Impact of Tannery Effluents on Aquatic Biota: Fish as Bioindicator for Sustainable Environmental Management

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Impact of Tannery Effluents on Aquatic Biota: Fish as Bioindicator

for Sustainable Environmental Management

Utpal Singha Roy1,2, Buddhadeb Chattopadhyay2, Subhra Kumar Mukhopadhyay2

1,2 Department of Zoology, P. R. Thakur Government College, Thakurnagar – 743287, West Bengal, India.

2 Ecotoxicology Laboratory, Government College of Engineering and Leather Technology, LB – III,

Salt Lake City, Kolkata – 700098, West Bengal, India

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Abstract

The present study which was conducted in East Kolkata Wetlands investigates the impact of tannery effluents on aquatic biota, focusing on fish (Labeo rohita, Catla catla, Cirrhinus mrigala, Oreochromis niloticus, Oreochromis mossambicus) as bioindicators for sustainable environmental management. Physicochemical analyses revealed distinct variations between wastewater-fed and uncontaminated fishponds, with parameters such as temperature, pH, dissolved oxygen, alkalinity and trace metal concentrations significantly influenced by effluent exposure. Wastewater-fed ponds exhibited elevated levels of toxic metals, notably exceeding recommended surface water limits, resulting in metal bioaccumulation primarily in fish liver and gills, while muscle and brain tissues showed lower concentrations. Fish demonstrated physiological adaptations, including increased metallothionein expression, which chelates heavy metals, mitigating toxicity and maintaining metal homeostasis. Despite metal stress and reduced total protein levels in tissues, fish yield remained high, indicating effective acclimatization mechanisms. Enzymatic biomarkers such as alkaline phosphatase, acid phosphatase, alanine aminotransferase and aspartate aminotransferase showed elevated activities, reflecting enhanced liver detoxification and stress responses. Additionally, oxidative stress markers, including superoxide dismutase and cytochrome P450, were significantly induced, suggesting reactive oxygen

Correspondence author email : srutpal@gmail.com

species generation due to metal overload. Immunotoxicological assessments revealed suppression of nonspecific cytotoxic cells, indicating compromised innate immunity linked to metal exposure. These findings highlight the dual role of fish as both indicators of environmental contamination and resilient organisms capable of physiological adjustments under heavy metal stress. The study underscores the importance of monitoring bioaccumulation patterns and biochemical responses in fish to evaluate aquatic ecosystem health and inform sustainable management strategies for wastewater-impacted environments.

Keywords

Bioindicator, Tannery Effluent, Heavy Metals, Metallothionein, Oxidative Stress

1. Introduction

The discharge of industrial effluents, particularly from tanneries, poses a significant threat to aquatic ecosystems worldwide. These effluents introduce a complex mixture of pollutants, including toxic heavy metals like chromium (Cr), lead (Pb), copper (Cu) and zinc (Zn) into receiving water bodies (Towill et al., 1978). The East Kolkata Wetlands (EKW), a Ramsar site, is a prime example of an urban wetland ecosystem that receives a substantial volume of untreated industrial and domestic wastewater from the Kolkata metropolitan area. This unique environment utilizes the nutrient-rich wastewater for large-

scale pisciculture, producing an estimated 10,915 metric tons of fish annually (Chattopadhyay et al., 2002). However, this practice exposes the aquatic biota, especially commercially important fish species, to the risk of contamination by hazardous metals.

Metals are persistent pollutants that can accumulate in various environmental compartments and biomagnify through the food chain, posing risks to both ecosystem health and human consumers (Robbins et al., 1977).

Fish, positioned at higher trophic levels, are particularly susceptible to metal bioaccumulation and are widely recognized as effective bioindicators for assessing aquatic pollution (Mansour and Sidky, 2002). The physiological and biochemical responses of fish to contaminants can provide early warning signals of environmental degradation (Lam and Gray, 2003). These responses include the induction of detoxification mechanisms, such as the synthesis of metal-binding proteins like metallothionein (MT) and the activation of antioxidant defence systems to combat oxidative stress (Nagalakshmi and Prasad, 1998; Cao et al., 2010).

