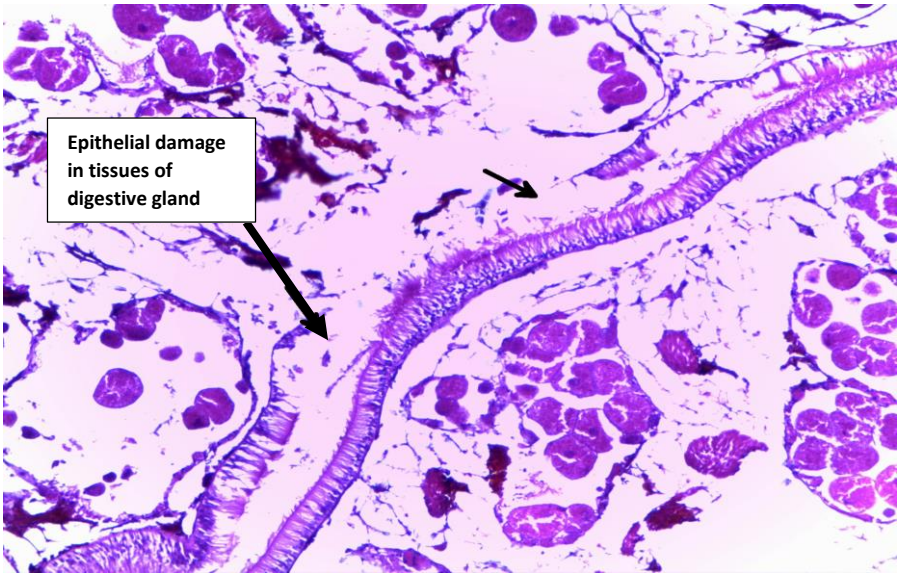

Plate VI: Histological image of digestive gland of Mussels from Site B (x100)

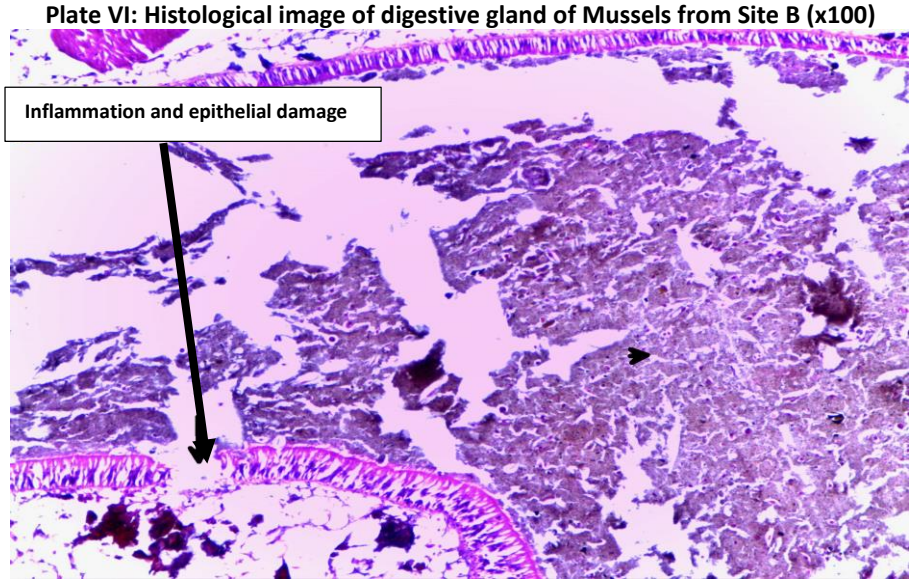

Plate VII: Histological image of digestive gland of Mussels from Site C (x100)

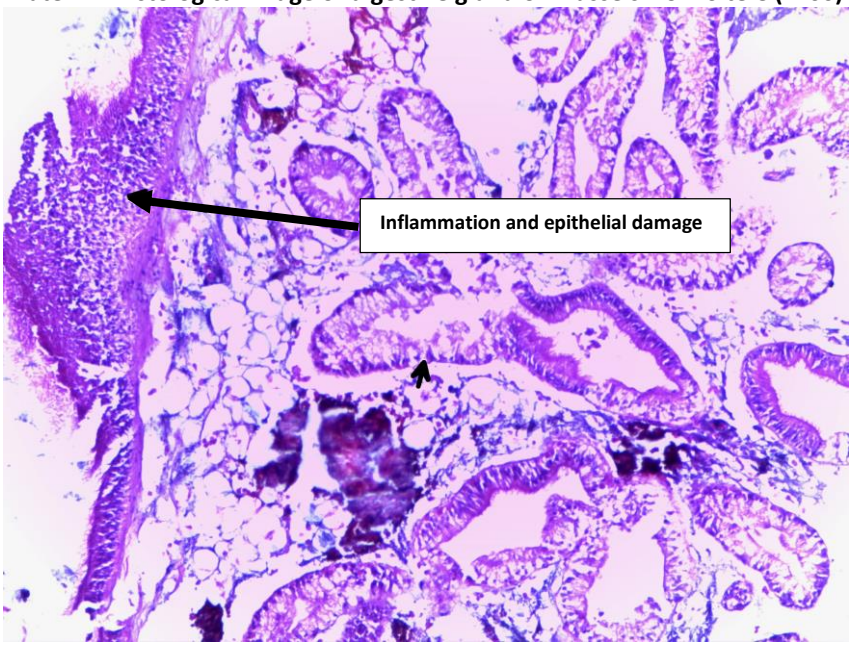

Plate VIII: Histological image of digestive gland of Mussels from Site D (x100)

CONCLUSION

In conclusion, the results indicate high concentrations of Cd, Cr, Cu, Ni and Pb in water and mussels from River Challawa. The study highlights the capability of heavy metals for bioaccumulation in the tissues of freshwater mussels and reveals moderate to severe histopathological alterations in mussel tissues. Hence, mussels are inevitably impacted by metal contaminants in water, therefore could act as biomarkers for water quality deterioration. The current study also revealed the vulnerability status of freshwater ecosystems in large cities and industrial areas due to heavy metal pollution. It is important to note that even low levels of heavy metals can contribute to the bioaccumulation of such elements with time in organisms that are in higher trophic levels in a food chain. This could pose risks and hazards to humans and ecosystems through direct contact or ingestion, contaminated drinking water, and reduced food quality among others. Necessary measures and policies should be implemented that will ensure provision of wastewater treatment plants, particularly at the industrial areas where effluents are being discharged into the river.

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