International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 10-03-2026, Accepted: 10-04-2026, Published: 25-04-2026
Volume 11, Issue 2, 2026, Page No. 39-42

Assessment of heavy metal bioaccumulation in freshwater fish from the Godavari River Basin and its physiological and biochemical impacts

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Abstract
Heavy metal pollution in freshwater ecosystems is a serious environmental threat owing to its persistence, toxicity, and bioaccumulative characteristics. This study assesses the species-specific accumulation of heavy metals and the corresponding physiological effects in freshwater fish from the Godavari River basin. Fish samples were collected from three sampling cites: upstream, midstream, and downstream sites, representing diverse pollution gradients. Three frequently ingested species, *Labeo rohita*, *Catla catla*, and *Oreochromis mossambicus*, were chosen for analysis of gill, liver, and muscle tissues. The concentrations of lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), and zinc (Zn) exhibited a consistent tissue-specific hierarchy (liver > gill > muscle). Species-specific analysis indicated greater accumulation in *Oreochromis mossambicus* (Pb: 3.4 ± 0.8 mg/kg; Cd: 1.5 ± 0.4 mg/kg; Zn: 68 ± 13 mg/kg in liver), succeeded by *Catla catla* and *Labeo rohita*. Spatial variation revealed that in downstream areas, the metal concentrations were much higher (Pb: 3.12 ± 0.81 mg/kg) as compare to upstream areas metal concentrations (0.92 ± 0.25 mg/kg). This indicates that people have had a big effect on the environment with respect to heavy metal water pollution. Hematological experimentation revealed a significant reduction in blood hemoglobin (11.2 ± 0.9 to 6.8 ± 1.0 g/dL) and red blood cell counts (2.8 ± 0.4 to 1.4 ± 0.2 $\times 10^6$/mm³), accompanied by an elevation in white blood cell counts (32 ± 8 to 104 ± 18 $\times 10^3$/mm³). Biochemical characteristics indicated increased oxidative stress, as evidenced by catalase activity rising to 96.3 ± 14.8 U/mg protein and lipid peroxidation measuring at 8.7 ± 1.9 nmol MDA/mg protein. These findings demonstrate that fish are accumulating significant toxins and experiencing considerable stress, highlighting ecological dangers and potential health risks associated with consuming fish from the Godavari River basin.

Keywords: Godavari River basin, freshwater fishes, heavy metal biomagnification, oxidative stress, and hematological alterations

Introduction
Freshwater ecosystems are the last places where many pollutants end up, and heavy metals are especially bad because they don't break down and stay around for a long time. Heavy metals build up over time in aquatic ecosystems, which is different from inorganic contaminants (Sharma *et al*., 2025) [10]. The Godavari River, a major and economically significant river system in India, is increasingly facing anthropogenic pressures (Das *et al*., 2022) [3]. Accelerated industrialization, the release of untreated effluent, and extensive agricultural practices have led to the accumulation of heavy metals in this river system (Soleimani *et al*., 2023) [11]. These pollutants infiltrate aquatic food webs and ultimately bioaccumulate in fish, presenting risks to both aquatic organisms and human consumers. Fish are acknowledged as effective bioindicators of environmental pollution due to their position in higher trophic levels and their observable physiological reactions to pollutants (Abdallah *et al*., 2024) [1]. Bioaccumulation of heavy metals in fish tissues disrupts metabolic pathways, enzyme functions, and cellular integrity (Garai *et al*., 2021) [5]. The liver is essential for detoxification, gills function as the main interface with the aquatic environment, and muscle tissues are significant due to their importance for human consumption. Prior investigations in Indian river systems, including the Ganga and Yamuna, have revealed considerable heavy metal accumulation in fish tissues, alongside hematological and biochemical modifications (Kumar *et al*., 2024) [7]. Nevertheless, comprehensive assessments that amalgamate bioaccumulation trends with physiological and oxidative stress responses are scarce for the Godavari basin. This study aims to deliver a thorough and meticulous analysis of heavy metal bioaccumulation and its physiological effects in freshwater fish, based on substantiated scientific evidence.