Despite the high pollution load, the wastewater-fed fisheries of the EKW exhibit high productivity, suggesting that the fish have developed remarkable acclimatization mechanisms. This study aims to bridge the gap in understanding these adaptive responses. The primary objectives were to: (1) compare the physicochemical parameters and heavy metal concentrations in wastewater-fed ponds versus uncontaminated sites, (2) quantify the bioaccumulation of Cr, Mn, Fe, Cu, Zn and Pb in various tissues of five commercially important fish species, (3) assess the physiological stress responses by measuring total protein levels, metallothionein expression and the activity of key enzymatic biomarkers (ALP, ACP, ALT, AST, SOD, CYP450) and (4) evaluate the immunotoxicological impact by measuring nonspecific cytotoxic cell (NCC) activity. This research provides a comprehensive ecotoxicological assessment of the EKW, highlighting the utility of fish as bioindicators and informing strategies for the sustainable management of such vital wetland ecosystems.

2. Materials and Methods

2.1. Study Area and Sampling Sites

The study was conducted in the East Kolkata Wetlands (EKW), India (lat. 22°332 - 22°402 N, long. 88°252 - 88°352 E), a Ramsar site receiving industrial and municipal wastewater. Samples were collected from large wastewater-fed fishponds (Bheris) near Chowbaga, which receive a complex mix of tannery effluent and municipal sewage. Control samples were obtained from an uncontaminated rural site in Ashoke Nagar, approximately 60 km from the EKW.

2.2. Sample Collection and Preparation

Abiotic Samples: Water and bottom sediment samples were collected from both wastewater-fed and control ponds. Water was collected in 1L acid-cleaned glass bottles, and sediments were collected using an Ekman grab from the upper 10 cm layer.

Biotic Samples: Five common freshwater fish species - Labeo rohita (rohu), Catla catla (catla), Cirrhinus mrigala (mrigel), Oreochromis niloticus (Nile cichlid) and Oreochromis mossambicus (Mozambique cichlid) were collected from both study sites (N=12 per species per site). Fish weighing 80 ± 20 g were euthanized and tissues (liver, gills, muscle, brain) were immediately dissected for analysis.

2.3. Physicochemical and Elemental Analysis

Physicochemical parameters of water (temperature, pH, dissolved oxygen, alkalinity, etc.) were measured on-site using standard kits and a multiparameter probe. For elemental analysis, water, sediment, and tissue samples were acid-digested following established protocols (Welz and Sperling, 1999). Concentrations of Cr, Mn, Fe, Cu, Zn and Pb were determined using a Perkin-Elmer AAnalyst-100 Atomic Absorption Spectrophotometer (AAS).

2.4. Biochemical and Immunological Assays

Total Protein: Measured according to the method of Lowry et al. (1951) using a UV-Vis spectrophotometer.

Metallothionein (MT): Quantified using a standard ELISA protocol (Derango and Page, 1996) with a primary antibody against metallothionein (Ab 36882) and an HRPtagged secondary antibody.

Enzymatic Biomarkers:

Cytochrome P450 (CYP1A1+1A2): Measured semiquantitatively via indirect ELISA in liver microsomal fractions following the method of Faller et al. (2003).

Superoxide Dismutase (SOD): Assayed using a Calbiochem SOD Assay Kit II, which detects superoxide radicals generated by xanthine oxidase.

Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP): Measured using standard enzyme assay kits (Erba, Mannheim) on a semi-auto analyzer.

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST): Measured using standard enzyme assay kits (Merck, Ecoline) following IFCC methods.

Nonspecific Cytotoxic Cells (NCCs): Estimated in liver and gill tissues via ELISA using a primary antibody that binds to the NCC receptor protein (NCCRP-1).

