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BIOACCUMULATION OF HEAVY METALS AND OXIDATIVE STRESS BIOMARKERS RESPONSE IN *Anodonta marginata* FROM RIVER CHALLAWA KANO STATE, 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 which include Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Manganese (Mn) 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 atomic absorption spectrophotometer. The oxidative stress biomarkers (Superoxide Dismutase, Catalase and Glutathione) were assessed using the tissues of mussels after homogenization. The water samples analyzed 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 levels of all the heavy metals in water, sediment and mussels between dry and wet seasons were not significantly different (p > 0.05). The trend of heavy metal levels in increasing order was Site B > Site C > Site A > Site D. The same trend in levels of SOD, CAT and GSH were observed which indicates antioxidant response as a result of heavy metal exposure. These results indicate that mussels stimulated the increase in antioxidant enzyme activities as an adaptive response to oxidative damage by metals. Such could pose a serious consequence given the massive population that relies on the river as a source of water, irrigation and fishing activities.

Keywords: Metals, mussels, oxidative stress.

INTRODUCTION

The term heavy metal refers to any metallic element that possesses an atomic number greater than 20 and a specific density greater than 5 $g.cm^{-3}$ (Raychuduri, 2021). Heavy metals may enter the aquatic ecosystem through natural as well as anthropogenic activities. The widespread contamination of aquatic bodies by these metals has engrossed worldwide attention due to their persistence and bio-accumulative nature (Zhang *et al*., 2016). 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 and Li, 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).

The characterization of the quality of aquatic systems requires specially designed biological methods for assessing their health status. Biomarkers are known to be useful tools for assessing environmental health (Jourmi *et al.*, 2012). Antioxidants represent the cellular defense mechanisms that counteract toxicity of reactive oxygen species (ROS), these mechanisms have been extensively investigated in sentinel organisms such as mussels (Jourmi *et al.*, 2012).

Among these antioxidant biomarkers are Superoxide dismutase (SOD) and Catalase (CAT), well-known antioxidant enzymes, which convert the ROS to hydrogen peroxide, and then to water respectively. The biological importance of SOD and CAT is more evident from various studies since H_2O_2 is the main cellular precursor of the hydroxyl radical (HO^-) which is a highly reactive and toxic form of ROS leading to oxidative damage to basic biological molecules (Jourmi *et al.*, 2012).

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. During the filter-feeding, they draw water from the posterior ventral side through the inhalant siphon, and the water passes through the gills and gets expelled through the exhalant siphon. Through this process, they filter large quantities of water, and the water filtering capacity of typical natural mussel beds has been calculated as 7–12 m^3, m^{-1}, h^{-1} (Krishnakumar *et al.*, 2018). As bivalves filter large quantities of seawater, their tissues absorb some of the contaminants present in water and food particles. Bivalves accumulate trace metals from the surrounding aquatic medium across the cellular membrane (dissolved source) and from food materials (dietary source) (El-Din Saleh and El-Adham, 2018). Historically, bivalve mollusks are considered valuable aquatic organisms for environmental monitoring and are used as biomonitors of chemical pollution of coastal waters (Kimbrough, 2008). Bivalves are widely distributed from the North Pole to the South Pole, sessile in nature, easy to sample and available in a suitable size for chemical analyses. Bivalves are also resistant to a wide range of contaminants and may thrive even in highly polluted environments. These qualities 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 bivalve tissues. 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 marine environment (Farrington *et al*., 2016).

Since Challawa river is one of the main water sources in Kano, comprising the upstream, where lesser human activities mainly fishing and sand dredging occur, midstream into which effluents are being discharged from Challawa and Sharada Industrial Areas, and the downstream where. human activities mainly irrigation and fishing occur. The major industries in Challawa are tanneries, textiles, foods and packaging. The effluents derived from the industries in the estate were connected by a canal and channeled directly into the river as a point source (Uzairu *et al*, 2014). These prompt the need to evaluate the complexities of such heavy metals which include Cadmium, Chromium, Copper, Manganese, Nickel and Zinc in Mussels, sediment, and water. Hence, this research aimed to assess the heavy metal bioaccumulation of mussels, water and sediment and to assess the oxidative stress biomarkers in mussels from River Challawa receiving raw industrial effluents.

