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Research Article

Linking Metal Contamination to Catalase Activity in *Labeo rohita*: Insights from the Indus River of Pakistan

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Abstract

This study examined catalase enzyme activity in relation to metal pollutant bioaccumulation in various body parts of *Labeo rohita* collected from different fishing sites along the Indus River, including Sukkur Barrage (SB), Guddu Barrage (GB), and Kotri Barrage (KB). A total of ten fish samples were captured from each site. The selected fish were dissected on-site to collect the liver, gills, muscle tissue, and kidneys. The extracted organs were stored in labeled polythene bags placed in crushed ice and transported to the laboratory for further analysis. Water samples from each site were also collected to measure the concentrations of heavy metals, including nickel (Ni), lead (Pb), cadmium (Cd), cobalt (Co), and mercury (Hg). The collected organ samples were prepared to assess catalase enzyme activity using a spectrophotometer at 240 nm. Both water and tissue samples were analyzed for metal content following standard procedures. Catalase activity in the liver, kidney, gills, and muscle tissues was highest in samples from SB, followed by GB, and lowest in KB. The concentration of heavy metals in both water and fish organs followed the order: Ni > Pb > Co > Cd > Hg. The findings suggest that measuring antioxidant enzyme activity and heavy metal bioaccumulation in fish tissues can serve as an early indicator of environmental pollution, helping to detect contamination levels before they reach thresholds that could harm aquatic life and disrupt ecosystem health. Monitoring enzyme activity in local fish populations can thus provide valuable insights into the ecological status of riverine systems..

Keywords: Antioxidants; heavy metals, catalase activity, *Labeo rohita*, Indus river

1. Introduction

The aquatic ecosystem's health and ecological integrity are severely impacted by the harmful contaminants, like heavy metals, that are emitted from different sources. In the recent decades, the pollution of aquatic ecosystems has become a major environmental concern [1]. Aquatic species require trace amounts of metals, which are extensively present in water for normal physiological and biological processes [2]. Aquatic system environmental contamination is a major worldwide issue. When commercial, industrial, and agricultural pollutants leak into the aquatic environment, it can have a variety of harmful effects on aquatic life. Fish have been seen to take up these contaminants through the food chain and directly from contaminated water [3].

Labeo rohita belongs to family Cyprinidae is the most culturable fish species in different areas of Pakistan due to its commercial values and good taste [4]. It occupies the major riverine system of Pakistan. *L. rohita* is of great commercial importance (owing to the ease of cultivation) and renowned for taste. Regarding the effects of heavy metal stress on this species, there is a dearth of information on antioxidant enzymes and nutritional composition.

Indus River in Pakistan is the major river system with seven barrages namely Jinnah, Tarbela, Chashma, Taunsa, Sukkur, Guddu and Kotri Barrage [5]. Pakistan's economic and agricultural growth have been greatly aided by the year-round availability of sufficient water. Heavy metals are considered to be major deadly contaminants for the aquatic ecosystem [6].

Heavy metals have ability to change the quality of freshwater and aquatic bodies especially fish, which sometimes metabolize metals to even more harmful derived compounds [7]. These metals accumulate and stored in the bodies of aquatic organisms enters into the humans ultimately by the food chain [8]. Aquatic species require trace amounts of metals, which are extensively present in water for normal physiological and biological processes [2].

Fish have an antioxidant enzyme system to protect them from oxidative damage. Reactive oxygen species (ROS) associated with oxidative stress can be very intracellular and can have deleterious effects on proteins, lipids, and DNA under physiological conditions in the body [9]. In actuality, oxidative stress a state in which the generation of the reactive oxygen species and antioxidant defense are out of balance can result in protein denaturation, lipid peroxidation, apoptosis, and ultimately destructive cell damage. According to Mishra et al. [10], in order to protect themselves from the harmful effects of ROS that are produced naturally, living things have evolved an antioxidant defense system that is divided into two distinct classes, the antioxidant system, that includes enzymes like catalase, glutathione reductase, superoxide dismutase and glutathione peroxidase and the non-enzyme antioxidants like glutathione, vitamin E and vitamin C. Additionally, these enzymes protect the organism against the harm that oxy-radicals can bring to proteins, nucleic acids and lipids [11].