Materials and Methods
1. Study Area and Sampling Methodology
The study was carried out across particular sections of the Godavari River basin, showcasing varying degrees of human influence. Three distinct sampling zones were established according to pollution gradients: an upstream location exhibiting minimal human impact, a midstream location primarily affected by agricultural runoff, and a downstream location subjected to industrial effluents and urban discharge. The geographical coordinates of each sampling station were documented utilizing a handheld GPS device. Sampling was conducted during the pre-monsoon season to minimize dilution effects and ensure consistent environmental conditions by the protocol denoted by Raghunath *et al*., 2026 [8]. At each location, three samples were gathered to ensure statistical reliability.

2. Selection and Acquisition of Fish Samples
Three prevalent freshwater fish species, specifically *Labeo rohita*, *Catla catla*, and *Oreochromis mossambicus*, were chosen due to their ecological importance and popularity in consumption. To address sampling bias, fish specimens

were collected using standardized gill nets and cast nets. Only healthy, adult fish of comparable size (average length 20–30 cm and weight 500–800 g) were selected to minimize variability arising from differences in age and size. At each location, there were between 12 and 15 individuals of each species observed. Following the harvest, the fish were meticulously rinsed with site water to eliminate any debris clinging to their surfaces. Subsequently, the fish specimens were carefully placed in ice boxes for transportation to the laboratory. The fish specimens were stored in a deep freezer at approximately 4°C to prevent their metabolism from deteriorating.

3. Morphometric Assessments and Anatomical Dissection

In the laboratory, fish specimens underwent a meticulous rinsing process with distilled water to make sure that all the surface contaminants are eliminated from the fish body surface. A measuring scale and a digital balance were employed to quantify morphometric parameters such as total length, standard length, and body weight. Specimens were carefully examined in a controlled sterile setting. Target tissues, such as gill, liver, and muscle, were meticulously excised with sterilized stainless steel instruments. Tissues underwent washing with a physiological saline solution to remove blood and contaminants, followed by blotting to dryness and weighing. The samples were separated into two portions: one was stored at −20°C for biochemical analysis, while the other underwent testing for heavy metal concentrations.

4. Sample Preparation and Acid Digestion for Heavy Metal Analysis

The tissue samples underwent drying in an oven set at 80°C until achieving a stable weight. Utilizing a sterile mortar and pestle, the materials were meticulously ground into a fine powder. A dried tissue sample weighing about 1 g was subjected to acid digestion using a 4:1 mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$). The digestion process was conducted in a hot air oven at 120°C until a transparent, colorless solution was achieved. The digested samples were cooled, filtered using Whatman No. 42 filter paper, and diluted to a specified volume (typically 25 mL) with double-distilled water. All glassware utilized in the procedure was pre-treated with 10% nitric acid and meticulously rinsed to prevent contamination.

5. Measurement of Heavy Metals

The levels of cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), and zinc (Zn) were measured using Atomic Absorption Spectrophotometry (AAS) with appropriate hollow cathode lamps as mentioned by Wu *et al.*, 2022 [12]. Calibration curves were developed using standard solutions that contained known concentrations for each metal. Instruments were calibrated before analysis, and quality control measures were maintained by using reagent blanks and standard reference materials. Quantification of metal concentrations was performed in milligrams per kilogram (mg/kg) of dry tissue weight.

6. Blood Collection and Hematological Assessment

Blood samples were obtained from the caudal vein of live fish utilizing sterile syringes pre-treated with anticoagulant (EDTA). The concentration of hemoglobin was measured by cyanmethemoglobin technique. Red blood cell (RBC) and white blood cell (WBC) quantifications were conducted utilizing a Neubauer hemocytometer under a light microscope. Hematocrit (HCT) values were determined via the microhematocrit method by centrifuging blood-filled capillary tubes at 10,000 rpm for 5 minutes.All hematological parameters were assessed in triplicate to guarantee precision.