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.

Kano is located in the northern part of Nigeria covering an area extending between latitudes 12° 40′ and 10° 30′ and longitudes of 7° 40′ and 9° 40′ (Uzairu *et al*, 2014).

Four (4) sampling sites were selected for this research. Each sampling site is 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: This site receives raw effluent from Challawa Industrial Area and is discharged into the river. They comprise mainly food, textile, agro-allied, plastics and tannery industries.

Site C: This site receives the discharge of raw industrial effluents typically from some textiles, tanneries and food industries through the Salanta stream.

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.

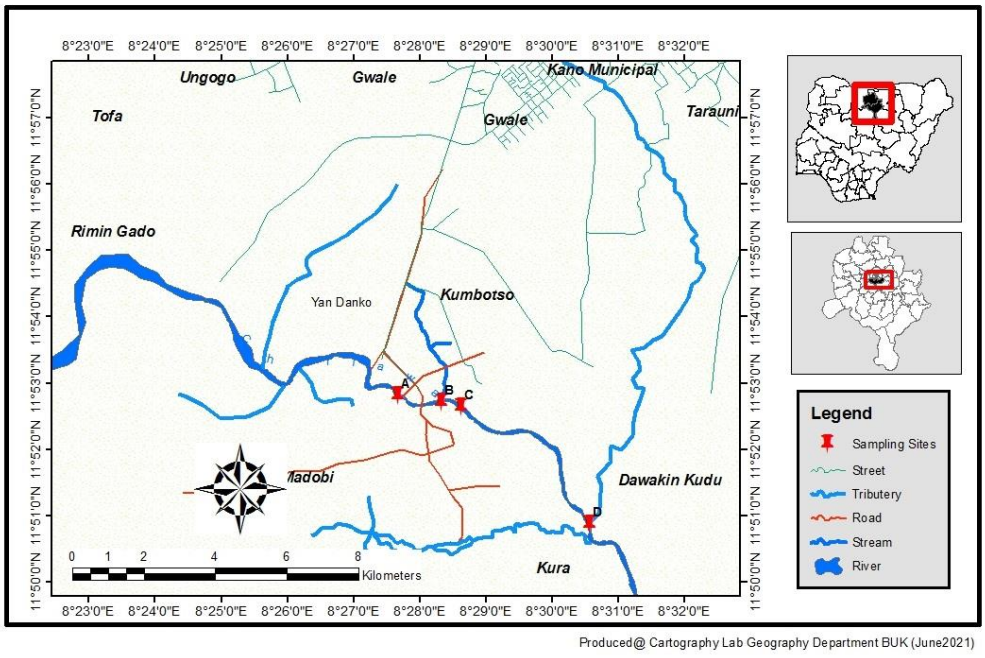

Fig 1: Map of River Challawa showing the Sampling Sites (Cartography Lab, Dept of Geography, 2021).

Sample Collection

All the samples for this research work were collected once, every month across 12 months in triplicates.

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°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 Thompson (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 sample digestion was conducted with the aid of 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 and lighter-colored solution were obtained. The digest was 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 the sediment was taken 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 digest was kept in a labeled 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 mussel tissue was taken 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 digest was kept in a labeled plastic container before analysis.

Determination of Heavy Metals

Atomic Absorption Spectrophotometer (Agilent Technologies model 200 series AA) was used to determine the levels of heavy metals analysis 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**)**.