Catalase exists as a key antioxidant enzyme in aerobic organisms to protect cells from oxidative damage. The enzyme functions to decompose hydrogen peroxide (H_2O_2) which is a reactive oxygen species into water and oxygen for prevention of harmful free radical accumulation [12]. Catalase activity serves as one of the body's primary defenses against oxidative stress because it demonstrates an organism's capacity to fight environmental pollutants together with heavy metals. The scientific community lacks detailed studies which explore antioxidant responses of *L. rohita* through catalase activity assessments. *L. rohita* serves as an important farmed species across South Asia particularly within Pakistan but scientists require more information about how this fish defends itself biochemically during environmental stress.

Fish ingest heavy metals mostly through their gills, skin, and diet [13]. Among the fish's most vital organs are the kidney, liver, gills and muscle tissues which regulate various processes including breathing, metabolism, digestion, and excretion. Several enzymes found in the body regulate the antioxidant activities of the liver. The liver tissue is incredibly active in both storing and absorbing heavy metals. It's possible that the liver's storage and detoxification of heavy metals from diet contribute to the liver's greater concentration of heavy metals [14]. The gills are regarded as the most significant organ among the others.

This study focused on establishing a link between metal accumulation in *L. rohita* organs alongside changes in catalase enzyme activity for understanding possible environmental effects on immune system

2. Materials and Methods

2.1. Fish sampling and extraction of organs

Using the gill nets, samples of *L. rohita* (500 g – 1500 g) were collected from various fishing sites including the Sukkur Barrage, Guddu Barrage and Kotri Barrage of the Indus River (10 samples from each location). At the specified sampling locations, the selected fish samples were dissected to remove the chosen organs (kidney, liver, gills and muscle tissues). The extracted organs were stored in the crushed dry ice boxes with polythene bags marked before being transferred to the laboratory for further analyses. In addition, the water samples of sampling sites were also collected both for physicochemical parameters and metal detection

2.2. Determination of physicochemical parameters

Numerous physicochemical characteristics such as pH, Temperature, CO_2, Ammonia, total hardness, alkalinity, Na, K, etc. of the water samples were measured using the techniques described in [15].

2.3. Preparation of homogenization of extracted organs

In laboratory, the extracted organs were divided into two groups (one for enzyme and second for metal detection). The part for catalase activity is homogenized in phosphate buffer (pH 7).

2.4. Analysis of catalase enzyme activity (assay)

Catalase activity was measured with the help of spectrophotometer at 240 nm by following the methods of Chance and Mehaly [16]. In order to prepare the buffer substrate solution, 10 mM H_2O_2 (0.442 mL) was dissolved in 60 Mm phosphate buffer. In a cuvette, 1.95 mL of buffered substrate solution was taken. Added 0.05 mL of enzyme extract and was placed inside a spectrophotometer, which was adjusted to zero at wavelength of 240 nm. 3 minutes was the reaction time, and the readings of absorbance were noted after 1-minute interval.

2.5. Detection of metals

To determine the presence of all selected metals, standard methods described in AOAC [17] were followed. 1g of weighted samples were placed into open-mouthed conical flask. After adding 30 mL of nitric acid, the flask was placed back on hot plate. 10 mL of perchloric acid were added after the mixture had boiled, and it was placed on the hot plate once more until 1 mL of colorless liquid remained. It was diluted with 100 mL of distilled water until it was perfectly clear. Filter paper was used to clear all of the particulate matter from digestion solution in this last volume before the mineral analysis. Using an atomic absorption spectrophotometer, the levels of selected heavy metals, including Zn, Co, Ni, Pb, and Cu, were measured in chosen organs.

2.6. Statistical analysis

The collected data's mean standard deviation (Mean ± SD) was calculated in order to calculate the statistical differences. Statistix 8.1 software was used for measuring correlation between physicochemical parameters. The statistical differences among different sites, fish organ tissues and CAT activity were measured at *$p<0.05$* by applying linear mixed model design in R software [18].