2.5. Statistical Analysis

Data were analyzed using multivariate statistical techniques including Principal Component Analysis (PCA), Cluster Analysis, and Analysis of Variance (ANOVA) to determine significant differences between groups ($p < 0.05$) (Quinn and Keough, 2002).

3. Results and Discussion

3.1. Physicochemical Conditions and Metal Contamination

The wastewater-fed ponds exhibited significantly different physicochemical characteristics compared to the uncontaminated ponds (Table 5.1). Notably, they had higher levels of dissolved oxygen (8.33±3.26 mg L^{-1} vs. 3.58±0.96 mg L^{-1}), alkalinity, total hardness, chloride, phosphate and nitrate.

The elevated nutrient levels supported higher gross primary productivity. The metal analysis revealed that concentrations of all six metals (Cr, Mn, Fe, Cu, Zn, Pb) were invariably higher in the water and sediment of wastewater-fed ponds compared to control sites, with most differences being statistically significant ($p < 0.05$). In contaminated water, concentrations of Cr, Mn, Fe, Zn and Pb exceeded the recommended limits for surface waters, indicating a substantial pollution load. PCA identified Fe and Zn as the principal components influencing metal bioavailability in the contaminated ponds.

The observed differences are attributable to the continuous influx of nutrient- and metal-rich wastewater. The higher dissolved oxygen in contaminated ponds, despite higher community respiration, is likely due to enhanced photosynthetic activity spurred by ample nutrients and high light penetration in the shallow waters (Wetzel, 2001). The sediments acted as a major sink for metals, with concentrations orders of magnitude higher than in the overlying water, a finding consistent with previous studies (DePinto and Martin, 1980). This sequestration immobilizes metals but also poses a long-term risk of release and uptake by benthic organisms (Robbins et al., 1977).

3.2. Bioaccumulation of Metals in Fish Tissues

Metal accumulation was significantly higher in fish from wastewater-fed ponds across all species. The general pattern of accumulation in tissues was liver > gills > muscle > brain, demonstrating the liver’s primary role in detoxification and storage (Shinn, 2009) and the gills’ role as a primary uptake site (Hayton and Barron, 1990). Muscle and brain tissues consistently showed the lowest concentrations, likely protected by physiological barriers like the blood-brain barrier (Wood et al., 2012).

The trend of metal concentration in the livers of contaminated fish was Fe > Zn > Mn > Cu > Cr > Pb. The accumulation of non-essential, toxic metals like Pb and Cr showed a strong positive relationship with their ambient concentrations (Farombi et al., 2007; Rauf et al., 2009). In contrast, the bioaccumulation of essential metals like Zn and Cu appeared to be more actively regulated by the fish, although their tissue levels were still elevated under exposure (Chatterjee et al., 2006). The feeding habits of the fish influenced accumulation patterns, with bottom-dwelling species (Cirrhinus mrigala) and detritivores (Oreochromis spp.) showing higher metal burdens than surface or column feeders (Mansour and Sidky, 2002; Rauf, 2009). These findings underscore the direct link between environmental contamination and biological uptake, confirming the role of these fish species as effective bioindicators.

3.3. Physiological and Biochemical Responses to Metal Stress

Despite the significant metal burden, fish from the EKW showed no external signs of toxicity, and fishery yields remain high. This resilience is attributed to robust physiological acclimatization mechanisms. However, underlying stress was evident. Total protein content was invariably lower in all tissues of fish from contaminated ponds, suggesting that proteins were being catabolized as an alternative energy source to cope with the metabolic cost of stress and detoxification. This depletion could result from increased autophagy, reduced protein synthesis or diversion of amino acids for cell repair (Moore, 1990).

Metallothionein (MT) Expression: A key adaptive response was the significant upregulation of MT expression in the contaminated fish, particularly in the liver and muscle. The expression pattern was liver > muscle > gill > brain. MT levels showed a significant positive correlation with tissue concentrations of Zn and Cu, the most potent inducers of this protein (Olsson, 1996). This increased MT synthesis allows for the effective chelation and detoxification of excess metals, preventing them from binding to and damaging other vital cellular components.