Determination Of Oxidative Stress Biomarkers

Whole soft tissues from each specimen (n = 3 for each site) were dissected out and immediately homogenized (1:3) in phosphate buffer 100 mM, p^H 7.4. Homogenates were then centrifuged at 9000 G at 4 °C for 30 min. After centrifugation, supernatants were collected and immediately used for the determination of enzyme activities. Catalase activity was measured following the decrease of absorbance at 240 nm due to H_2O_2 consumption according to Aebi (1983). An extinction coefficient for H_2O_2 of 40 M^{-1} cm^{-1} (Abel, 1974) was used in the calculation. The obtained supernatant was used for the assay of superoxide dismutase (SOD) activity, which was based on its ability to inhibit the oxidation of epinephrine by superoxide anion (Aksnes and Njaa, 1981). The enzyme activities were assayed with an SP 1800 UV/VIS Spectrophotometer.

Glutathione activity was analyzed by preparing tissue samples by washing them twice with PBS. 0.1 g of the sample was added into a homogenizer, 1 mL reagent was added (the proportion of tissue and reagents are kept constant) and this was fully ground on ice (using liquid nitrogen gave a better grinding effect). Centrifugation was done at 8000 x g for 10 minutes at 4 ^oC, the supernatant was also placed at 4 ^oC. The spectrophotometer was then preheated for 30 minutes and adjustment was made to a wavelength of 412 nm with distilled water.

DATA ANALYSES

Two-way analysis of variance (ANOVA) was applied to determine the significant mean differences in oxidative stress biomarkers and heavy metals levels in mussels, water and sediments 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 fish 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.

RESULTS AND DISCUSSION

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. (Vilia-Elena, 2006, Cheang *et al.*, 2021).

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 - 0.850 1mg/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, 2020). 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) and Ismawati *et al.* (2021) 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).

Manganese concentration in water ranged between 0.335 and 0.417 mg/L with a mean of 0.378 mg/L. The highest concentration of Mn was observed in site B with the least value reported in site D which could be a result of the industrial discharge. These values are within the permissible limits of 0.4 mg/L. Udiba *et al.* (2014) reported 0.24 mg/L as the concentration of Mn in water from Challawa dam which is within the permissible limits of WHO (2008). According to Atanasov *et al.* (2013), levels of Mn from the Tundzha River ranged from 0.021 mg/l to 0.186 mg/l which could be explained by the fact that wastewater from settlements, industrial enterprises, agricultural activities and probably by mining in the region of Tvarditsa town flows into it.

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 0.05 mg/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).

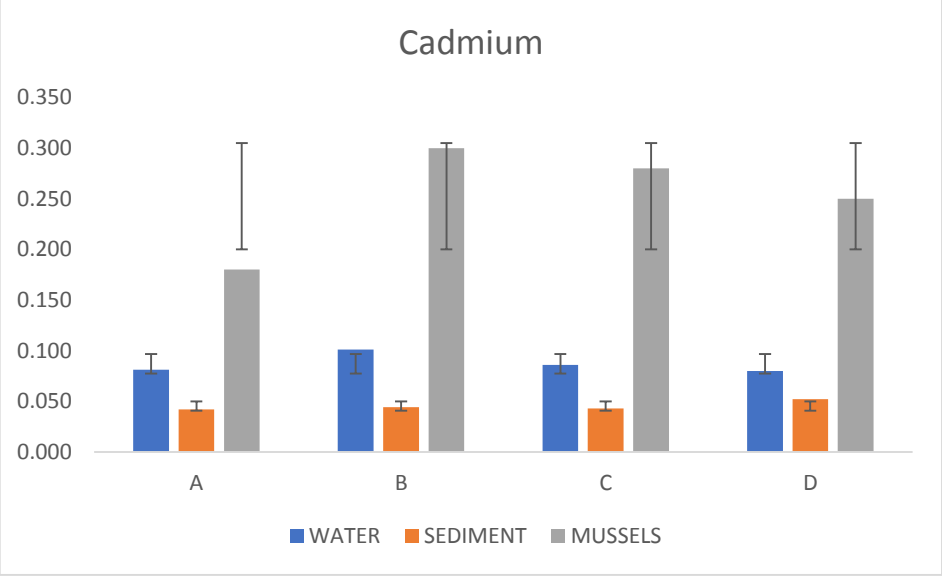

Fig 2: Mean Levels of Cadmium in Water (mg/L), Sediment (mg/kg) and Mussels (mg/kg) from River Challawa

