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Seasonal Variations of Heavy Metal Distribution and Bioaccumulation Patterns with Health Risk Assessment in Commercial Freshwater Fish from Karanja Reservoir, India

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ABSTRACT
Seasonal variations in metal contamination and the potential human exposure through fish consumption is poorly understood for many Indian reservoirs. This study aims to assess the concentrations, seasonal variations and bioaccumulation patterns of ten heavy metals (As, Cd, Co, Cu, Cr, Fe, Hg, Ni, Pb and Zn) in different tissues (all the fins, gills, muscles, liver and whole body) of five commercially important species of fishes: *Channa striata*, *Labeo rohita*, *Mystus cavasius*, *Notopterus synurus and Oreochromis mossambicus* along with surface and bottom water collected from Karanja Reservoir, Bidar, Karnataka (India), during January 2023 to December 2024. In addition, this study evaluates the potential human health risks posed by metal contamination through fish consumption which has not been documented earlier for this reservoir. Heavy metal concentrations varied significantly among fish tissues and between water samples. The bioaccumulation patterns and order of occurrence were also assessed. Comparative analyses with the WHO and Indian standards revealed that Cd, Ni and Pb levels in both fish and water exceeded permissible limits, while Cr in water was also higher than WHO standards. Whereas As, Co, Cu, Ni, Pb and Zn concentrations generally remained within the permissible limits. Bioaccumulation pattern varied among species and tissues; Fe showed the highest bioaccumulation in the fins of *N. synurus*, whereas the lowest was recorded in the muscles of *O. mossambicus*, remaining below threshold values. Health risk assessment indices (Estimated Daily Intake- EDI, Target Hazard Quotient- THQ and Hazard Index- HI) indicated that non-carcinogenic risks from fish consumption were within acceptable safety thresholds. Carcinogenic risk (CR) values for As, Cd, Cr, Ni and Pb were below the critical limit, suggesting negligible cancer risk, except for As, which showed a potential carcinogenic threat. Overall, the findings highlight the need for regular monitoring of heavy metal contamination and public awareness regarding safe fish consumption from the Karanja Reservoir.

INTRODUCTION
Water, the elixir of life, is necessary for all life forms; it is a precious resource that is limited for humans and other living organisms, but it can also become a source of disease when polluted. Understanding the primary causes of water contamination and the diseases linked to it is important for developing strategies to control pollution and preserve water quality **(Thirumala & Kiran, 2018)**. Water pollution management is often a government responsibility aimed at protecting the environment for public welfare **(Rakhecha, 2020)**. Freshwater covers only about 2.5% out of the Earth's total water, but only half of the water, i.e. about 1.2% is available for human utilization **(Wetzel, 2001)**.

Although nearly every aspect of human development depends either directly or indirectly on water, the quality of freshwater is under threat due to increased urbanization, agricultural expansion and industrial development. These pressures have led to a global decline in the quality and condition of freshwater resources **(UNESCO, 2021; Elhaddad *et al*., 2022)**. Over recent decades, concern has grown over the contamination of aquatic environments by heavy metals, which pose serious risks to public health, particularly through drinking water supplies. The persistence of these toxic metal ions, which do not degrade easily, leads to long-term exposure and bioaccumulation in aquatic organisms **(Salaah & El-Gaar, 2020)**. Furthermore, heavy metals in fish and other aquatic species can enter the food chain, ultimately affecting human health. Edible fish, in particular, are key in transferring these contaminants to higher trophic levels, including humans, as they accumulate metals in their tissues **(Salaah *et al*., 2022)**.

The United States Environmental Protection Agency (USEPA) has listed thirteen heavy metals as hazardous which include arsenic (As), antimony (Sb), beryllium (Be), chromium (Cr), silver (Ag), cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), mercury (Hg), thallium (Ti), nickel (Ni) and selenium (Se) **(USPEA, 1989)**. These metals are known to cause damage to the structure and function of biomolecules **(McCormick *et al*., 2005)**. Furthermore, metals act as endocrine disruptors, interfering with hormone metabolism, synthesis, and transport **(Manjappa & Puttaiah, 2005)**. Heavy metals are undegradable and accumulate in water, sediments, and aquatic organisms, leading to the pollution of aquatic ecosystems **(Kumar & Kumar, 2018; Farsani *et al*., 2019)**.

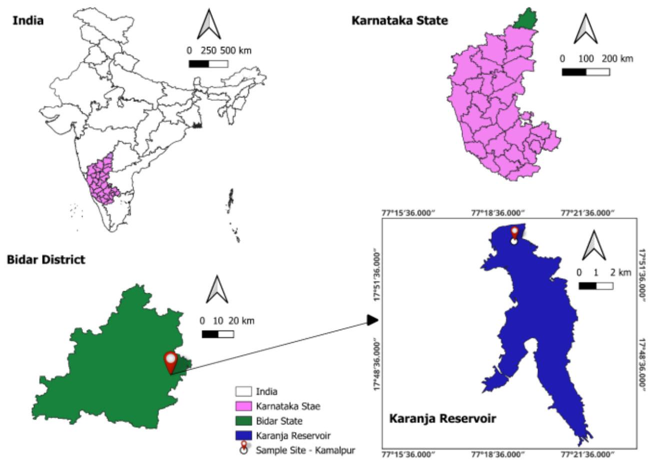
Fish occupies higher trophic levels in the aquatic food chain, absorbing pollutants through both dietary and non-dietary pathways, through the digestive tract, as well as via permeable membranes such as muscles and gills, allowing contaminants to accumulate in significant quantities across various concentrations of heavy metals that can be thousands of times higher in fish compared to their surrounding environment **(Siraj *et al*., 2014; Traina *et al*., 2018; Rajeshkumar & Li, 2018)**. Numerous studies have highlighted that different parts of a fish's body accumulate specific metals in varying amounts. Notably, gills and the liver are crucial organs for monitoring the accumulation of metals, with gills being especially important since they are directly exposed to water **(Saha *et al*., 2016; Atique Ullah *et al*., 2017; Maurya *et al*., 2019; Salaah & El-Gaar, 2020; Salaah *et al*., 2022)**. The metal concentrations in the gills reflect the level of contamination in the surrounding water **(Burger *et al*., 2002)**. As explained by **Newman (1998)**, heavy metals enter fish through two primary pathways: 1) via the gills, where dissolved metals are absorbed during respiration and membrane exchange and 2) through the ingestion of contaminated food or sediment, where metals are absorbed through the digestive system.

Data from the last **Census of India (2011)** revealed that pollution in Bidar District increased by 13.37% between 2001 and 2011, putting further strain on local water resources. Human activities have increasingly damaged the local lakes affecting their natural ecological functions and biodiversity. Reservoirs are at higher risk because their slow-moving water allows harmful metals to build up. The Karanja Reservoir, an important source of water for drinking and agriculture in Bidar faces this problem, A study by **Majagi *et al*. (2008)** even found heavy metals in the reservoir with some concentrations exceeding the safe permissible limits. Therefore, the primary objective of this study is to examine the distribution and seasonal variations of heavy metal accumulation in water and selected freshwater fish from the Karanja Reservoir in Bidar, Karnataka (India) and to address the associated carcinogenic and non-carcinogenic health risks.

MATERIALS AND METHODS

Study area

Karanja Reservoir is a manmade freshwater lake located near Byalhalli Village in Bhalki Taluk, Bidar District, of Karnataka (India). The reservoir lies at 17°51'00" N latitude and 77°20'00" E longitude. The total catchment area of the reservoir is 625.75sq. km and maximum depth of the reservoir is 10m. Seasonal water samples were collected from the Kamalpur location, situated at 17°52'57"N and 77°19'25"E (Fig.1). This collection site is considered to be a potential source of pollution as it influenced by the agricultural runoff and industrial waste. The reservoir is primarily constructed for irrigation and potable water supply, while also facilitating several ecological activities, such as fisheries and aquatic biodiversity **(Shaikh & Zodape, 2025)**.



(Source: QGIS Software)

Fig. 1. Map showing the location of the study area Karanja Reservoir, Bidar, Karnataka (India)

Ethics approval

All necessary permissions for the collection of fish samples were obtained from the Karnataka State Biodiversity Board. The research was conducted under ethical approval granted by letter No. TEC/C06/Research/2023-24/01/651.

Collection of samples

Water samples

Five litres of each water samples were collected seasonally from January 2023 to December 2024. The surface and bottom water were collected 10m away from the bank of the collection site of the reservoir. Bottom water was specifically collected from a depth of 8-10 m below the water surface. The water samples were collected in pre-cleaned polypropylene bottles and stored in an ice box at 4°C and transported to the laboratory for further analysis.

