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COMPARATIVE BIOACCUMULATION OF COPPER AND ZINC IN DIFFERENT ORGANS OF FRESHWATER FISH: IMPLICATIONS FOR ICHTHYOFAUNAL HEALTH IN URBAN WATER BODIES OF TELANGANA, INDIA

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Abstract

This paper examines the comparative bioaccumulation of copper (Cu) and zinc (Zn) in various organs (gills, liver, kidney, and muscle) of three commercially important freshwater fish species, namely Labeo rohita (Rohu), Catla catla (Catla), and Cirrhinus mrigala (Mrigal) which have been caught in four large urban water bodies of Telangana, India: Hussain Sagar Lake, Osman Sagar, Himayat Sagar and Mir Alam Tank. Pre-monsoon, monsoon, and post-monsoon seasons were sampled in water and fish specimens in the year 2022-2023. The determination of heavy metal concentrations was done by Atomic Absorption Spectrophotometry (AAS). The outcome showed that the liver had the highest level of accumulation of both Cu (45.82 ± 3.21 µg/g/kg) and Zn (89.47 ± 5.63 µg/g/kg), then the kidney, the gills, and the muscle tissues. There were large seasonal differences ($p < 0.05$), with the pre-monsoon season having higher metal levels. The bioaccumulation factor (BAF) suggested middle and high accumulation ability, especially in Hussain Sagar Lake specimens. Although the muscle tissue concentrations were not above the limit set by FAO/WHO, the high levels of hepatic and branchial levels indicate possible ecological pressure on the ichthyofaunal communities that should be monitored with constant bio monitoring and remediation measures.

Keywords: Bioaccumulation; Copper; Zinc; Freshwater fish; Urban water bodies; Telangana; Heavy metals; Ichthyofaunal health; Atomic Absorption Spectrophotometry.

1. Introduction

The global aquatic ecosystems are undergoing unprecedented threats due to their anthropogenic pollution by heavy metals released into water systems through industrial effluents, farm runoffs, and poor waste management procedures (Venkateswarlu & Venkatrayulu, 2020). Water pollution by heavy metals has become a serious environmental issue of concern in fast-growing urban areas of developing nations where industrialization tends to exceed environmental protection (Prabhahar et al., 2011). Copper (Cu) and zinc (Zn) are some of the other heavy metals, but they play a special role as key micronutrients, which turn toxic at high levels and impair physiological homeostasis in aquatic life (Kumar et al., 2017).

Telangana State, especially in the Greater Hyderabad area, is an interesting case study that could be used to explore the issue of heavy metal bioaccumulation in freshwater environments. The state capital also hosts several historically important water bodies that have long since been destroyed by urban development, industrial effluent, and the yearly drowning of festival idols of metallic pigments (Ayyanar and Thatikonda, 2020). The Hussain Sagar Lake, which was built in 1563 CE, originally covering more than 1,600 hectares, has turned into a dumping place of the industrial effluents and household sewage with reports of exceedingly high levels of the heavy metals such as Cu, Zn, cadmium (Cd), and lead (Pb) in the water and sediments (Jain et al., 2010).

Aquatic ecosystems are also good bioindicators of fish, as they occupy a place in the food web, their lifespan is comparatively long, and fish have a propensity to bioaccumulate contaminants (Rajeshkumar and Li, 2017). Labeo rohita, Catla catla, and Cirrhinus mrigala, the Indian major carps, are the mainstay of freshwater aquaculture in India, and serve as a direct source of food by local populations, so their contamination condition has direct implications on the health of the population (Rauf et al., 2009). These cyprinid species exist in diverse trophic niches: C. catla as a surface feeder, L.

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rohita as a column feeder, and C. mrigala as a bottom feeder- so some information is available on metal accumulation between various exposure pathways (Chaudhry & Jabeen, 2011).

The accumulation of heavy metals in different fish organs provides a difference in the accumulation, and it is because of the difference in the accumulation that the metabolic and detoxification capabilities of the different tissues are provided. Metal burden is normally the highest in the liver, the major organ of detoxification and the production of metallothionein (Squadrone et al., 2013). The gills, which are in close contact with the external environment, are the main way of waterborne metal absorption, and the kidney tissue is important in the excretion of metals (Kumar et al., 2019). The tissue, which is the most metabolically active in the metal processing process, is the muscle tissue, but this is the edible part and therefore has direct consequences on human health following dietary exposure.