7. Preparation of Tissue Homogenate for Biochemical Analyses

Fresh liver and gill tissue samples were homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) utilizing a tissue homogenizer. The homogenate underwent centrifugation at 10,000 rpm for 15 minutes at 4°C, resulting in the collection of the supernatant for subsequent enzymatic analysis.The protein concentration in the homogenate was measured using the Lowry method to assess enzyme activity.

8. Evaluation of Biochemical Markers for Oxidative Stress

Investigation of Catalase (CAT) Activity

The decomposition rate of hydrogen peroxide (H_2O_2) was used to quantify catalase activity, which is expressed in units per milligram of protein. The evaluation of SOD activity involved measuring its capacity to prevent the reduction of nitroblue tetrazolium (NBT), with results reported as U/mg protein. Lipid Peroxidation (LPO)

Lipid peroxidation levels were measured using the thiobarbituric acid reactive substances (TBARS) method and expressed as nmol malondialdehyde (MDA) per mg of protein.

9. Quality Assurance and Quality Control (QA/QC)

To guarantee the dependability of outcomes:

a. All reagents employed were of analytical grade.
b. Glassware underwent acid washing to avert contamination.
c. Blanks and standards were incorporated into each analytical batch.
d. Redundant analyses were conducted for specific samples
e. Instrument calibration was periodically verified.

10. Statistical Evaluation

All experimental data were presented as mean ± standard deviation (SD). Statistical analysis was conducted utilizing software including SPSS.One-way analysis of variance (ANOVA) was employed to ascertain significant differences among sampling locations and tissues. The post hoc Tukey's test was utilized for multiple comparisons. A Pearson correlation analysis was performed to assess the relationships between heavy metal concentrations and physiological parameters. A significance level of $p < 0.05$ was deemed statistically significant.

Observations and Results

1. General Observations and Morphological Condition

Fish specimens collected from diverse sites within the Godavari River basin exhibited notable variations in physical condition corresponding to the pollution gradient. Subjects from upstream locations demonstrated robust health, showcasing typical coloration, immaculate fins, and striking red gill filaments. In contrast, fish collected from downstream regions displayed clear signs of stress, including pale gills, increased mucus secretion, and slight enlargement of liver tissue upon examination. The morphometric analysis indicated that the selected specimens were within a comparable size range, thereby minimizing size-related bias in bioaccumulation. The total length exhibited an average variation between 23.4 ± 2.1 cm and 27.8 ± 1.8 cm, whereas body weight was noted to fluctuate from 540 ± 60 g to 760 ± 85 g among various species.

2. Tissue-wise Heavy Metal Bioaccumulation

The quantitative analysis of heavy metals demonstrated a distinct pattern of accumulation across various tissues. The liver demonstrated the greatest concentration of metals when compared to the other tissues examined, with the gills and muscle tissues following closely behind. This pattern was consistently observed across all examined metals, underscoring the liver's functions in metabolism and detoxification. The highest concentration of lead (Pb) was observed in liver tissue, recorded at 3.21 mg/kg, indicating a notable potential for accumulation. Cadmium (Cd), known for its high toxicity, exhibited a propensity for preferential accumulation in the liver, with concentrations exceeding 1.3 mg/kg. Zinc (Zn), a vital trace element, was observed in relatively high concentrations across all tissues, with liver levels exceeding 60 mg/kg. Muscle tissues, although showing comparatively lower concentrations, still held detectable levels of all metals, which is noteworthy from a human consumption perspective.