Fish samples

Five samples of each five fish species (Fig. 2A–E), commonly consumed by the local population, were randomly collected from Karanja Reservoir. The fishes were placed in clean polyethylene bags, labelled and transported in ice-stored containers at −8°C to the Department of Zoology, C.S.'s S.S. & L.S. Patkar-Varde College, Goregaon (West), Mumbai. The preliminary identification of the species was carried out using the FAO manual and final authentication, and confirmation was done at the Zoological Survey of India (ZSI), Hyderabad (India). Biometric measurements were recorded (Table 1) before dissection. Each specimen was dissected using stainless-steel knives and forceps to separate different tissues including fins (pectoral, dorsal, pelvic, anal and caudal), gills, liver, muscles and whole body. The whole fish was also cut into small pieces for uniform processing. All tissues were rinsed with deionized water to remove surface debris, blotted dry with filter paper and oven-dried at 110°C for 2 days. The dried samples were ground into a fine powder using a mortar and pestle, and the powder was stored at −20°C until further use.

A. *Channa striata* (Bloch, 1793)

B. *Labeo rohita* (Hamilton, 1822)

C. *Mystus cavasius* (Hamilton, 1822)

D. *Notopterus synurus* (Bloch and Schneider, 1801)

E. *Oreochromis mossambicus* (Peters, 1852)

Fig. 2. (A-E): Photographs of the fish species collected from Karanja Reservoir, Bidar, Karnataka (India)

Table 1. The ecological and morphometric characteristics of selected fish species collected from Karanja Reservoir, Bidar, Karnataka (India) (2023-2024)

Scientific name	Common & local name	Habitat	Feeding behavior	IUCN status	Length (cm) and weight (gm) relationship
Channa striata	Striped Snakehead, Murrel	Still or slow-moving waters, mid-column or bottom dweller	Carnivorous, feeds on smaller fish and invertebrates	Least concern	24 ± 9.30 cm; 210 ± 7.57 gm
Labeo rohita	Rohu, Rohu	Inhabits flowing and standing water, middle and bottom layers	Herbivorous, feeds on phytoplankton and zooplankton	Least concern	20 ± 9.35 cm; 510 ± 6.55 gm
Mystus cavasius	Gangetic mystus, Teengra	Bottom dweller, prefers clear or slightly turbid water	Carnivorous, feeds on insects, crustaceans, small fish and invertebrates	Least concern	15 ± 3.10 cm; 33 ±5.44 gm
Notopterus synurus	Bronze featherback, Chamberi	Bottom dweller, prefers slow flowing or stagnant water	Carnivorous, feeds on small fish, insects and crustaceans	Least concern	20 ± 6.99 cm; 100 ± 16.58 gm
Oreochromis mossambicus	Mozambique Tilapia, Chilapi	All levels of water column depending on food availability and water conditions	Omnivorous, feeds on algae, plankton, detritus and small invertebrates	Vulnerable	22 ± 10.09 cm; 120 ± 14.07 gm

Sample digestion

10mL of each water sample was taken in a digestion flask and 10mL of conc. HNO_3 was added as suggested by **Zodape and Tayade (2016)**. 1gm of each dried tissue powder from each fish species was transferred into a Kjeldahl flask and digested following the procedure described by **Ehi-Eromosele and Okiei (2012)** and **Zodape and Tayade (2016)**.

Instruments

Heavy metal concentration was determined using Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES), (Model: ARCOS Simultaneous ICP Spectrometer) at Sophisticated Analytical Instrument Facility (SAIF), I.I.T. Bombay, Powai, Mumbai - 400 076.

Bioaccumulation factor (BAF)

The bioaccumulation factors (BAF) are the ratios of heavy metal concentrations in fish organs to water. BAF was calculated using the formula suggested by **Maurya *et al*. (2019)**.

$$\text{BAF} = \frac{\text{Concentration of heavy metals in fish tissue}}{\text{Concentrations of heavy metals in water}}$$

Quantitative health risk assessment

Estimated daily intake (EDI): The EDI of heavy metals was calculated using the following formula suggested by **Maurya *et al*. (2019)**:

$$\text{EDI } (\mu g/kg/day) = \frac{C}{\text{FIR} \times \text{BW}}$$

Where, C represents the mean heavy metal concentration in fish tissue (ppm = µg/kg) on a dry weight basis. A conversion factor of 4.8 is used for dry to wet weight **(Rahman *et al*., 2012)**. Food Ingestion Rate (FIR) refers to the daily consumption of freshwater fish (gm/day). The average FIR of fish is 49gm/ day **(USEPA, 2011)**. Body weight (BW) represents the average weight which is 70kg for adults **(USEPA, 2012)**.

Target hazard quotient (THQ): The THQ estimates the non-carcinogenic risk level associated with exposure to heavy metals **(Islam *et al*., 2015)**. It is calculated using the following formula suggested by the **USEPA (2012)**:

$$\text{THQ} = \frac{\text{Efr} \times \text{ED} \times C}{\text{RfD} \times \text{BW} \times \text{ATn}} \times 10^{-3}$$

Where, exposure frequency (Efr) is 365 days/year; exposure duration (ED) is 70 years (for the purpose of this study); reference dose (RfD) evaluates the health risk associated with fish consumption where As (0.0003), Cd (0.001), Co (0.0003), Cr (0.003), Cu (0.04), Fe (0.7), Hg (0.0001), Ni (0.02), Pb (0.0035) and Zn (0.3), and averaging time (ATn) represents the average exposure duration for non-carcinogenic effects (365 days x ED) **(USEPA, 2012; WHO, 2017)**.

Hazard index (HI): HI is estimated as the sum of all individuals non-carcinogenic risk (THQ) for all metals (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb and Zn) using the equation:

$$\text{HI} = \sum \text{THQ}_s$$

Carcinogenic risk (CR): The carcinogenic risk (CR) was calculated to evaluate the possibility of cancer in an individual over the lifetime for the exposure of cancer-causing agents. CR was calculated as using the following formula:

$$CR = \frac{Efr \times ED \times EDI \times CSF}{ATn} \times 10^{-3}$$

Here, CSF is the oral slope factor of cancer-causing agents (mg/kg/day), which is only available for As (1.5), Cd (6.1), Cr (0.5), Ni (0.91) and Pb (0.0085), as set by the **USEPA (2000)**.

Statistical analysis

The statistical analysis was carried out using two-way ANOVA (R Studio Software, Version: 2024.12.1) to evaluate the effects of seasons on heavy metal concentration in fish tissues and water. The significant effects were calculated by performing Tukey's HSD post-hoc test to identify pairwise differences. Statistical significance was considered at $P < 0.005$, while $P \geq 0.005$ was treated as non-significant

RESULTS

The findings of the current study are tabulated and values evaluated are depicted in Tables (2-9b) in addition to Graph (1). Results are discussed in the succeeding section.

Table 2. The mean ± S.D. (n = 5) seasonal variations of selected heavy metal concentrations (ppm) in different tissues of *Channa striata* collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Pre-Monsoon Season										
Tissues	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Zn**
Fins	0.012 ± 0.008	0.011 ± 0.001	0.023 ± 0.001	0.917 ± 0.023	0.346 ± 0.001	0.816 ± 0.001	ND	0.563 ± 0.006	1.066 ± 0.005	1.121 ± 0.012
Gills	0.011 ± 0.034	0.042 ± 0.002	0.011 ± 0.015	0.572 ± 0.002	0.158 ± 0.003	0.978 ± 0.032	ND	0.627 ± 0.060	0.653 ± 0.004	0.995 ± 0.004
Muscles	0.019 ± 0.001	0.042 ± 0.011	0.09 ± 0.001	0.246 ± 0.019	0.311 ± 0.006	0.354 ± 0.002	ND	0.117 ± 0.009	1.851 ±0.003	1.134 ± 0.009
Liver	0.011 ± 0.009	0.013 ± 0.019	0.043 ± 0.018	ND	0.819 ± 0.007	0.215 ± 0.014	ND	0.431 ± 0.009	0.255 ± 0.018	1.346 ± 0.005
Whole Body	0.012 ± 0.017	0.071 ± 0.001	0.012 ± 0.032	0.802 ± 0.022	0.199 ± 0.003	0.124 ± 0.015	ND	0.761 ± 0.006	1.826 ± 0.012	2.223 ± 0.064
Monsoon Season										
Fins	0.016 ± 0.001	0.029 ± 0.002	0.016 ± 0.006	0.188 ± 0.043	0.241 ± 0.002	0.727 ± 0.011	ND	0.124 ± 0.001	1.858 ± 0.009	2.723 ± 0.002
Gills	0.043 ± 0.004	0.092 ± 0.009	0.045 ± 0.016	0.466 ± 0.002	0.723 ± 0.019	0.498 ± 0.021	ND	0.497 ± 0.015	1.584 ± 0.001	0.982 ± 0.009