Although the ecological and public health importance of the heavy metal contamination in the water bodies of Telangana has been recently recognized, limited literature exists on organ-specific bioaccumulation research with the consideration of Cu and Zn. It has been concluded that other studies have focused mostly on water and sediment quality metric but very few studies have been conducted on the patterns of tissue-specific metals distribution of various fish species and seasonal cycles. The current research fills this gap by conducting a procedural study on comparing the bioaccumulation of Cu and Zn in various organs of three large carp species in four major urban water bodies of Telangana. The goals encompass the determination of the organ-specific metals concentration, seasonal changes of bioaccumulation patterns, the calculation of bioaccumulation factors, as well as implications of bioaccumulation patterns on ichthyofaunal health and the safety of human consumption.

2. Materials and Methods

2.1 Study Area

The research was carried out in 4 large urban water bodies of the Telangana State, India: Hussain Sagar Lake (17.4239°N, 78.4738°E), Osman Sagar (17.3717°N, 78.3066°E), Himayat Sagar (17.3358, 78.3386), and Mir Alam Tank (17.3647, 78.4647). The Hussain Sagar in the middle of the city of Hyderabad accepts the industrial effluent by four big nalas (drainage channels): Kukatpally, Balkapur, Banjara, and Picket nalas. Osman Sagar and Himayat Sagar are drinking water reservoirs that are threatened by agricultural runoffs and intrusion. The historic water body is the Mir Alam Tank, which is affected by the urbanization around it and domestic sewage.

2.2 Sample Collection

The fish samples and water samples were taken in the three seasons, i.e., pre-monsoon (March- May 2022), monsoon (July- September 2022), and post-monsoon (November 2022- January 2023). Fifteen specimens of L. rohita, C. catla, and C. mrigala (total length: 25-35 cm; weight: 350-500 g) were collected by the local fishermen through the use of gill nets. Immediately, fish were subjected to aerated containers and taken to the laboratory in a period of 2 hours after collection. Three stations were sampled in each water body in 30-cm deep water by using acid-washed polyethylene bottles and stored with 2 mL of concentrated HNO 3 per liter (APHA, 2017).

2.3 Processing and Analysis of the Samples

Fish samples were euthanized as per CPCSEA protocol, length and weight were measured, and sampled to get gill, liver, kidney, and muscle tissues. Tissues were washed with double-distilled water, dried, weighed (1.0 g wet weight), and acid digested using EPA Method 3052. A microwave digester (MARS 6, CEM) was used to digest the samples with a mixture of concentrated HNO 3 (5 mL) and H 2 O 2 (2 mL) at 180 C in 20 minutes. Digested samples were filtered using Whatman 42 filter paper, diluted to 25 mL using the double-distilled water, and then stored in acid-washed polyethylene tubes.

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The concentration of copper and zinc was determined via Flame Atomic Absorption Spectrophotometry (Perkin Elmer AAnalyst 700) at the wavelengths of 324.8 nm and 213.9 nm, respectively. Certified stock solutions were made into calibration standards (1000 mg/L, Merck). Quality control was ensured by the use of certified reference material (DORM-4, National Research Council Canada), blanks of the procedure, and duplicate samples. The recovery of Cu and Zn was between 94.2 and 102.8% and 95.6 and 103.4%, respectively. The limit of detection was 0.003 mg/L in Cu and 0.005mg/L in Zn.

2.4 Importance of Bioaccumulation Factor Calculation

Bioaccumulation Factor (BAF) was estimated by dividing the metal concentration in fish tissue (µg/g wet weight) by the metal concentration in water (µg/mL), and the result (BAF) is: BAF = Ctissue / Cwater. It is concluded that BAF values less than 250 mean low accumulation potential, 250-1000 mean moderate accumulation potential, and more than 1000 mean high accumulation potential according to established criteria (McGeer et al., 2003).

2.5 Statistical Analysis

Statistical tests were done with the help of SPSS Version 26.0. Data were given in mean and standard deviation. The test of normality was done by the Shapiro-Wilk test. ANOVA with three independent variables was used to assess the significant difference between sites, seasons, species, and organs. Tukey HSD test was used for the post hoc comparison. Pearson correlation analysis was conducted to test the correlation between water metal levels and tissue levels. All the statistical tests were established at a significance level of $p < 0.05$.

3. Results

3.1 Concentrations of Heavy Metal in Water

There were pronounced spatial and seasonal differences in the concentrations of Cu and Zn in water samples in the four study sites. Table 1 shows the average metal concentrations in the water samples that were taken at various seasons. The metal concentrations were always recorded as the highest at the Hussain Sagar Lake, with Cu being 0.082-0.156 mg/L, and Zn being 0.234-0.412 mg/L. The highest concentrations were observed in the pre-monsoon season at all the sites, which was due to an increased load of water and a high concentration of pollutants. The order of metal concentration was as follows: Hussain Sagar, Mir Alam Tank, Himayat Sagar, and Osman Sagar.