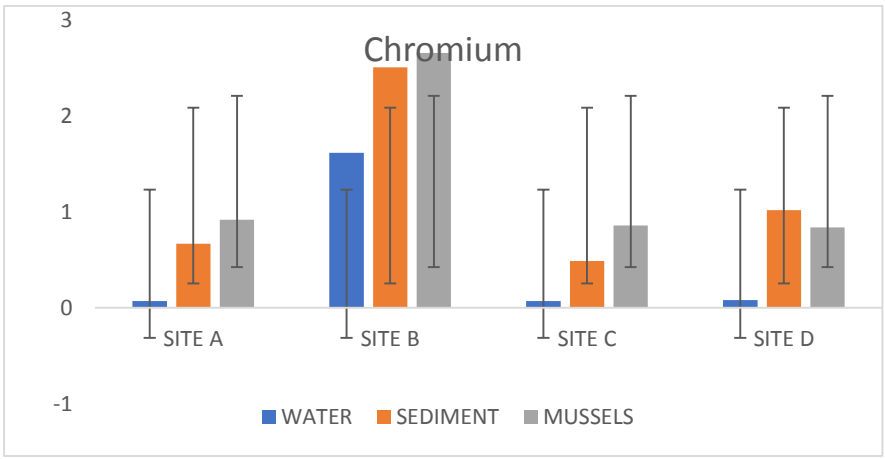

Fig 3: Mean Levels of Chromium in Water (mg/L), Sediment (mg/kg) and Mussels (mg/kg) across Sampling Sites from River Challawa

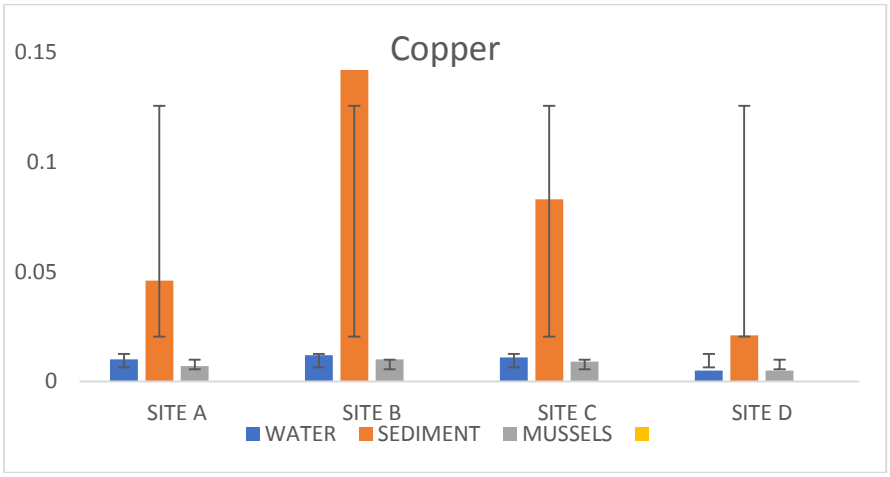

Fig 4: Mean Levels of Copper in Water (mg/L), Sediment (mg/kg) and Mussels (mg/kg) across Sampling Sites from River Challawa