This mechanism is crucial for maintaining metal homeostasis and explains the fish’s ability to tolerate high environmental metal loads (Wicklund-Glynn and Olsson, 1991).

Enzymatic Biomarkers of Stress: The activities of several key enzymes were significantly elevated in the livers of contaminated fish, indicating cellular stress and enhanced metabolic activity related to detoxification.

Oxidative Stress: The induction of cytochrome P450 (CYP1A1+1A2) and superoxide dismutase (SOD) was significantly higher in contaminated fish. This suggests the generation of reactive oxygen species (ROS) due to metal overload and the presence of other organic pollutants. The antioxidant enzyme SOD is a primary defence against ROS and its elevated activity reflects an active response to mitigate oxidative damage (Pinto et al., 2003).

Hepatotoxicity and Metabolism: The activities of ALT and AST, well-known markers of liver damage, were significantly higher, likely due to leakage from damaged hepatocytes or increased synthesis under stress (Aich et al., 2011). Similarly, the elevation of ALP and ACP activities suggests alterations in lysosomal membrane integrity and an increased functional demand on the liver to manage the toxicant load (Atli and Canli, 2007; Sastry and Gupta, 1979).

3.4. Immunotoxicological Effects

A significant finding of this study was the suppression of the innate immune system. The expression of nonspecific cytotoxic cells (NCCs), which are functionally equivalent to mammalian natural killer cells, was significantly lower in both the liver and gills of all fish species from the wastewater-fed ponds. NCC activity showed a significant negative correlation with tissue Cu concentrations in the liver and Cr in the gills. This immunosuppression indicates that while the fish have adapted to survive, their resistance to pathogens may be compromised. This finding is consistent with studies demonstrating that chronic exposure to metals and other pollutants can impair immune function, making fish more susceptible to disease (Zelikoff, 1993, 1994).

Table 1. Summary of Metal Concentrations and Biomarker Responses in Fish from

Wastewater-Fed vs. Uncontaminated Ponds

High environmental contamination and biological uptake. Bioaccumulation Pattern

Liver > Gills > Muscle > Brain Liver is the primary site for metal storage and detoxification. Total Protein Significantly lower in all tissues Metabolic stress, catabolism of protein for energy. Metallothionein (MT) Significantly higher expression, especially in liver

Parameter Finding in Wastewater-Fed

Ponds

Metal Concentrations Significantly higher in water, sediment and all fish tissues

Key adaptive mechanism for metal detoxification. CYP450 and SOD Significantly higher activity in liver

Indicates oxidative stress from metals and organic pollutants. ALP, ACP, ALT, AST Significantly higher activity in liver

Liver stress, potential cellular damage and enhanced metabolism. Nonspecific Cytotoxic Cells (NCC)

Significantly lower expression in liver and gills

Compromised innate immunity and increased disease susceptibility.

4. Conclusion

This study demonstrates that fish inhabiting the wastewater-fed ponds of the East Kolkata Wetlands are under significant physiological stress due to chronic exposure to high levels of heavy metals and other pollutants. Despite this, they exhibit remarkable resilience, maintaining high productivity through effective acclimatization strategies. The primary adaptive mechanism is the significant upregulation of metallothionein, which efficiently sequesters and detoxifies excess metals. However, this adaptation comes at a cost, as evidenced by reduced total protein levels, elevated activities of stress-related enzymes indicating oxidative stress and hepatotoxicity and a compromised innate immune system.

The findings highlight the dual nature of these fish, they are resilient survivors in a contaminated environment but also sensitive bioindicators whose biochemical and physiological changes reflect the health of the aquatic ecosystem. The suppression of the immune system is a particular concern, as it could lead to increased disease susceptibility and eventual population decline if pollution

Implication

levels exceed the threshold of their adaptive capacity. This research underscores the critical need for continuous monitoring of biomarkers in fish to assess the ecological integrity of wastewater-impacted systems and to

formulate sustainable management and conservation strategies for vital resources like the EKW.

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