Muscles	0.007 ± 0.001	0.012 ± 0.017	0.102 ± 0.006	0.928 ± 0.020	0.313 ± 0.007	0.278 ± 0.006	ND	0.766 ± 0.009	0.767 ± 0.001	2.618 ± 0.003
Liver	0.016 ± 0.019	0.092 ± 0.019	0.027 ± 0.009	0.973 ± 0.009	0.23 ± 0.005	0.787 ± 0.038	ND	0.312 ± 0.003	1.604 ± 0.019	1.312 ± 0.005
Whole Body	0.03 ± 0.008	0.015 ± 0.005	0.081 ± 0.009	0.998 ± 0.026	0.207 ± 0.001	0.925 ± 0.009	ND	0.747 ± 0.006	0.758 ± 0.015	2.217 ± 0.001
Post-Monsoon Season										
Fins	0.007 ± 0.001	0.083 ± 0.002	0.07 ± 0.006	0.859 ± 0.001	0.481 ± 0.014	0.232 ± 0.018	ND	0.243 ± 0.001	0.628 ± 0.004	2.125 ± 0.004
Gills	0.005 ± 0.004	0.013 ± 0.007	0.02 ± 0.015	0.719 ± 0.006	0.819 ± 0.032	0.248 ± 0.008	ND	0.178 ± 0.021	1.86 ± 0.001	1.264 ± 0.009
Muscles	0.044 ± 0.010	0.018 ± 0.016	0.113 ± 0.060	0.979 ± 0.004	0.243 ± 0.007	0.963 ± 0.027	ND	0.748 ± 0.009	0.65 ± 0.016	2.515 ± 0.003
Liver	0.013 ± 0.017	0.093 ± 0.001	0.113 ± 0.009	ND	0.216 ± 0.001	0.826 ± 0.01	ND	0.432 ± 0.004	1.145 ± 0.021	2.346 ± 0.034
Whole Body	0.043 ± 0.007	0.017 ± 0.009	0.073 ± 0.009	ND	0.112 ± 0.001	0.159 ± 0.028	ND	0.421 ± 0.006	0.804 ± 0.001	2.162 ± 0.001
ND=Not detected (below detectable level)										

Table 3. The mean ± S.D. (n = 5) seasonal variations of selected heavy metal concentrations (ppm) in different tissues of *Labeo rohita* collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Pre-Monsoon Season										
Tissues	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Zn**
Fins	0.026 ± 0.020	0.018 ± 0.006	0.011 ± 0.002	0.517 ± 0.014	0.031 ± 0.002	0.711 ± 0.033	ND	0.485 ± 0.021	1.135 ± 0.005	1.192 ± 0.003
Gills	0.019 ± 0.008	0.033 ± 0.001	0.026 ± 0.005	0.116 ± 0.012	0.172 ± 0.015	0.341 ± 0.018	ND	0.584 ± 0.001	1.043 ± 0.007	1.003 ± 0.001
Muscles	0.008 ± 0.014	0.017 ± 0.007	0.003 ± 0.001	0.164 ± 0.001	0.312 ± 0.009	0.335 ± 0.005	ND	0.117 ± 0.001	1.742 ± 0.032	0.783 ± 0.011
Liver	0.093 ± 0.011	0.019 ± 0.027	0.061 ± 0.014	0.004 ± 0.006	0.641 ± 0.004	0.214 ± 0.011	ND	0.117 ± 0.002	1.235 ± 0.012	1.732 ± 0.089
Whole Body	0.011 ± 0.005	0.081 ± 0.001	0.021 ± 0.015	0.118 ± 0.019	0.118 ± 0.015	0.114 ± 0.031	ND	0.734 ± 0.017	0.973 ± 0.004	1.523 ± 0.006
Monsoon Season										
Fins	0.016 ± 0.020	0.031 ± 0.006	0.018 ± 0.002	0.211 ± 0.003	0.213 ± 0.004	0.672 ± 0.003	ND	0.216 ± 0.025	1.376 ± 0.007	1.732 ± 0.002
Gills	0.038 ± 0.005	0.071 ± 0.004	0.032 ± 0.006	0.451 ± 0.011	0.641 ± 0.012	0.116 ± 0.024	ND	0.361 ± 0.001	1.434 ± 0.002	0.962 ± 0.001
Muscles	0.013 ±	0.048 ±	0.016 ±	0.351 ±	0.317 ±	0.253 ±	ND	0.183 ±	0.561 ±	1.812 ±

	0.043	0.007	0.006	0.001	0.007	0.104		0.005	0.032	0.019
Liver	0.015 ± 0.011	0.083 ± 0.006	0.013 ± 0.015	0.031 ± 0.012	0.311 ± 0.032	0.482 ± 0.021	ND	0.218 ± 0.009	0.227 ± 0.012	2.865 ± 0.006
Whole Body	0.021 ± 0.004	0.019 ± 0.045	0.017 ± 0.003	0.721 ± 0.021	0.201 ± 0.019	0.643 ± 0.022	ND	0.428 ± 0.014	0.531 ± 0.021	1.873 ± 0.018
Post-Monsoon Season										
Fins	0.028 ± 0.067	0.054 ± 0.006	0.172 ± 0.003	0.421 ± 0.032	0.572 ± 0.011	0.232 ± 0.009	ND	0.128 ± 0.043	0.841 ± 0.007	2.116 ± 0.002
Gills	0.016 ± 0.019	0.064 ± 0.004	0.068 ± 0.011	0.259 ± 0.002	0.819 ± 0.006	0.261 ± 0.006	ND	0.153 ± 0.001	0.657 ± 0.006	2.421 ± 0.001
Muscles	0.037 ± 0.010	0.015 ± 0.023	0.142 ± 0.018	0.157 ± 0.001	0.145 ± 0.001	0.874 ± 0.010	ND	0.316 ± 0.006	0.453 ± 0.030	0.517 ± 0.015
Liver	0.023 ± 0.017	0.077 ± 0.008	0.173 ± 0.015	0.027 ± 0.016	0.21 ± 0.029	0.723 ± 0.037	ND	0.521 ± 0.015	1.116 ± 0.004	1.492 ± 0.006
Whole Body	0.032 ± 0.004	0.011 ± 0.002	0.035 ± 0.003	0.231 ± 0.032	0.151 ± 0.018	0.112 ± 0.019	ND	0.362 ± 0.015	0.743 ± 0.003	1.983 ± 0.017
ND=Not detected (below detectable level)										

Table 4. The mean ± S.D. (n = 5) seasonal variations of selected heavy metal concentrations (ppm) in different tissues of *Mystus cavasius* collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Pre-Monsoon Season										
Tissues	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Zn**
Fins	0.021 ± 0.004	0.018 ± 0.011	0.043 ± 0.003	0.194 ± 0.023	0.126 ± 0.011	0.248 ± 0.008	ND	0.159 ± 0.015	1.141 ± 0.003	2.765 ± 0.019
Gills	0.064 ± 0.004	0.027 ± 0.006	0.048 ± 0.007	0.293 ± 0.011	0.031 ± 0.043	0.241 ± 0.002	ND	0.542 ± 0.004	1.327 ± 0.001	1.012 ± 0.021
Muscles	0.054 ± 0.013	0.031 ± 0.002	0.027 ± 0.004	0.385 ± 0.011	0.139 ± 0.001	0.119 ± 0.007	ND	0.174 ± 0.007	0.784 ± 0.005	1.633 ± 0.032
Liver	0.073 ± 0.017	0.028 ± 0.016	0.076 ± 0.009	0.117 ± 0.005	0.124 ± 0.032	0.241 ± 0.012	ND	0.185 ± 0.026	0.252 ± 0.021	1.006 ± 0.011
Whole Body	0.051 ± 0.002	0.032 ± 0.011	0.025 ± 0.024	0.115 ± 0.003	0.035 ± 0.011	0.194 ± 0.003	ND	0.643 ± 0.030	0.365 ± 0.006	0.254 ± 0.031
Monsoon Season										
Fins	0.046 ± 0.001	0.032 ± 0.017	0.038 ± 0.014	0.186 ± 0.001	0.237 ± 0.018	0.186 ± 0.034	ND	0.218 ± 0.006	0.623 ± 0.004	1.656 ± 0.019
Gills	0.021 ± 0.004	0.036 ± 0.006	0.142 ± 0.017	0.427 ± 0.019	0.145 ± 0.017	0.472 ± 0.003	ND	0.184 ± 0.004	0.314 ± 0.003	1.745 ± 0.022
Muscles	0.035 ± 0.013	0.039 ± 0.004	0.164 ± 0.001	ND	0.258 ± 0.009	0.175 ± 0.017	ND	0.185 ± 0.001	1.362 ± 0.029	1.768 ± 0.034
Liver	0.042 ± 0.020	0.057 ± 0.018	0.031 ± 0.024	0.143 ± 0.002	0.173 ± 0.023	0.187 ± 0.001	ND	0.226 ± 0.032	1.863 ± 0.021	2.051 ± 0.001
Whole Body	0.027 ± 0.025	0.027 ± 0.006	0.117 ± 0.007	0.163 ± 0.023	0.153 ± 0.018	0.482 ± 0.029	ND	0.324 ± 0.064	1.341 ± 0.006	0.351 ± 0.019