3. Results and discussion

3.1. Water physico-chemistry analysis

Various physicochemical parameters including temperature (°C), pH, electrical conductivity ($mScm^{-1}$), dissolved oxygen (mgL^{-1}), calcium (mgL^{-1}), carbon dioxide (mgL^{-1}), potassium (mgL^{-1}), magnesium (mgL^{-1}), total ammonia (mgL^{-1}), sodium (mgL^{-1}) and total hardness of sampling sites by following the standard methods described in [15]. The correlation coefficient among various physicochemical parameters of different sites water samples were given in the table 1 to 3 in the present study.

3.2. Comparative catalase activity in different tissues of *L. rohita*

Water pollution has serious negative effects on aquatic ecosystems, water quality, as well as the environment, human health, and food security. It is caused by toxins found in both point and non-point sources, including fertilizers, pesticides, sediment, and more [19]. Dumping of industrial wastes into water resources may cause water pollution that is very dangerous for the aquatic life. Heavy metals that are accumulated and stored in the bodies of aquatic organisms consequently by the food chain ultimately enters into the humans [8]. Different metals are deteriorating water from river largely [20].

High catalase activity was noted in the liver (144.6±4.29), gills (176.67±1.33), kidney (238.67±2.67) and muscle (118 ±6.55) tissues of fish captured from Sukkur Barrage as compared to other sites. The order of catalase activity as site wise was noted as Sukkur Barrage > Guddu Barrage > Kotri Barrage in this study (Fig 1). According to Ahmed et al. [21], oxidative stress in aquatic species is caused by heavy metal contamination in the water, which ultimately results in an organism's mortality. Reactive oxygen species (ROS) are controlled by the antioxidant defense mechanism, which also shields an organism from oxidative stress [22]. Elevated renal CAT activity is associated with increased production of ROS or oxidative stress by metal mixture in fish [21].

Further, redox-inactive metals (Pb, Cd and Hg) and redox active metals (Cu, Cr and Fe) can cause significant increases in rate of ROS production and followed by a situation known as oxidative stress that becomes the reason of several dysfunctions in DNA, proteins and lipids [22,23]. Antioxidant enzymes, especially CAT and superoxide dismutase are affected in the presence of cadmium because it displaces copper and iron from these enzymes.

Table 1. Correlation coefficients among physicochemical parameters of Sukkar Barrage.

Parameters	Ca	DO	EC	K	Mg	NH_3	Na	TH	Temperature
Ca									
DO	0.972								
EC	-0.32	-0.09							
K	-0.96	-0.86	0.57						
Mg	0.00	-0.23	-0.94	-0.27					
NH3	-0.61	-0.40	0.94	0.80	-0.79				
Na	0.86	0.95	0.18	-0.69	-0.50	-0.13			
TH	0.00	0.23	0.94	0.27	-1.00	0.7924	0.50		
pH	-0.86	-0.72	0.75	0.97	-0.50	0.9245	-0.50	0.50	0.0

Note: TH = Total Hardness; NH3 = Total Ammonia; DO = Dissolved Oxygen; EC = Electrical conductivity; Na = Sodium; K = Potassium; Ca = Calcium; Mg = Magnesium

Table 2. Correlation coefficients among physicochemical parameters of Guddu Barrage.

Parameters	Ca	DO	EC	K	Mg	NH_3	Na	TH	Temperature
Ca									
DO	-0.99								
EC	0.27	0.27							
K	-0.49	0.50	-0.69						
Mg	1.00	-0.99	-0.27	-0.49					
NH3	0.92	-0.90	0.05	-0.72	0.92				
Na	0.49	-0.49	-0.97	0.49	0.49	0.18			
TH	-0.99	1.00	0.27	0.50	-0.99	-0.90	-0.49		
pH	0.99	-0.97	-0.27	-0.48	0.99	0.94	0.49	-0.9	0.00

Note: TH = Total Hardness; NH3 = Total Ammonia; DO = Dissolved Oxygen; EC = Electrical conductivity; Na = Sodium; K = Potassium; Ca = Calcium; Mg = Magnesium

Due to direct binding of cadmium with CAT active site, its enzymatic activity inhibited. Inhibition of CAT activity is related to the binding of heavy metal ions to thiol (-SH) groups of the enzyme. As a result, ROS increased in number [24].