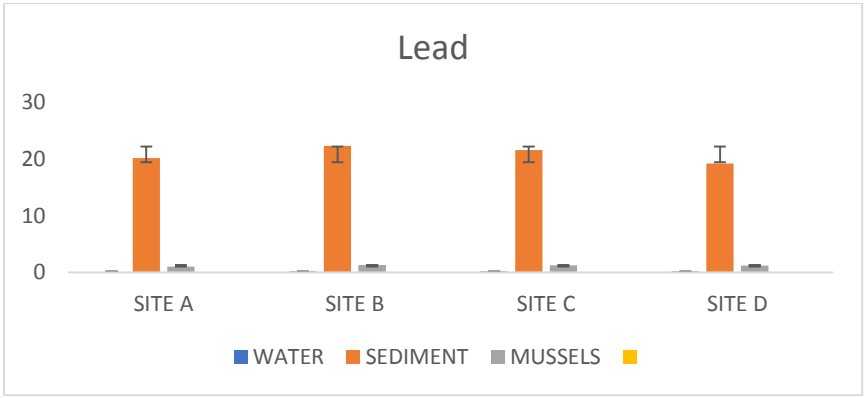

Fig 5: Mean Levels of Lead in Water (mg/L), Sediment (mg/kg) and Mussels (mg/kg) across Sampling Sites from River Challawa

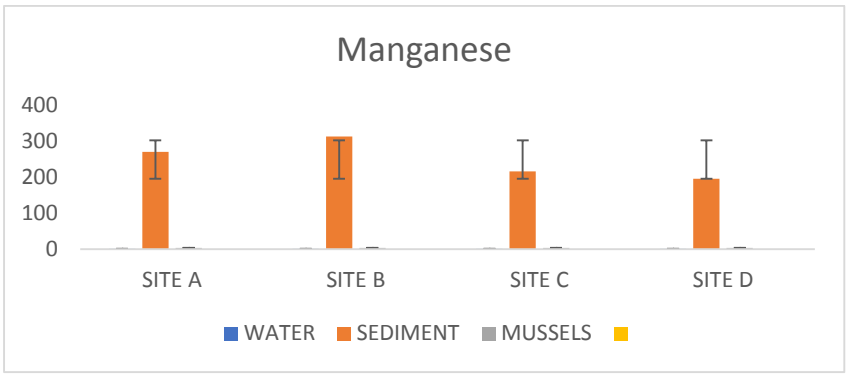

Fig 6: Mean Levels of Manganese in Water (mg/L), Sediment (mg/kg) and Mussels (mg/kg) across Sampling Sites from River Challawa

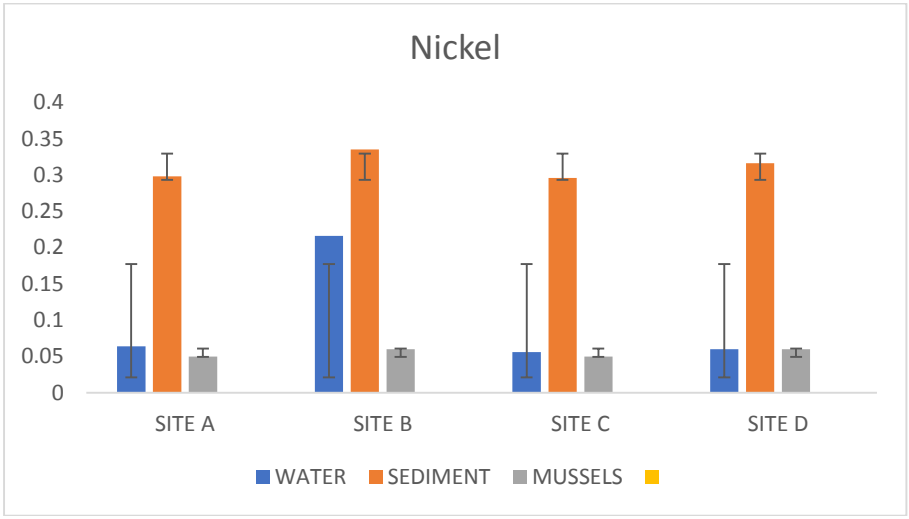

Fig 5: Mean Levels of Nickel in Water (mg/L), Sediment (mg/kg) and Mussels (mg/kg) across Sampling Sites from River Challawa

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

Seasons	Cd	Cr	Cu	Mn	Ni	Pb
Rainy	0.086±0.01[a]	0.589±0.96[a]	0.010±0.006[a]	0.381±0.11[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]	0.375±0.09[a]	0.105±0.100[a]	0.125±0.087[a]

Means followed by superscripts with same letters across the columns are not 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.