Post-Monsoon Season										
Fins	0.082 ± 0.006	0.056 ± 0.017	0.024 ± 0.018	0.236 ± 0.001	0.185 ± 0.006	0.563 ± 0.003	ND	0.137 ± 0.004	1.564 ± 0.018	1.577 ± 0.018
Gills	0.093 ± 0.016	0.043 ± 0.006	0.039 ± 0.018	0.137 ± 0.019	0.586 ± 0.001	0.164 ± 0.003	ND	0.293 ± 0.007	0.621 ± 0.001	2.532 ± 0.022
Muscles	0.032 ± 0.001	0.042 ± 0.009	0.142 ± 0.001	0.377 ± 0.009	0.146 ± 0.011	0.367 ± 0.012	ND	0.343 ± 0.001	1.167 ± 0.012	1.476 ± 0.011
Liver	0.033 ± 0.002	0.065 ± 0.018	0.174 ± 0.024	0.011 ± 0.007	0.224 ± 0.026	0.321 ± 0.002	ND	0.521 ± 0.008	1.156 ± 0.001	0.863 ± 0.024
Whole Body	0.017 ± 0.006	0.063 ± 0.006	0.041 ± 0.009	0.154 ± 0.036	0.152 ± 0.026	0.183 ± 0.034	ND	0.166 ± 0.054	0.754 ± 0.032	1.165 ± 0.009
ND=Not detected (below detectable level)										

Table 5. The mean ± S.D. (n = 5) seasonal variations of selected heavy metal concentrations (ppm) in different tissues of *Notopterus synurus* collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Tissues	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Zn**
Pre-Monsoon Season										
Fins	0.054 ± 0.004	0.048 ± 0.017	0.047 ± 0.004	0.175 ± 0.033	0.062 ± 0.020	0.982 ± 0.016	ND	0.344 ± 0.003	1.573 ± 0.034	0.426 ± 0.004
Gills	0.021 ± 0.012	0.019 ± 0.002	0.076 ± 0.002	0.294 ± 0.010	0.135 ± 0.001	0.241 ± 0.033	ND	0.642 ± 0.017	1.745 ± 0.002	1.009 ± 0.001
Muscles	0.023 ± 0.023	0.033 ± 0.018	0.084 ± 0.054	0.286 ± 0.006	0.463 ± 0.008	0.162 ± 0.002	ND	0.162 ± 0.011	0.854 ± 0.001	1.573 ± 0.008
Liver	0.047 ± 0.001	0.027 ± 0.004	0.091 ± 0.006	0.187 ± 0.019	0.263 ± 0.023	0.345 ± 0.028	ND	0.186 ± 0.012	1.257 ± 0.010	2.001 ± 0.011
Whole Body	0.046 ± 0.007	0.036 ± 0.007	0.022 ± 0.011	0.135 ± 0.029	0.032 ± 0.021	0.189 ± 0.011	ND	0.648 ± 0.005	0.742 ± 0.022	0.257 ± 0.010
Monsoon Season										
Fins	0.082 ± 0.008	0.042 ± 0.001	0.019 ± 0.004	0.194 ± 0.033	0.056 ± 0.029	0.853 ± 0.033	ND	0.213 ± 0.009	1.335 ± 0.005	1.653 ± 0.019
Gills	0.048 ± 0.021	0.028 ± 0.002	0.026 ± 0.002	0.425 ± 0.002	0.379 ± 0.010	0.523 ± 0.004	ND	0.379 ± 0.017	0.218 ± 0.002	2.462 ± 0.001
Muscles	0.046 ± 0.014	0.053 ± 0.017	0.127 ± 0.008	0.372 ± 0.009	0.324 ± 0.054	0.196 ± 0.009	ND	0.176 ± 0.011	0.434 ± 0.001	1.673 ± 0.014
Liver	0.063 ± 0.001	0.088 ± 0.005	0.047 ± 0.015	0.138 ± 0.012	0.184 ± 0.026	0.281 ± 0.024	ND	0.227 ± 0.004	0.863 ± 0.011	2.032 ± 0.019
Whole Body	0.028 ± 0.007	0.046 ± 0.016	0.175 ± 0.001	0.147 ± 0.020	0.208 ± 0.031	0.563 ± 0.001	ND	0.359 ± 0.006	1.475 ± 0.004	1.351 ± 0.020
Post-Monsoon Season										
Fins	0.043 ± 0.008	0.017 ± 0.002	0.043 ± 0.002	0.186 ± 0.010	0.258 ± 0.007	0.463 ± 0.022	ND	0.157 ± 0.004	1.645 ± 0.005	1.578 ± 0.016
Gills	0.062 ± 0.004	0.056 ± 0.009	0.075 ± 0.002	0.374 ± 0.006	0.628 ± 0.008	0.118 ± 0.006	ND	0.148 ± 0.016	0.741 ± 0.002	2.415 ± 0.001
Muscles	0.048 ± 0.015	0.094 ± 0.017	0.162 ± 0.006	0.381 ± 0.011	0.133 ± 0.004	0.541 ± 0.017	ND	0.351 ± 0.011	1.113 ± 0.001	1.843 ± 0.012

Liver	0.033 ± 0.001	0.074 ± 0.005	0.159 ± 0.012	0.072 ± 0.027	0.216 ± 0.021	0.492 ± 0.033	ND	0.553 ± 0.002	1.176 ± 0.020	0.764 ± 0.010
Whole Body	0.037 ± 0.001	0.062 ± 0.002	0.038 ± 0.001	0.287 ± 0.011	0.151 ± 0.034	0.187 ± 0.023	ND	0.153 ± 0.005	0.382 ± 0.007	1.125 ± 0.007
ND=Not detected (below detectable level)										

Table 6. The mean ± S.D. (n = 5) seasonal variations of selected heavy metal concentrations (ppm) in different tissues of *Oreochromis mossambicus* collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Tissues	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Zn**
Pre-Monsoon Season										
Fins	0.028 ± 0.016	0.047 ± 0.001	0.174 ± 0.009	0.268 ± 0.001	0.392 ± 0.011	0.732 ± 0.029	ND	0.328 ± 0.005	1.349 ± 0.007	1.285 ± 0.007
Gills	0.029 ± 0.018	0.034 ± 0.011	0.043 ± 0.016	0.631 ± 0.017	0.058 ± 0.012	0.573 ± 0.009	ND	0.472 ± 0.010	0.782 ± 0.012	0.935 ± 0.007
Muscles	0.018 ± 0.019	0.022 ± 0.011	0.085 ± 0.005	0.197 ± 0.019	0.361 ± 0.003	0.179 ± 0.013	ND	0.575 ± 0.002	1.893 ± 0.005	1.721 ± 0.002
Liver	0.026 ± 0.036	0.045 ± 0.001	0.045 ± 0.019	ND	0.135 ± 0.009	0.367 ± 0.008	ND	0.461 ± 0.001	1.245 ± 0.006	0.276 ± 0.001
Whole Body	0.016 ± 0.006	0.051 ± 0.009	0.029 ± 0.001	0.053 ± 0.009	0.173 ± 0.022	0.627 ± 0.001	ND	0.143 ± 0.006	0.832 ± 0.021	1.239 ± 0.010
Monsoon Season										
Fins	0.034 ± 0.018	0.039 ± 0.001	0.042 ± 0.019	0.242 ± 0.006	0.571 ± 0.001	0.438 ± 0.027	ND	0.394 ± 0.001	1.903 ± 0.017	2.254 ± 0.032
Gills	0.025 ± 0.004	0.013 ± 0.014	0.164 ± 0.010	0.165 ± 0.018	0.164 ± 0.015	0.775 ± 0.009	ND	0.735 ± 0.019	1.254 ± 0.001	1.373 ± 0.002
Muscles	0.021 ± 0.003	0.017 ± 0.011	0.016 ± 0.013	0.648 ± 0.032	0.127 ± 0.006	0.458 ± 0.011	ND	0.758 ± 0.003	0.652 ± 0.009	0.542 ± 0.067
Liver	0.031 ± 0.002	0.024 ± 0.012	0.067 ± 0.003	0.753 ± 0.001	0.436 ± 0.002	0.562 ± 0.005	ND	0.147 ± 0.001	1.841 ± 0.007	1.452 ± 0.003
Whole Body	0.032 ± 0.005	0.033 ± 0.009	0.025 ± 0.002	0.141 ± 0.002	0.541 ± 0.019	0.195 ± 0.032	ND	0.264 ± 0.010	1.854 ± 0.012	1.943 ± 0.009
Post-Monsoon Season										
Fins	0.041 ± 0.018	0.044 ± 0.006	0.147 ± 0.016	0.126 ± 0.016	0.145 ± 0.004	0.868 ± 0.014	ND	0.169 ± 0.001	0.853 ± 0.014	1.876 ± 0.034
Gills	0.026 ± 0.004	0.059 ± 0.019	0.076 ± 0.005	0.525 ± 0.026	0.468 ± 0.009	0.659 ± 0.016	ND	0.634 ± 0.016	1.137 ± 0.005	1.832 ± 0.002
Muscles	0.016 ± 0.008	0.026 ± 0.013	0.127 ± 0.017	0.952 ± 0.006	0.043 ± 0.034	0.476 ± 0.011	ND	0.237 ± 0.003	0.542 ± 0.010	1.377 ± 0.001
Liver	0.011 ± 0.006	0.029 ± 0.008	0.151 ± 0.001	0.159 ± 0.001	0.235 ± 0.002	0.341 ± 0.002	ND	0.194 ± 0.001	0.428 ± 0.007	2.152 ± 0.004
Whole Body	0.012 ± 0.007	0.038 ± 0.022	0.146 ± 0.002	0.274 ± 0.006	0.063 ± 0.001	0.116 ± 0.022	ND	0.266 ± 0.010	1.058 ± 0.020	0.962 ± 0.009
ND=Not detected (below detectable level)										