3.3. Comparative level of selected heavy metals in water samples

Concentration of selected metals was found as Ni (0.01 ppmL^{-1}), Cd (0.0006 ppmL^{-1}), Pb (0.0025 ppmL^{-1}), Co (0.0005 ppmL^{-1}) and Hg (0.00005 ppmL^{-1}) in water sample taken from Sukkur Barrage. Similarly, the concentration of selected metals was found as Ni (0.014 ppmL^{-1}), Cd (0.0001 ppmL^{-1}), Pb (0.0045 ppmL^{-1}), Co (0.00073 ppmL^{-1}) and Hg (0.00001 ppmL^{-1}) in water sample taken from Guddu Barrage. The heavy metal concentration was found as Ni (0.0189 ppmL^{-1}), Cd (0.0001 ppmL^{-1}), Pb (0.0045 ppmL^{-1}), Co (0.0093 ppmL^{-1}) and Hg (0.00001 ppmL^{-1}) in water sample taken from Kotri Barrage. Order of heavy metals at all sites were observed as Ni> Pb> Cd> Co> Hg (Fig 2).

3.4. Comparative concentration of heavy metals in various body tissues of *L. rohita*

The results for selected heavy metals in different tissues of *L. rohita* indicated that there was a higher amount of Ni compared to other metals. Order of selected heavy metals was observed as Ni> Pb> Co> Cd> Hg (Fig 3). The results of this study indicate that, in comparison to other organs, fish liver and kidneys acquire a large amount of metals. It suggests that the kidney and liver of fish are more actively involved in their metabolic processes than the muscles and gills, which only gather little amounts of metals. Ni is a major contaminant in aquatic settings, and its toxicity can damage the immunological and reproductive systems as well as induce respiratory cancer. These complications must be considered, and more investigation into aspects such as other pollutants, metabolic variations across species, and possible interactions between Cu and other trace elements that may impact Cu accumulation in fish tissues, is necessary. It is generally known that plants and terrestrial animals require nickel as a nutrient, and there is growing evidence that fish also likely require nickel [25]. But it can be dangerous in high doses [26]. Pb and Cd are hazardous heavy metals that come naturally through erosion and manmade activities that he adopted for his own benefits such as pesticides.

Table 3. Correlation coefficients among physicochemical parameters of Kotri Barrage.

Parameters	Ca	DO	EC	K	Mg	NH_3	Na	TH	Temperature
Ca									
DO	-0.89								
EC	-0.98	0.81							
K	-0.18	0.52	0.00						
Mg	-0.180	-0.47	0.00	-0.98					
NH3	-0.98	0.81	1.00	0.0	0.00				
Na	-0.18	0.49	0.00	0.99	-0.99	0..0000			
TH	0.50	-0.71	-0.32	-0.93	0.94	-0.32	-0.94		
Temperature	0.18	-0.47	0.00	-0.98	1.00	0.00	0.99	0.94	
pH	0.17	-0.33	0.00	-0.87	0.94	0.00	-0.919	0.89	0.94

Note: TH = Total Hardness; NH3 = Total Ammonia; DO = Dissolved Oxygen; EC = Electrical conductivity; Na = Sodium; K = Potassium; Ca = Calcium; Mg = Magnesium