Table 1: Mean Heavy Metal Concentration in Fish Tissues (mg/kg dry weight)

Metal	Gill (Mean ± SD)	Liver (Mean ± SD)	Muscle (Mean ± SD)
Pb	1.62 ± 0.48	3.21 ± 0.72	0.86 ± 0.29
Cd	0.56 ± 0.17	1.38 ± 0.41	0.24 ± 0.09
Cr	1.12 ± 0.36	2.64 ± 0.68	0.71 ± 0.22
Hg	0.22 ± 0.08	0.61 ± 0.19	0.14 ± 0.05
Zn	28.5 ± 6.4	64.2 ± 12.5	19.3 ± 5.8

3. Site-wise Variation in Heavy Metal Concentration

A significant spatial variation in metal concentration was noted among sampling locations. Downstream locations consistently exhibited elevated metal concentrations relative to upstream and midstream sites. The downstream region exhibited heavy metal concentrations that were roughly 2 to 3 times higher than those observed at upstream sites. This rise clearly reflects the influence of anthropogenic contributions, such as industrial waste and urban runoff. The increase in zinc and lead was notably significant, indicating their widespread presence in the environment.

Table 2: Site-wise Heavy Metal Concentration in Fish (mg/kg, combined tissue average)

Site	Pb	Cd	Cr	Hg	Zn
Upstream	0.92 ± 0.25	0.28 ± 0.10	0.76 ± 0.21	0.12 ± 0.04	18.6 ± 4.2
Midstream	1.84 ± 0.46	0.62 ± 0.18	1.52 ± 0.39	0.27 ± 0.09	34.8 ± 7.5
Downstream	3.12 ± 0.81	1.46 ± 0.39	2.84 ± 0.74	0.58 ± 0.16	61.5 ± 11.2

4. Hematological Observations

Hematological analysis indicated substantial physiological changes in fish subjected to elevated metal concentrations. A notable decrease in hemoglobin levels and red blood cell counts was observed, alongside an elevation in white blood cell counts. Fish from downstream locations demonstrated a notable decrease in hemoglobin (up to approximately 40%), signifying compromised oxygen transport capability. The decrease in red blood cell count further corroborates the existence of anemia-like conditions. In contrast, WBC counts surged over threefold, indicating a vigorous immune response to environmental stressors.

Table 3: Hematological Parameters in Fish from Different Sites

Parameter	Upstream	Midstream	Downstream
Hb (g/dL)	11.2 ± 0.9	8.7 ± 1.1	6.8 ± 1.0
RBC ($\times 10^6$/mm^3)	2.8 ± 0.4	2.1 ± 0.3	1.4 ± 0.2
WBC ($\times 10^3$/mm^3)	32 ± 8	58 ± 12	104 ± 18
HCT (%)	36 ± 4	28 ± 3	20 ± 3

5. Biochemical and Oxidative Stress Responses

Biochemical assays revealed a significant elevation in antioxidant enzyme activity and lipid peroxidation levels in fish subjected to contaminated environments. The activities of catalase and superoxide dismutase exhibited a progressive increase from upstream to downstream sites, signifying the activation of antioxidant defense mechanisms. Lipid peroxidation levels exhibited a marked elevation in downstream fish, indicating substantial oxidative damage to cellular membranes.

Table 4: Oxidative Stress Biomarkers in Fish Tissues

Parameter	Upstream	Midstream	Downstream
CAT (U/mg protein)	32.4 ± 6.2	58.7 ± 9.5	96.3 ± 14.8
SOD (U/mg protein)	26.8 ± 5.4	49.2 ± 8.1	78.6 ± 12.3
LPO (nmol MDA/mg protein)	2.1 ± 0.6	4.8 ± 1.2	8.7 ± 1.9

6. Relationship between Metal Load and Physiological Stress

A significant correlation was noted between heavy metal concentration and physiological parameters. An increase in metal load was associated with a decrease in hematological indices and an elevation in oxidative stress markers. Inversely proportional relationships between metal concentration and Hb/RBC validate the detrimental effects of metals on hematological physiology. Positive correlations between WBC and LPO signify stress-induced immune activation and oxidative injury.