Table 7. The mean ± S.D. (n = 5) seasonal variations of selected heavy metal concentrations (ppm) in surface and bottom water collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Heavy Metals	Pre-Monsoon		Monsoon		Post-Monsoon	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
As	0.021 ± 0.004	0.011 ± 0.002	0.034 ± 0.002	0.067 ± 0.006	0.041 ± 0.001	0.024 ± 0.001
Cd	0.064 ± 0.001	0.942 ± 0.001	0.058 ± 0.004	0.104 ± 0.003	ND	0.101 ± 0.002
Co	0.062 ± 0.006	0.711 ± 0.006	0.081 ± 0.008	0.073 ± 0.009	0.017 ± 0.008	0.032 ± 0.001
Cr	0.389 ± 0.003	0.921 ± 0.002	ND	0.471 ± 0.006	0.433 ± 0.001	0.637 ± 0.001
Cu	0.221 ± 0.005	0.298 ± 0.001	0.147 ± 0.002	0.314 ± 0.002	0.116 ± 0.004	0.512 ± 0.001
Fe	0.023 ± 0.009	0.028 ± 0.007	0.032 ± 0.007	0.124 ± 0.004	0.029 ± 0.002	0.044 ± 0.003
Hg	ND	ND	ND	ND	ND	ND
Ni	0.633 ± 0.005	0.895 ± 0.003	0.476 ± 0.003	0.746 ± 0.006	0.598 ± 0.001	0.363 ± 0.001
Pb	1.218 ± 0.006	1.102 ± 0.006	0.732 ± 0.001	1.764 ± 0.003	1.764 ± 0.003	1.865 ± 0.002
Zn	2.012 ± 0.008	1.124 ± 0.003	1.013 ± 0.006	2.011 ± 0.007	1.211 ± 0.003	2.215 ± 0.004

ND=Not detected (below detectable level)

Table 8. The permissible limits of heavy metals (ppm) in fishes and freshwater samples set by the **WHO (2011) / BIS (2012)**

Sample	As	Cd	Co	Cr	Cu	Fe	Hg	Ni	Pb	Zn
Fish	0.1	0.05	-	1	10	1 - 3	0.5	0.1	0.3	40 – 50
Water	0.01	0.003 - 0.005	-	0.05	1 - 2	0.3	0.006	0.002 – 0.007	0.3	3 - 5

Table 9a: The bbioaccumulation factor (BAF) of selected heavy metals in different fish and their tissues collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Fish	*Channa striata*					*Labeo rohita*					*Mystus cavasius*				
Heavy Metals	F	G	M	L	WB	F	G	M	L	WB	F	G	M	L	WB
As	0.354	0.596	0.707	0.404	0.0213	0.707	0.737	0.576	1.323	0.646	1.505	1.798	1.222	1.495	0.0716
Cd	0.194	0.232	0.113	0.312	0.0259	0.162	0.265	0.126	0.282	0.175	0.167	0.167	0.177	0.236	0.1243
Co	0.223	0.156	0.625	0.375	0.0417	0.412	0.258	0.330	0.506	0.150	0.215	0.469	0.682	0.576	0.0816
Cr	1.378	1.233	1.510	0.683	0.4523	0.806	0.579	0.471	0.043	0.751	0.432	0.601	0.535	0.190	1.1962
Cu	1.328	2.114	1.078	1.573	0.3905	1.015	2.030	0.963	1.445	0.585	0.682	0.948	0.675	0.648	0.5242
Fe	12.679	12.314	11.393	13.057	0.3030	11.536	5.129	10.443	10.136	6.207	7.121	6.264	4.721	5.350	0.9710
Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ni	0.501	0.702	0.879	0.633	0.4847	0.447	0.592	0.332	0.461	0.821	0.277	0.549	0.378	0.502	1.7069
Pb	0.900	1.038	0.828	0.761	0.8511	0.849	0.794	0.698	0.653	0.569	0.843	0.573	0.840	0.829	2.5166
Zn	1.245	0.676	1.308	1.044	1.6585	1.052	0.915	0.649	1.270	1.122	1.251	1.103	1.018	0.818	6.0245

***Note*: *F* = Fins; *G* = Gills; *M* = Muscles; *L* = Liver; *WB* = Whole Body; *ND* = Not Detected**

Table 9b: Bioaccumulation Factor (BAF) of selected heavy metals in different fish and their tissues collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

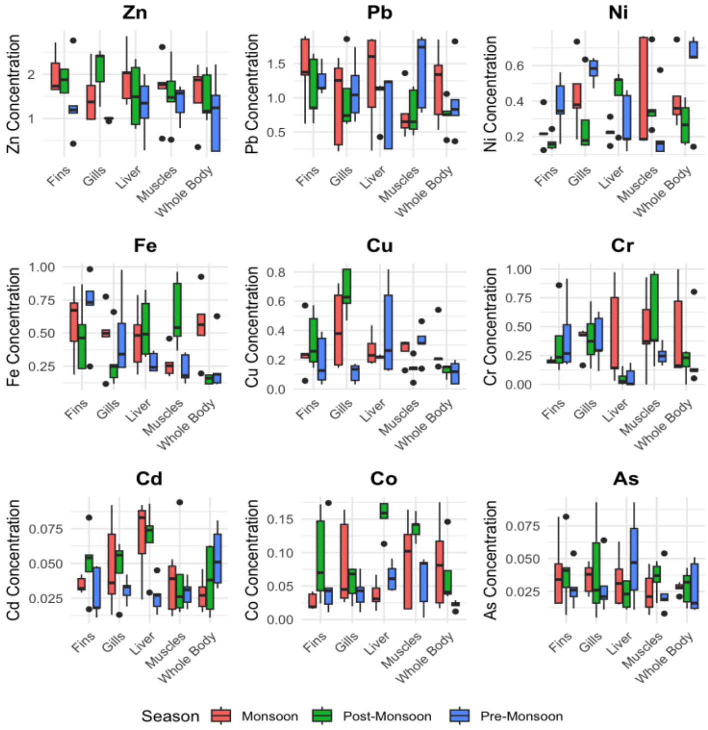
Fish	***Notopterus synurus***					***Oreochromis mossambicus***				
Heavy Metals	**F**	**G**	**M**	**L**	**WB**	**F**	**G**	**M**	**L**	**WB**
As	**1.808**	**1.323**	**1.182**	**1.444**	**1.121**	**1.040**	**0.808**	**0.556**	**0.687**	**0.606**
Cd	**0.169**	**0.162**	**0.284**	**0.298**	**0.227**	**0.205**	**0.167**	**0.102**	**0.154**	**0.192**
Co	**0.223**	**0.363**	**0.764**	**0.609**	**0.482**	**0.744**	**0.580**	**0.467**	**0.539**	**0.410**
Cr	**0.389**	**0.767**	**0.729**	**0.278**	**0.399**	**0.446**	**0.927**	**1.261**	**0.640**	**0.328**
Cu	**0.468**	**1.420**	**1.144**	**0.825**	**0.486**	**1.378**	**0.858**	**0.660**	**1.002**	**0.966**
Fe	**16.414**	**6.300**	**6.421**	**7.986**	**6.707**	**14.557**	**14.336**	**7.950**	**9.071**	**6.700**
Hg	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Ni	**0.385**	**0.630**	**0.371**	**0.521**	**0.625**	**0.480**	**0.992**	**0.846**	**0.432**	**0.363**
Pb	**1.154**	**0.685**	**0.608**	**0.835**	**0.659**	**1.040**	**0.804**	**0.782**	**0.891**	**0.949**
Zn	**0.763**	**1.228**	**1.062**	**1.001**	**0.570**	**1.130**	**0.864**	**0.759**	**0.810**	**0.865**