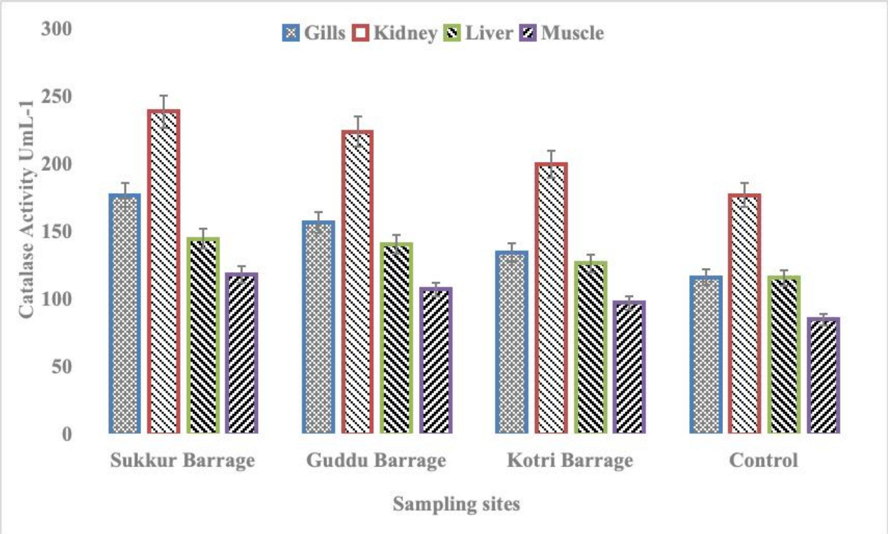

Figure 1. Comparative catalase a ctivity in different tissues of *L. rohita..*

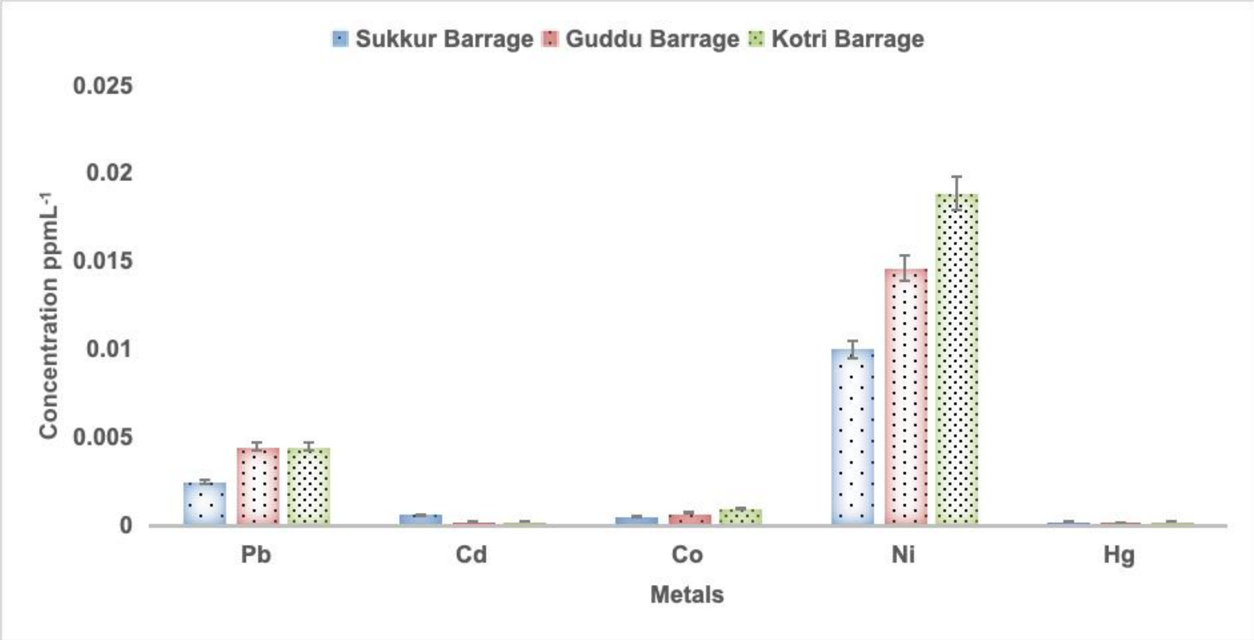

Figure 2. Comparative level of selected heavy metals in water samples.