Manganese concentration in sediment had a mean value of 247.95 mg/L. Similar to Mn in water, the highest value of 312.47 was also recorded in site B. Muneer *et al.* (2022) reported a range of 5.403 - 9.581 mg/kg of Mn from Mangla lake in Kashmir. These values are far below those obtained in this study. Huang *et al.* (2021) have reported that Mn is lethal to aquatic organisms, such as invertebrates and fishes long-term and that sublethal toxicity of Mn (II) in sediment showed that the growth and reproduction of *C. elegans* are inhibited.

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 Sediments across Seasons

Seasons	Cd	Cr	Cu	Mn	Ni	Pb
Rainy	0.045± 0.006[a]	0.045± 0.006[a]	0.076± 0.039[a]	247.85± 61.27[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]	248.08± 60.61[a]	0.311± 0.04[a]	21.04± 4.99[a]

Mean followed by superscript with the same letters across the columns are not 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.* (2012) 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 Karlsson *et al.* (2012) 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. Novakor *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.

Manganese in mussels ranged between 3.23 and 3.66 mg/kg with a mean of 3.39 mg/kg. This value is less than 6.637 mg/kg obtained by Hossain *et al.* (2022) from the fish market of Bangladesh but higher than the range of 0.055 - 0.121 mg/kg obtained by Muneer *et al.* (2022) from Mangla dam in Kashmir. Levels of Mn in the fish species from River Challawa revealed *Clarias gariepinus* has the highest concentration of 6.07 µg/g which has exceeded the WHO (1989) guideline of 0.01 ppm and FEPA (2003) limits of 0.05 ppm (Taoheed and Said, 2014).

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 defenses, 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.* (2017) 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, there 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 Seasons

Seasons	Cd	Cr	Cu	Mn	Ni	Pb
Rainy	0.25±0.07^{a}	0.25±0.07^{a}	0.007±0.004^{a}	3.39±0.68^{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}	3.39±0.61^{a}	0.06±0.01^{b}	1.17±0.26^{a}

Mean followed by superscript with the same letter across the columns are not significant at *p>0.05*

Oxidative Stress Biomarkers

Superoxide Dismutase (SOD)

Levels of SOD ranged between 12.15 and 39.43 µ/ml from sites C and B respectively. The highest mean value recorded was in site B with 35.22 µ /ml. Lowest mean value recorded was in site C with a value of 16.48 µ/ml. There were significant differences in the values of SOD across all sites except between sites C and D (Table 4.11). However, there was no significant variation (p > 0.05) in means of SOD across wet and dry seasons as shown in table 5.

The activity of SOD was elevated across all sites with the highest level at site B (23.10 µ /mol). This could be as a result of heavy metals in all sites with site B having the highest concentration. The accumulation of heavy metals might have triggered the production of superoxide anions. This results to the induction of SOD whose primary role is to accelerate the dismutation of the toxic superoxide radical (O^{2-}) produced during oxidative processes to Hydrogen peroxide and Oxygen. This is in agreement to the findings of Musa and Imam (2021). Biochemically, the first and the most effective antioxidant enzyme that acts against the free radical formation process is SOD (Ighodaro and Akinloye, 2018; Yang *et al.*, 2019). The significant increase in SOD values in this study may be a response of the first defense system to ROS induced by xenobiotics in the tested water as indicated by Ighodaro and Akinloye (2018), Sedeno-Diaz and Lopez-Lopez (2022). Similarly, the nutrients from agricultural runoff imply the presence of other agrochemicals, their metabolites, and byproducts could also form potential ROS. These results are in agreement with Castañeda-Chávez and Lango-Reynoso (2021), Sedeno-Diaz and Lopez-Lopez (2022).