Note: ***F*** **= Fins;** ***G*** **= Gills;** ***M*** **= Muscles;** ***L*** **= Liver;** ***WB*** **= Whole Body;** ***ND*** **= Not Detected**

Table 10. Target hazard quotient (THQ), carcinogenic risk (CR), estimation of daily intake (EDI µg/kg/day) and hazardous index (HI) of selected heavy metals in different fish muscles collected from Karanja Reservoir, Bidar, Karnataka (2023-2024)

Heavy Metal	***Channa striata***			***Labeo rohita***			***Mystus cavasius***			***Notopterus synurus***			***Oreochromis mossambicus***		
	THQ	**CR**	**EDI**	**THQ**	**CR**	**EDI**	**THQ**	**CR**	**EDI**	**THQ**	**CR**	**EDI**	**THQ**	**CR**	**EDI**
As	0.0537	1.159E-07	0.0773	0.0450	9.727E-08	0.0648	0.1003	2.167E-07	0.1445	0.0910	1.966E-07	0.1310	0.0427	9.223E-08	0.0615
Cd	0.0168	4.919E-07	0.0806	0.0182	5.329E-07	0.0874	0.0261	7.645E-07	0.1253	0.0420	1.230E-06	0.2016	0.0151	4.427E-07	0.0726
Co	0.2357	-	0.3394	0.1251	-	0.1801	0.2590	-	0.3730	0.2893	-	0.4166	0.1773	-	0.2554
Cr	0.1673	1.205E-06	2.4091	0.0523	3.763E-07	0.7526	0.0572	4.116E-07	0.8232	0.0807	5.813E-07	1.1626	0.1398	1.006E-06	2.0126
Cu	0.0051	-	0.9710	0.0045	-	0.8669	0.0032	-	0.6082	0.0054	-	1.0282	0.0031	-	0.5947
Fe	0.0005	-	1.7842	0.0005	-	1.6363	0.0002	-	0.7392	0.0003	-	1.0046	0.0004	-	1.2466
Hg	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND	ND	-	ND
Ni	0.0190	1.660E-06	1.8245	0.0072	6.268E-07	0.6888	0.0082	7.155E-07	0.7862	0.0080	7.002E-07	0.7694	0.0183	1.599E-06	1.7573
Pb	0.2178	3.110E-08	3.6590	0.1836	2.622E-08	3.0845	0.2208	3.153E-08	3.7094	0.1600	2.285E-08	2.6880	0.2058	2.939E-08	3.4574
Zn	0.0049	-	7.0190	0.0024	-	3.4843	0.0038	-	5.4499	0.0040	-	5.6986	0.0028	-	4.0757
HI	**0.7207**	-	-	**0.4388**	-	-	**0.6788**	-	-	**0.6807**	-	-	**0.6053**	-	-

ND **= Not Detected**



Graph 1. Seasonal variation in heavy metal bioconcentrations across fish tissue types

Boxplots display the distribution of nine metals (Zn, Pb, Ni, Fe, Cu, Cr, Cd, Co, As) across five tissue types (fins, gills, liver, muscles, whole body) during monsoon (red), post-monsoon (green), and pre-monsoon (blue) seasons. Each panel represents one metal, showing median values, interquartile ranges, and potential outliers. Notable seasonal trends were observed for Zn, Cu, Co, and Cd, while tissue-specific accumulation was pronounced for Cu (gills) and Fe (fins).

DISCUSSION

Contamination of heavy metals in different fish species and water samples

(Table 2-7 & Graph 1) show the concentration of heavy metals in the fish species and water samples collected from Karanja Reservoir, Bidar, Karnataka (India).

Arsenic (As): As is one of the most potential toxic heavy metals present in the environment and originates from both natural and anthropogenic processes **(Saha *et al*., 2016).** According to U.S. Food and Drug Administration **(USFDA, 1993)**, about 90 percent of total human exposure of As instigates from fish and other seafood. From our study amongst all the fish species, the mean concentration of As was found the highest in the liver of *L. rohita* (0.093 ± 0.011 ppm) in the pre-monsoon season and in the gills of *M. cavasius* during the post-monsoon season whereas the lowest mean concentration of As was recorded (0.005 ± 0.004 ppm) in the gills of *C. striata* during the post-monsoon season. In the case of water samples, the bottom water showed the highest mean amount for As (0.067 ± 0.006 ppm) in monsoon season and the lowest (0.011 ± 0.002 ppm) in the pre-monsoon season. The concentration of As in all the fish samples and their tissues was found within the permissible limits (0.1 ppm).

However, in the case of water samples, it showed above the permissible limit (0.01 ppm) as prescribed by the **WHO (2011)** and **BIS (2012)**.

Cadmium (Cd): Cd is a highly toxic element capable of causing severe toxicity even when it is present at a very low concentration of ∼1 mg/kg **(Friberg *et al*., 1971)**. The accumulation of Cd in the human body may give rise to hepatic, pulmonary, renal, skeletal, reproductive effects and even cancer **(Ahmed *et al*., 2015)**. The highest mean concentration of Cd (0.094 ± 0.017 ppm) was found in the muscles of *N. synurus* during the post-monsoon season whereas the lowest mean concentration of Cd was recorded in the fins (0.011 ± 0.001 ppm) of *C. striata* during the pre-monsoon season and in the whole body (0.011 ± 0.002 ppm) of *L. rohita* during the post-monsoon season respectively. In the case of water samples, the mean concentration of Cd was found at its highest value in the bottom water (0.942 ± 0.001 ppm) in the pre-monsoon season, whereas the lowest recorded value for Cd was found in the surface water (0.058 ± 0.004 ppm) during the monsoon season. From the above result, it was found that the concentration of Cd in fish and water samples was found beyond the permissible limits (0.05 ppm), as prescribed by the **WHO (2011)** and **BIS (2012)**.

Cobalt (Co): Co is not often freely available in the environment, but when Co is not bound to soil or sediment particles the uptake by plants and animals is higher resulting in its accumulation. Co is beneficial for humans because it is a part of vitamin B12. However, too high concentration of cobalt may damage human health. It can cause sterility, hair loss, vomiting, bleeding, diarrhoea, coma and even death **(Zodape, 2014)**. The mean concentration of Co was found to be the highest (0.175 ± 0.001 ppm) in the whole body of *N. synurus* throughout the monsoon season, whereas the lowest mean concentration of Co was recorded in the muscles (0.003 ± 0.001 ppm) of *L. rohita* during the pre-monsoon season. Amongst the water samples, the mean concentration of Co was recorded at its highest value in the bottom water (0.711 ± 0.006 ppm) during pre-monsoon season, while the lowest mean concentration of Co was found in surface water (0.017 ± 0.008 ppm) during post-monsoon season. It is worthy to mention that Co is not regulated by any international or national agencies. Thus, values of Co were compared with those recorded in prior research; it was found that the current outcomes are higher than those reported by **Zodape (2014)**.

Chromium (Cr): The presence of Cr in the diet is of great importance due to its active involvement in lipid metabolism and insulin function **(Ahmed *et al*., 2015)**. The mean concentration of Cr was at its highest (0.998 ± 0.026 ppm) in the whole body of *C. striata* during the monsoon season, whereas the lowest recorded mean concentration of Cr was observed in the liver (0.004 ± 0.006 ppm) of *L. rohita* during the pre-monsoon season. In water samples, the highest mean Cr was recorded in the bottom water (0.921 ± 0.002 ppm) during the pre-monsoon season and the lowest in surface water (0.389 ± 0.003 ppm) during the same season. The concentration of Cr in all the fish samples and their tissues was found within the acceptable limits (1ppm); however, in the case of the water samples, the mean concentration of Cr was found above the permissible limit (0.05 ppm) as prescribed by the **WHO (2011) and BIS (2012)**.