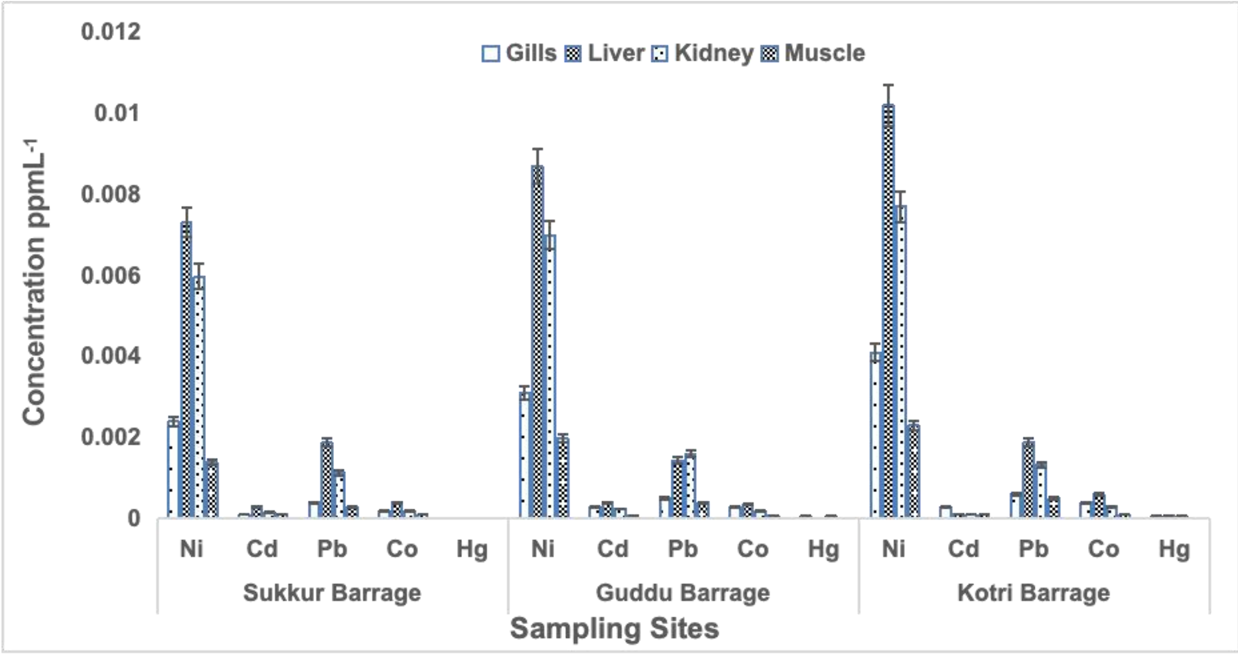

Figure 3. Comparative concentration of heavy metals in various body tissues of *L. rohita*

Due to increasing population and high demand of routine products, a lot of toxic pollutants produce on daily basis which does not disposed properly on safe level get released in open environment and get dumped in freshwater to a toxic level [27]. The current study found that, as compared to other places, the selected organs of the fish obtained from Sukkur Barrage had greater catalase activity. The presence of pollutants in the aquatic environment is the cause of the increased Catalase level found in our investigation at Sukkur Barrage. When contaminants are taken up by a fish from its environment in dissolved form, it causes oxidative stress in various body regions [28]. when a result of this oxidative stress, antioxidant activity will rise when ROS are first broken down into fewer ROS [29]. The presence of metals in both water sample and organs of fish may be due to mining, electroplating, and smelting industries in nearby region from where they get runoff into freshwater [30, 31]. Heavy metals such as Cd, Pb, Hg, and arsenic (As) are known to induce oxidative stress in aquatic organisms by generating ROS [32, 33]. Habib et al. [34] stated that metal pollution in water is mostly due to human activities by investigate both water and fish organs from the Chashma Barrage. Similarly, Chatha et al. [35] stated that heavy metals have an adverse effect on both the aquatic ecosystem and human population. Aquatic species are helpful to investigate the water quality [36].

4. Conclusion

Aquatic pollution, particularly from heavy metals poses a significant threat to both aquatic life and human health. The findings showing that some tissues contained metal concentrations that are exceeding if not controlled from recommended safe levels. The water can possibly maintain fish farming operations when under strict management followed. The inferences also showing that heavy metals trigger alterations in fish antioxidant defenses which triggered enhanced catalase activity. The study demonstrated that measuring of catalase activity represents a crucial tool for environmental monitoring because it functions as a biomarker of ecological pollution along with its correlates of health hazards to aquatic organisms. Further studies must analyze how heavy metals change across different seasons and locations as researchers need to understand how prolonged metal exposure affects fish quality while employing several biomarkers to determine environmental health properly. It is necessary to work on developing remediation methods and strengthening regulatory rules that control heavy metal pollutants entering freshwater ecosystems.

Data availability statement

The data supporting the results of this study can be obtained from the corresponding author upon request.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Ethical approval

Not applicable (N/A)

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Authors Contribution

TA conceptualized the study, NM conducted the study, MHB and MA assisted during trial and data collection, HN, AY, NM and ET analyzed the data, NM and TA wrote the article and HN reviewed the article.

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