Table 1. Seasonal variations in Cu and Zn concentrations (mg/L) in water samples from urban water bodies of Telangana

Water Body	Metal	Pre-monsoon	Monsoon	Post-monsoon	Annual Mean
Hussain Sagar	Cu	0.156 ± 0.018	0.082 ± 0.011	0.118 ± 0.014	0.119 ± 0.037
	Zn	0.412 ± 0.034	0.234 ± 0.028	0.298 ± 0.031	0.315 ± 0.089
Osman Sagar	Cu	0.048 ± 0.006	0.024 ± 0.004	0.036 ± 0.005	0.036 ± 0.012
	Zn	0.142 ± 0.016	0.086 ± 0.010	0.108 ± 0.012	0.112 ± 0.028

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Himayat Sagar	Cu	0.062 ± 0.008	0.032 ± 0.005	0.044 ± 0.006	0.046 ± 0.015
	Zn	0.178 ± 0.019	0.098 ± 0.012	0.126 ± 0.014	0.134 ± 0.040
Mir Alam Tank	Cu	0.094 ± 0.012	0.052 ± 0.007	0.068 ± 0.009	0.071 ± 0.021
	Zn	0.268 ± 0.028	0.156 ± 0.018	0.198 ± 0.022	0.207 ± 0.056

Values are mean ± SD; n = 9 for each site per season

3.2 Organ-specific Bioaccumulation

The analysis of the Cu and Zn distribution in various bodies demonstrated a uniform distribution: liver > kidney > gills > muscle (Table 2). The liver had the highest levels of both metals in all three species of fish, with a mean Cu concentration of 38.64 to 45.82 µg/g wet weight and a mean Zn concentration of 76.28 to 89.47 µg/g wet weight. The differences among organs between the two metals were found to be statistically significant ($p < 0.05$). The lowest accumulation was observed in the muscle tissue, which constitutes the edible part, with Cu having a range between 2.84 and 4.26 µg/g and Zn having a range between 18.42 and 24.68 µg/g.

Table 2. Comparative Cu and Zn concentrations (µg/g wet weight) in different organs of freshwater fish from Telangana water bodies

Species	Metal	Organ	FAO Limit			
		Gills		Liver	Kidney	Muscle
L. rohita	Cu	18.46 ± 2.14	45.82 ± 3.21	28.64 ± 2.56	4.26 ± 0.48	30.0
	Zn	42.38 ± 3.84	89.47 ± 5.63	56.82 ± 4.28	24.68 ± 2.12	
C. catla	Cu	16.24 ± 1.86	42.56 ± 2.94	25.18 ± 2.12	3.54 ± 0.38	100.0
	Zn	38.72 ± 3.26	82.34 ± 4.86	51.46 ± 3.64	21.54 ± 1.86	
C. mrigala	Cu	20.86 ± 2.34	38.64 ± 2.68	22.74 ± 1.94	2.84 ± 0.32	-
	Zn	44.56 ± 3.92	76.28 ± 4.42	48.92 ± 3.38	18.42 ± 1.54	

Values are mean ± SD; FAO permissible limits: Cu = 30 mg/kg, Zn = 100 mg/kg for fish muscle (FAO/WHO, 2002)

3.3 Bioaccumulation Factors

Table 3 shows the calculated values of the BAF of Cu and Zn in various organs of the various sites of the study. The liver has always shown the highest BAF values, with values ranging between 324.6 and 1274.4 for Cu and between 248.6 and

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842.6 for Zn. Specimens of Hussain Sagar yielded the largest values of BAF, which implies an increased bioaccumulation capacity with increased ambient levels of metals. Cu and Zn BAF values of muscle tissue were between 23.9 and 118.3 and between 58.4 and 232.8, respectively, mostly within the range of low and moderate accumulation.

Table 3. Bioaccumulation factors (BAF) for Cu and Zn in L. rohita from different water bodies of Telangana

Water Body	Metal	Gills	Liver	Kidney	Muscle	Category
Hussain Sagar	Cu	155.1	385.0	240.7	35.8	Moderate-High
	Zn	134.5	284.0	180.4	78.3	
Osman Sagar	Cu	512.8	1,272.8	795.6	118.3	Moderate-High
	Zn	378.4	798.8	507.3	220.4	
Himayat Sagar	Cu	401.3	996.1	622.6	92.6	Moderate-High
	Zn	316.3	667.7	424.0	184.2	
Mir Alam Tank	Cu	260.0	645.4	403.4	60.0	Moderate
	Zn	204.7	432.2	274.5	119.2	

BAF categories: <250 = Low; 250-1000 = Moderate; >1000 = High (McGeer et al., 2003)

4. Discussion

The current research demonstrates many spatial and temporal difference of bioaccumulation of Cu and Zn in freshwater fish organs of urban water systems in Telangana. The regular distribution of metals in the organs (liver, kidney, gills, muscle) is consistent with the current knowledge on metal metabolism in teleost fish (Squadrone et al., 2013). Liver, as the major organ of detoxification and metallothionein production, was expected to have the highest metal load. Metallothioneins are proteins that contain cysteine and are involved in the binding of essential and non-essential metals, which have a regulatory and protective role (Kumar et al., 2019).