Copper (Cu): Cu is a heavy metal that is both essential and toxic to living organism as its concentration significantly influences how it functions as both essential and toxic. Cu is also required for the formation of haemoglobin, while high intake of Cu can damaged the liver and kidney **(Zodape, 2014).** The highest mean concentration of Cu was found in gills (0.819 ± 0.032 ppm) and liver (0.819 ± 0.032 ppm) in *C. striata* during the post-monsoon and pre-monsoon season, respectively, whereas in *L. rohita*, the same highest mean concentration (0.819 ± 0.006 ppm) was recorded during the pre-monsoon season. The lowest mean concentration of Cd was recorded in *M. cavasius* gills (0.031 ± 0.043 ppm) in the pre-monsoon season and in the fins (0.031 ± 0.002 ppm) of *L. rohita* during the pre-monsoon season. For water samples, the Cu showed the highest mean concentration in the bottom water (0.512 ± 0.001 ppm) during post-monsoon season and the lowest mean concentration of Cu was recorded in surface water (0.116 ± 0.004 ppm) during post-monsoon season. From our study, the highest and lowest mean concentration of Cu in the case of fish (10 ppm) and water samples (2 ppm) were found to be within the acceptable permissible limits as prescribed by the **WHO (2011)** and **BIS (2012)**.

Iron (Fe): The ingestion of large quantities of Fe results in haemochromatosis, a condition in which normal regulatory mechanisms do not operate effectively, leading to tissue damage because of its accumulation. This condition rarely develops from simple dietary overloading **(Zodape & Tayade, 2016)**. The mean concentration of Fe was recorded to be the highest in *N. synurus* fins (0.982 ± 0.016 ppm) during the pre-monsoon season while the lowest recorded mean concentration of Fe was found in the whole body of *L. rohita* (0.112 ± 0.019 ppm) in the post-monsoon season. For the water samples, the mean concentration of Fe was at its highest value in the bottom water during monsoon season (0.124 ± 0.004 ppm) and the lowest mean was recorded in the surface water during pre-monsoon season (0.023 ± 0.009 ppm). The mean concentration of Fe in all the fish and water samples was found within the permissible limits (1- 3 and 0.3ppm), respectively, as prescribed by the **WHO (2011)** and **BIS (2012)**.

Mercury (Hg): Hg is a non-essential heavy metal and cannot be excreted easily. It could be retained in the tissues for long periods resulting in behavioral and cognitive changes, neurological impairment and lesions; during pregnancy, mercury can pass through the placenta to the foetus and may affect the development of central nervous system **(Authman *et al*., 2015)**. In the present study, the mean concentration of Hg was found below the detectable limit. Therefore, it is concluded that Hg may be absent in all the fish tissue samples and may not be present in the water samples. The permissible limit of Hg in fishes is 0.006 ppm and in water samples it is 0.5 ppm, as prescribed by the **WHO (2011)** and **BIS, (2012)**.

Nickel (Ni): Ni is an essential metal in very small amount, but it can be extremely dangerous if it is consumed more than the permissible amount. In our present study, the highest mean concentration of Ni was recorded (0.766 ± 0.009 ppm) in the muscles of *C. striata* in the monsoon season whereas, the lowest mean concentration of Ni was found in the muscles (0.117 ± 0.009) of *C. striata* and in muscles (0.117 ± 0.001) and liver (0.117 ± 0.002) of *L.*

rohita in the pre-monsoon season respectively. In case of water samples, the bottom water recorded the highest mean of Ni concentration during pre-monsoon (0.895 ± 0.003 ppm) and the lowest during post-monsoon season (0.363 ± 0.001 ppm). From our results, the mean concentration of Ni was found to be higher than the permissible limit in the different tissues of fish samples (0.1 ppm) and water (0.07 ppm) samples, as prescribed by the **WHO (2011)** and **BIS (2012)**.

Lead (Pb): Pb is a highly toxic heavy metal that can lead to severe health complications whenever it is consumed directly or indirectly. Lead is a non-essential heavy metal and endures numerous adverse health effects including neurotoxicity and nephrotoxicity **(García-Lestón *et al*., 2010)**. Based on our results, it was found that the recorded mean concentration of Pb was found the highest in the fins of *O. mossambicus* (1.903 ± 0.017 ppm) and the lowest mean concentration of Pb was observed in the gills of *N. synurus* (0.218 ± 0.002 ppm) in the monsoon season. However, in the case of water samples, the highest mean recorded concentration of Pb was found in bottom water during post-monsoon season (1.865 ± 0.002 ppm) and the lowest mean concentration of Pb was found in the surface water during monsoon season (0.732 ± 0.001 ppm). The current result indicates that the concentration of Pb in all the fish and water samples are above the permissible limits (0.3 ppm) prescribed by the **WHO (2011) and BIS (2012)**.

Zinc (Zn): Zn is a crucial metal for all living organisms and for enzymes like ferritin, flavin iron, transferrin and carbonic anhydrase **(Maurya *et al*., 2019)**. In our study, the mean concentration of Zn was the highest in the liver of *L. rohita* (2.865 ± 0.006 ppm) during the monsoon season while the lowest recorded mean concentration of Zn (0.254 ± 0.031 ppm) was found in the whole body of *M. cavasius* during the pre-monsoon season. In case of water samples, the highest mean concentration of Zn was observed in the bottom water during post-monsoon season (2.215 ± 0.004 ppm) and the lowest mean concentration of Zn was found in the surface water during monsoon season (1.013 ± 0.006 ppm). Concerning the present study, it was found that the levels of Zn are below the permissible limits in fishes (40-50 ppm) and in water (3-5 ppm), as prescribed by the **WHO (2011) and BIS (2012)**.

Overall ranking of heavy metals in different fish species and water samples

Across all five fish species and seasons, the metal ranking from the highest to the lowest is as follows: **Zn > Pb > Fe > Ni > Cr > Cu > Co > Cd > As > Hg.**

***Channa striata*:** Zn > Pb > Cr > Fe > Ni > Cu > Co > Cd > As > Hg.

***Labeo rohita*:** Zn > Pb > Fe > Ni > Cu > Cr > Co > Cd > As > Hg.

***Mystus cavasius*:** Zn > Pb > Ni > Fe > Cr > Cu > Co > Cd > As > Hg.

***Notopterus synurus*:** Zn > Pb > Fe > Ni > Cr > Cu > Co > Cd > As > Hg.

***Oreochromis mossambicus*:** Zn > Pb > Fe > Ni > Cr > Cu > Co > Cd > As > Hg.

The overall ranking of heavy metals in both surface and bottom waters from the highest to the lowest is represented as: **Zn > Pb > Ni > Cr > Cu > Cd > Co > Fe > As > Hg.**

Statistical analysis of heavy metals in fish and water

No significant results were detected since the level of As remained stable across seasons and tissues, indicating low variability or uniform distribution in fish tissues. While, Co showed significant differences toward season and tissues, with post-monsoon > monsoon (P= 0.0084), pre-monsoon < post-monsoon (P= 0.0002). Elevated Co concentrations were recorded in post-monsoon liver and muscles vs. monsoon fins and gills. It was noticed that pre-monsoon whole body < post-monsoon liver/muscles. Co accumulation is strongly influenced by both season and tissue. Liver and muscle tissues act as major accumulation sites, especially in the post-monsoon season. Cd showed only significant seasonal effect across pre-monsoon < post-monsoon (P= 0.0431). Cd levels increase toward the post-monsoon season, potentially due to runoff or sediment remobilization. Cr showed no significant effects for season and tissues as Cr concentration appeared stable, with no strong season-tissue-driven variability. Cu showed significant difference against season and tissues. It was recorded that the whole body < gills (P= 0.0016). Multiple significant differences involving post-monsoon: gills, which consistently had the highest Cu levels. Gills are a primary site for Cu accumulation, with enhanced uptake post-monsoon possibly due to higher dissolved Cu in water or gill-mediated filtration. Fe showed significant differences against season and tissues. The whole body had significantly less Fe than fins (P= 0.0168) post-monsoon: whole body < pre-monsoon: fins (P= 0.0177). Fe accumulates more in fins, particularly in pre-monsoon and significantly lower in whole body later in the season. Ni showed significant interaction across season and tissue. Pre-monsoon: gills > post-monsoon: fins (P= 0.0376). Pre-monsoon: whole body > post-monsoon: fins (P= 0.0275). Ni uptake is influenced by both tissue type and season. Gills and the wholebody tissues show elevated Ni level during pre-monsoon, likely due to higher environmental exposure or physiological activity. Pb showed no significant differences across season, tissue or their interaction. Pb exposure and accumulation remained relatively consistent across conditions, suggesting a stable baseline input. Lastly, Zn levels were significantly lower in the pre-monsoon season compared to both monsoon (P= 0.0131) and post-monsoon (P= 0.0229). No differences were observed across tissues or interactions. Remarkably, zinc accumulates more in aquatic systems post-monsoon, potentially due to increased runoff or biological uptake activity.

Metals like As, Cd, Co, Fe, Ni, and Zn (P-values > 0.25), showing no clear variation by either water depth or season under these conditions. Cr showed highly significant differences by both water and season. Cr concentration is strongly dependant on both depth and seasonal changes. Cu showed a relatively high F value (10.847) for seasonal differences, but it was not significant (P= 0.21) suggesting only a possible seasonal effect. Pb also displayed a large F-value (19.446) for water depth, hinting that surface or bottom water may affect its concentration (P= 0.142).