Table 5: Correlation between Heavy Metals and Biological Parameters

Parameter	Pb	Cd	Cr	Hg
Hb	−0.74	−0.69	−0.71	−0.66
RBC	−0.77	−0.72	−0.74	−0.68
WBC	+0.72	+0.75	+0.70	+0.65
LPO	+0.82	+0.78	+0.80	+0.73

7. Integrated Observations

The findings collectively indicate that heavy metal contamination results in:

- Notable bioaccumulation in piscine tissues
- Modifications in hematological indices signifying physiological stress
- Activation of antioxidant defense mechanisms
- Elevated oxidative damage at heightened pollution levels

Fish from the downstream areas of the Godavari River basin exhibited the greatest susceptibility, underscoring the influence of human activities on aquatic ecosystems.

Discussion

This study demonstrates notable species-specific differences in heavy metal bioaccumulation in freshwater fish from the Godavari River basin, alongside a general tissue-specific hierarchy (liver > gill > muscle). Of the species examined, *Oreochromis mossambicus* demonstrated a relatively greater accumulation of the majority of metals, succeeded by *Catla catla* and *Labeo rohita*. This variation can be attributed to differences in feeding behavior, habitat preference, and metabolic activity. *Oreochromis mossambicus* exhibited elevated concentrations of metals in its liver (Pb: ~3.4 ± 0.8 mg/kg, Cd: ~1.5 ± 0.4 mg/kg, Cr: ~2.9 ± 0.7 mg/kg, Hg: ~0.65 ± 0.2 mg/kg, Zn: ~68 ± 13 mg/kg), indicating its proficiency in metal absorption and retention. This species exhibits a notable vulnerability to metals that are sequestered in sediment, a consequence of its omnivorous and benthic feeding behaviors (Gohell, 2025).

The physiological stress noted in this species, indicated by diminished hemoglobin levels and elevated oxidative stress markers, implies a greater probability of contamination. In contrast, *Catla catla* exhibited moderate levels of metals in its liver, with concentrations measured as follows: Pb: ~3.0 ± 0.7 mg/kg, Cd: ~1.3 ± 0.3 mg/kg, Cr: ~2.6 ± 0.6 mg/kg, Hg: ~0.58 ± 0.18 mg/kg, and Zn: ~62 ± 11 mg/kg. As a surface and column feeder, its exposure is comparatively lower than that of bottom feeders, resulting in marginally diminished metal concentrations relative to O (Saleem *et al*., 2022) [9].

Mugil cephalus. Nonetheless, the detected hematological changes suggest that even moderate exposure levels considerably affect physiological functions. Conversely, *Labeo rohita* demonstrated comparatively diminished metal accumulation (Pb: ~2.8 ± 0.6 mg/kg, Cd: ~1.2 ± 0.3 mg/kg, Cr: ~2.4 ± 0.5 mg/kg, Hg: ~0.55 ± 0.15 mg/kg, Zn: ~58 ± 10 mg/kg in hepatic tissue).This may pertain to its feeding behaviors and ecological role, which diminish direct contact with heavily contaminated sediments (Yadav *et al*., 2024).

Nonetheless, the detection of quantifiable metal concentrations and corresponding oxidative stress responses verifies that all species are impacted (Ben *et al*., 2023) [2]. The variation among species suggests that ecological behavior significantly influences metal bioaccumulation (Edo *et al*., 2024) [4]. The elevated accumulation in *Oreochromis mossambicus* underscores its efficacy as a bioindicator species, whereas the detection of metals in the consumable tissues of all species raises apprehensions about food safety in the Godavari River basin.

Conclusion

Heavy metal contamination in freshwater ecosystems represents a significant environmental challenge. Evidence indicates that fish inhabiting polluted waters accumulate metals in a tissue-specific manner and exhibit measurable physiological and biochemical alterations. These changes not only affect fish health but also pose potential risks to human populations consuming contaminated fish. Continuous monitoring and effective management strategies are essential to mitigate the impact of heavy metal pollution.

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