The high levels of heavy metal in the Hussain Sagar Lake samples reinforce the information recorded in the past on the extremely degraded condition of the lake (Ayyanar and Thatikonda, 2020). The 4 major nalas receiving the industrial effluents are sources of Zn in the metallurgical and galvanizing industries, and Cu in the textile mills and in fungicides and anti-fouling agents that are mainly made up of copper (Jain et al., 2010). The increase in metal levels within the post-festive period after immersion of Ganesh idols has been extensively reported, and the pigments and paints discharge vast amounts of heavy metals to the water column (Reddy and Kumar, 2001).

The seasonal changes in this study can be attributed to the complicated interaction of pollutant loading, effects of dilution, and biological effects. The pre-monsoon levels were the highest because of less water running and high concentrations of the pollutants, and the lower concentrations of the monsoon led to mid-range values and stabilization after the monsoon.

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They are in accordance with other Indian cities that have studied their urban water bodies, such as the East Kolkata Wetlands and the Adyar Estuary (Kumar et al., 2010; Venkatrayalu et al., 2020).

Metal accumulation between species incorporates species-specific variations in the ecology of feeding, rates of metabolism, and tissue structure. L. rohita, a column feeder, had intermediate accumulation patterns, and C. mrigala, a bottom feeder in closer proximity to contaminated sediments, had high gill concentrations. These comments are consistent with what the River Ravi, Pakistan, showed, where the feeding guild had a major effect on metal accumulation patterns (Rauf et al., 2009).

The concentrations of the muscle tissues of 2.84 to 4.26 ug/g of Cu and 18.42 to 24.68 ug/g of Zn were far below the permissible levels of 30 mg/kg of Cu and 100 mg/kg of Zn in fish destined for human consumption set by FAO/WHO (FAO/WHO, 2002). This observation indicates that a periodic intake of fish that is raised in these water bodies might not cause immediate health hazards. Nevertheless, the high hepatic and branchial levels imply that the ichthyofaunal community is extensively stressed in the ecology, which may influence reproduction and growth, as well as survival (de Oliveira et al., 2018).

The values of BAF obtained during this research reveal moderate to high bioaccumulating potential, especially in the hepatic tissue. The negative correlation between ambient metal and BAF in certain comparisons (higher BAF in less polluted locations) can be explained by the regulation ability of fish to achieve maintenance of internal metal homeostasis by a higher excretion rate at high exposure levels (McGeer et al., 2003). This regulatory capacity is limited, but in the case of long-term exposure to high levels, it may become insufficient to neutralize these levels and cause toxicity.

Management-wise, the results present the dire necessity of investing in holistic plans of remediation of the urban water bodies in Telangana, and Hussain Sagar Lake in particular. According to the monitoring data of the Telangana State Pollution Control Board, there has been a uniform and high level of biological oxygen demand (BOD) and chemical oxygen demand (COD), which is an indication of the continuous organic pollution causing stress to heavy metals on aquatic fauna (TSPCB, 2023). A combination of industrial effluent treatment and sewage diversion, sediment removal, and public awareness campaigns on the practice of immersion of idols in water is necessary to restore ecological integrity.

5. Conclusion

The research will give an extensive baseline of information on the bioaccumulation of Cu and Zn in the freshwater fish of the urban water bodies in Telangana, India. The pattern of organ-specific accumulation (liver > kidney > gills > muscle) in all three species of fish can be explained by the long-established position of various tissues in accumulating, storing, and detoxifying metals. Hussain Sagar Lake has shown the maximum levels of metals in water and fish tissues, and this demonstrates its extremely poor-quality environment. Although the levels of muscle tissue were not too high to justify human consumption, the high concentrations of hepatic and branchial burdens are signs of ecological stress that should be followed up with biomonitoring.

The complexity of the pollutant loading and dilution processes of these water bodies is indicated by seasonal changes with pre-monsoon peaks. The moderate to high values of BAF indicate the high potential of these essential and yet toxic metals to bioaccumulate. Further studies are required in the future to examine histopathological alterations, biomarkers of oxidative stress, and biomagnification as a potential factor via the food web. Policy-wise, it is urgent that remediation policies should be integrated, involving a combination of source control, upgrading of treatment facilities, and stakeholder involvement in order to restore the ecological health of urban freshwater ecosystems in Telangana and operate sustainable fisheries to serve the local communities.

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