Bioaccumulation factor (BAF) in selected fish and their different tissues

According to **Hatem *et al*. (2015)**, the bioaccumulation factor (BAF) indicates the extent to which metals accumulate in the fish tissue from the surrounding water. In this study, BAF values of five fish species and their tissues were evaluated (Table 9a, b)and it was found that, *N. synurus* exhibited the highest BAF, particularly for Fe in fins, while *O. mossambicus* showed the lowest BAF for Cd in muscles. Overall, Fe displayed consistently high BAF values across species and tissues, whereas Cd showed the lowest. The prominent tissue-specific bioaccumulation was also observed in the gills (Cu), fins (Fe) and liver/muscles (Co). The lack of significant variation in Pb, Cr and As was observed suggesting either steady background exposure or low bioavailability and low bioaccumulation. According to prior research **(Ozmen *et al*., 2008; Ahmed *et al*., 2019a)**, the bioaccumulation patterns differ because of the size, tissue metabolism, the conditions of its habitat and different routes of exposures. As a result, these factors vary from one species to another species, as every fish species has a unique bioaccumulation pattern of uptake in response to the metals **(USEPA 1989; Jefrey & Alison 1993; Ahmed *et al*., 2019b)**.

Health risk assessment

The accumulation of heavy metal in fish can have a direct impact on the health of those people who consume fish on a regular basis and who live near the fishing area or far away. To understand and manage this risk, it is important to perform health risk assessment for fish originated from contaminated resources. Health risk evaluations assume that most substances have a non-cancerous effect and a threshold response **(Yadav *et al*., 2019)**.

Non-carcinogenic risk (EDI, THQ and HI)

Consuming contaminated fish with heavy metals can cause a serious risk to health, hence it is necessary to analyze the contaminants before the fishes reach the consumers **(Feng *et al*., 2020)**. EDI was calculated using the oral reference dose (RfD), which is a useful indicator to assess both non-carcinogenic and carcinogenic risk associated eith eating fish that contains heavy metals **(Liu *et al*., 2018)**. Additionally, EDI indicates the daily quantity of each metal that a person would consume and help to ensure that consumption intake remains below level that could cause harm **(Abtahi *et al*., 2017)**.

Estimated daily intake of metals (EDI):

The EDI of heavy metals through the consumption of five edible fish species from Karnataka is presented in Table (10). Among the heavy metals, Zn contributed the highest proportion to daily intake, while As contributed the lowest. These findings are consistent with earlier reports **(Saha *et al*., 2016; Atique Ullah *et al*., 2017)**. Furthermore, the EDI values for all studied metals across different fish species are below the recommended permissible limits, indicating that consumption of these fish does not pose any significant health risk to the local population.

Target hazard quotient (THQ):

The muscle concentration was assessed in different fish species to calculate the THQ for the people living in Karnataka (Table 10). The THQ values of selected heavy metals showed that the highest THQ was recorded for Co (0.2893) in *N. synurus* whereas the lowest Fe (0. 0002) was found in *M. cavasius*. Eating freshwater fish contaminated with heavy metals can seriously affect human health **(Ezemonye *et al*., 2018; RajeshKumar and Li, 2018)**. The acceptable THQ guideline value is 1 **(USEPA, 2011)**. In our study, all the THQ values for the selected heavy metals across the muscles in different fish species are below the threshold levels (THQ <1). Therefore, it can be concluded that the long- term consumption of these fishes by local population will not pose any significant health risk. It is assumed that THQ depends on the fish size and weight. For fish, the smaller the size and the lighter the weight, the higher the bioaccumulation capacity, and THQ value may be greater than 1. Whereas larger size and heavier weight of fish show low THQ. Additionally, an organism's size and age are important factors that influence metal accumulation and the associated health risks **(Farkas *et al*., 2003; Ahmed *et al*., 2016)**. All the fish samples are collected from the same habitat; however, differences in feeding habits and developmental stages, rather than age, can cause variation in their size and metal accumulation **(Farkas *et al*., 2003)**. In our study, all the fish had THQ value less than 1, even though their average weight was above 200gm. Because THQ and HI do not have a specific dose relationship, they are not regarded as an immediate measure of potential hazard **(USEPA, 1989)**. Our findings contradict the study carried out by **Noman *et al.* (2022)**, who reported that larger size fish accumulates more metals in their tissues and may therefore pose a greater risk to human health. However, our study did not specifically focus on size-based metal accumulation as all the fish samples were within an average weight range of 210 ±7.57 to 510 ±6.55gm, which is consistent with the low THQ values, indicating a minimal health risk to humans (THQ < 1).

Hazardous index (HI):

The HI was calculated for all heavy metals in different fish species and in their muscles from Karanja Reservoir (Table 10). The highest HI was recorded in *C. striata* (0.7207) and the lowest in *L. rohita* (0.4388). Our result showed that the combined value of THQ for HI of all the estimated metals were below 1. Therefore, it is deduced that the HI is under the permissible limit (HI < 1) as set by the **USEPA (2011)**. Consequently, the consumption of these fish species from the Karanja Reservoir are under threshold hazardous limit, posing no significant risk to human health.

Carcinogenic risk (CR)

Carcinogenic heavy metals are found in both inorganic and organic forms; where organic is considered deadlier than the inorganic form **(Means 1989; Zhong *et al*., 2018)**. Chronic exposure to these metals can damage multiple systems, including the circulatory system, digestive system, respiratory tract, nervous system and may lead to failure of vital organs such as brain, kidney and liver **(Mandal & Suzuki 2002; Kibria *et al*., 2016)**. Prolonged exposure to Cd can cause disruption of circulatory system **(Fang *et al*., 2014)**. Given that the health risk depends on both metal concentration and total amount of fish consumed, future assessments should account for metal bioavailability and cumulative dietary intake upon evaluating the safety of fish consumption. In our study, we measured As, Cd, Cr, Ni and Pb metals across the five fish species in their muscles as these are known as carcinogens (Table 10). The calculated CR values were between As (9.727×10^{-8}) and Cr (1.006×10^{-8}). Generally, the values of CR lower than 10^{-6} is considered negligible when it is at or below 10^{-6}, whereas CR between 10^{-6} to 10^{-4} is considered acceptable, but any value above 10^{-4} represents a non-tolerable risk that could lead to carcinogenic effect in humans and other organisms **(Means 1989; Baki *et al*., 2018)**.

All the measured CR values were well below 10^{-6}, indicating a negligible carcinogenic risk from the consumption of these fish species. Therefore, based on the current exposure levels, consumers are not expected to face any significant carcinogenic threat from their consumption.

CONCLUSION

From the present findings, the concentrations of Cd, Ni, Pb and Cr were found to be high in different tissues of fish samples and in water samples collected from Karanja Reservoir, Bidar, Karnataka (India). It can be assumed that the collection site under investigation might be receiving outfalls from industrial waste and sewage from the neighboring towns and villages. The levels of other heavy metals such as As, Co, Cu, Ni, Pb and Zn were within permissible limits in fish tissues and water samples. Seasonal variations indicated that metal contamination in both fish tissues and water was more dominant during the pre- and post-monsoon seasons. Among the analyzed metals, Zn exhibited the highest concentration across all fish species and their tissues as well as in surface and bottom water samples, followed by Pb in the studied samples. These elemental toxicants may be transferred to humans through fish consumption and drinking water, posing potential health risks due to their cumulative effects in the body.

Health risk assessments (EDI, THQ, HI) revealed that, in all fishes, EDI value was the highest for Zn and the lowest for As. The THQ was found to be the highest for Co (0.2893) in *N. synurus* and the lowest for Fe (0. 0002) in *M. cavasius*, while the HI was the highest in *C. striata* (0.7207) and the lowest in *L. rohita* (0.4388). All values were low and well below the safety threshold limit, indicating negligible non-carcinogenic risk. The estimation of non-carcinogenic risk indicates that the intake of individual heavy metals through fish consumption is safe for human health. The CR indices for As, Cd, Cr, Ni and Pb also remains below the critical level, indicating no significant carcinogenic risk to consumers. However, the CR value for As indicates a potential cancer risk through prolonged fish consumption.

Overall, the results suggest that the fish and water from Karanja Reservoir are not heavily burdened with metals at the present. Nevertheless, continuous monitoring is essential, as future industrial and agricultural activities in the region may increase contamination levels. Therefore, it is recommended that fish and water from the reservoir be periodically monitored to prevent excessive trace metal intake by humans and to maintain the ecological health of the aquatic environment. Implementation of strict waste disposal and effluent management practices is strongly advised to ensure environmental safety and the protection of aquatic life.

DECLARATION OF COMPETING INTEREST:

The authors declare that they have no known competing financial interests that could have influence the work reported in this manuscript.

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