Catalase (CAT)

Levels of CAT recorded in mussels from River Challawa had the highest mean of 39.78 µ/ml in site B, while the lowest mean of 5.26 u/ml was reported in site A. There were significant variations across all sites ($p < 0.05$), however, differences between wet and dry seasons were not significant ($p > 0.05$).

Levels of CAT recorded in mussels from River Challawa had the highest mean of 39.78 µ/ml in site B, while the lowest mean of 5.26 µ/ml was reported in site A. This could be as a result of higher levels of pollutants in the site, which contributed to higher levels of SOD, therefore, resulting to higher level of CAT. Activities of CAT and SOD are two indicators of oxidative stress. There is usually a rise of ROS and reactive metabolites as a result of the interactions between different enzyme systems, including detoxifying enzymes (Akinsanya *et al.*, 2020). CAT degrades hydrogen peroxide that SOD produces by the dismutation of superoxide ion in periods of prolonged stress (Batista *et al.*, 2014). According to Akinsanya *et al.* (2020), increased CAT activity can prevent the potential toxicity of free radicals which consequently may protect the mussels from oxidative damage.

Glutathione (GSH)

Levels of GSH in mussels from River Challawa ranged from 3.45 µg/ml in site A to 21.45 µg/ml in site B. Differences in means between the sites were significant except between sites A and C ($p < 0.05$). Differences in means of GSH across wet and dry seasons were not significant ($p > 0.05$).

Levels of GSH in mussels from River Challawa ranged from 3.45 µg/ml in site A to 21.45 µg/ml in site B. GSH showed an elevated level in all the sites and GSH is well understood to be a substrate for the activity of GST. The increase recorded in GSH formation in a high level suggests an adaptation and protective mechanism by this biomolecule against oxidative stress induced by heavy metal and pesticide residues which agrees with the findings of Musa and Imam (2021) and Farombi *et al*. (2007).

Table 4: Mean Concentrations of Oxidative Stress Enzymes in Mussels across Sites

Sites	SOD (u/ml)	CAT (u/ml)	GSH (µg/ml)
Site A	23.10 ± 2.68^{b}	5.26 ± 1.71^{d}	7.75 ± 2.02^{bc}
Site B	35.22 ± 1.80^{a}	39.78 ± 3.33^{a}	18.06 ± 1.57^{a}
Site C	16.48 ± 1.40^{c}	26.88 ± 2.65^{b}	8.37 ± 0.83^{b}
Site D	16.79 ± 1.26^{c}	24.72 ± 1.68^{c}	7.45 ± 0.89^{c}

Means followed by superscripts with different letters across the columns are significant at *p<0.05*

Table 5: Mean Concentrations of Oxidative Stress Enzymes in Mussels across Seasons

Seasons	SOD(u/ml)	CAT(u/ml)	GSH(µg/ml)
Dry	23.06 ± 7.73^{a}	24.70 ± 12.77^{a}	10.62 ± 4.69^{a}
Wet	22.08 ± 8.02^{a}	23.40 ± 12.46^{a}	10.11 ± 4.65^{a}

Means followed by superscripts with different letters across the columns are significant at *p<0.05*

CONCLUSION

The results from this study confirmed the presence of heavy metals (Cd, Cr, Cu, Mn, Ni and Pb) in River Challawa water, sediment and in the freshwater mussel *Anodonta marginata.* Similarly, as a result of pollutants and their accumulation in the tissues of mussels, the oxidative stress biomarkers showed a considerable level of antioxidant activity. The results from this study also confirm that mussels are good indicators for assessing aquatic pollution, particularly when searching for trace elements. 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 also pose risks and hazards to humans and ecosystems through direct contact or ingestion, contaminated drinking water, and reduced food quality among others. Pollution of the environment with traces of heavy metals from anthropogenic sources should therefore not be ignored. Government should enforce laws 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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