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Bioaccumulation of Lead and Chromium in *Cyprinus carpio* (L.) from the Small Waterbodies Located in Rice Paddy Fields of Western Ghats, India

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ABSTRACT

Small water bodies located in agricultural fields in the Western Ghats have potential as sites for fish culture. However, these water bodies are contaminated with agrochemicals, which affect both the water and sediment and result in bioaccumulation in fish. This study investigated the bioaccumulation of lead (Pb) and chromium (Cr) in *Cyprinus carpio*, reared in two small water bodies (S1 and S2). The concentration of Pb followed the pattern: water < fish tissue < sediment, with 0.4, 28.3, and 71.4% of the total Pb estimated in each, respectively. The concentration of Cr showed a similar pattern: water < fish tissue < sediment, with 0.2, 6.7, and 93.1% of the total Cr recorded, respectively. Fish from site 1 contained 12.4 ± 4.85mg Pb/kg dry weight, while those from site 2 had 7.8 ± 1.81mg Pb/kg dry weight. Similarly, Cr levels were 14.0 ± 3.10mg/ kg dry weight in fish from site 1 and 11.1 ± 2.40mg/ kg dry weight in fish from site 2. These concentrations of both Pb and Cr exceed the permissible limits recommended by the World Health Organization (WHO). Accumulation of both Pb and Cr in fish was found to increase with fish growth ($P < 0.05$). The presence of elevated levels of these metals in food fish raised in such water bodies may pose significant health risks to humans.
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INTRODUCTION

Heavy metals are known for their toxicity, persistence, non-biodegradability, and bioaccumulation in piscine species **(Giri *et al.*, 2021)**. Lead (Pb) and chromium (Cr) are natural components of the Earth's crust, found at trace levels in aquatic environments. Pb exhibits several oxidation states in the environment **(Kasthuri & Chandran, 1997)** and is abundantly present in aquatic ecosystems. High concentrations of Pb are associated with anthropogenic activities **(Kim & Kang, 2016)**. Cr exists in both trivalent and hexavalent forms **(Kamila *et al.*, 2023)**. These heavy metals can easily accumulate and bio-magnify through water, sediment, and aquatic organisms, thereby harming aquatic ecosystems **(Ahmed *et al.*, 2019; Giri *et al.*, 2021)**.

Pb accumulation in fish tissues induces the production of reactive oxygen species and causes oxidative stress, leading to neurotoxicity. It also acts as an immunotoxicant by affecting fish immunity **(Lee *et al.*, 2019)**. Exposure to and accumulation of Pb result in a wide range of toxic effects on physiological, behavioral, and biochemical functions in animals; it also damages the central nervous system (CNS), peripheral nervous system (PNS), hematopoietic system, cardiovascular system, and organs such as the liver and kidney **(Hsu & Guo, 2002; Yousafzai & Shakoori, 2008)**.

Cr naturally occurs as trivalent and hexavalent forms; the poor permeability of Cr [III] renders it non-toxic, whereas Cr [VI] can cross biological membranes and cause several adverse health effects in fish **(Kamila *et al.*, 2023)**. The toxic effects of Pb and Cr arise from bioaccumulation and metal uptake **(Eroglu *et al.*, 2015)**. Fish are exposed to heavy metals in aquatic environments through direct contact, diet, and gastrointestinal absorption.

The agricultural fields of the Western Ghats include many small, perennial or seasonal, and natural or manmade water bodies at lower elevations. These water bodies serve as potential sources for aquaculture ventures. Due to favorable productivity and ease of monitoring, small-scale fish farming is practiced in these areas. Fish species such as *Cyprinus carpio*, *Catla catla*, *Labeo rohita*, and *Cirrhinus cirrhosis* are cultivated in composite farming systems within these water bodies, which contribute to local food security.

As these water bodies are embedded within agroecosystems, the continuous discharge of agricultural runoff and leaching processes introduce heavy metals into them. The presence of heavy metals in fish has been extensively studied over recent decades **(Rajeshkumar & Li, 2018)**. Studies indicate that the degree of metal bioaccumulation in fish depends on the metal type, fish species, and water chemistry. Additionally, sediment serves as a significant source of contaminants, especially for bottom-feeding fish species **(Görür *et al.*, 2012; Petrovic *et al.*, 2013; Rajeshkumar & Li, 2018)**.

Cyprinus carpio is a bottom-feeding fish that spends most of its time near the sediment in water bodies. In the study area, annual carp production ranged from 790 to 1285kg/ ha. Due to intensive agricultural activities, Pb and Cr concentrations in the study area were reported to range from 11.27 to 84mg/ kg, and 22 to 256mg/ kg, respectively **(Hegade *et al.*, 2023)**. Although these fish provide a crucial nutritional source for local communities, no specific studies have addressed the bioaccumulation of Pb and Cr in *C. carpio* cultivated intensively in these water bodies.

Therefore, this paper aimed to investigate the bioaccumulation of Pb and Cr in *C. carpio* in relation to heavy metal concentrations in the water and sediment.

MATERIALS AND METHODS

1. Study area

The research was carried out in two perennial standing water bodies - S1 (14°01’05” N, 75°18’43” E alt: 694 m asl) and S2 (13°43’36” N, 75°37’55” E alt: 637 m asl), each approximately 3-9 acres in size, surrounded by rice paddy fields in the Western Ghats of Karnataka State (Fig. 1). In these water bodies, *C. carpio* is farmed alongside other species as part of a composite fish farming practice. The potential sources of pollution in these water bodies are agricultural runoff. The total drainage area of S1 and S2 is 33.5 and 45 acres, respectively. *C. carpio* was cultivated in both water bodies using the same broodstock.

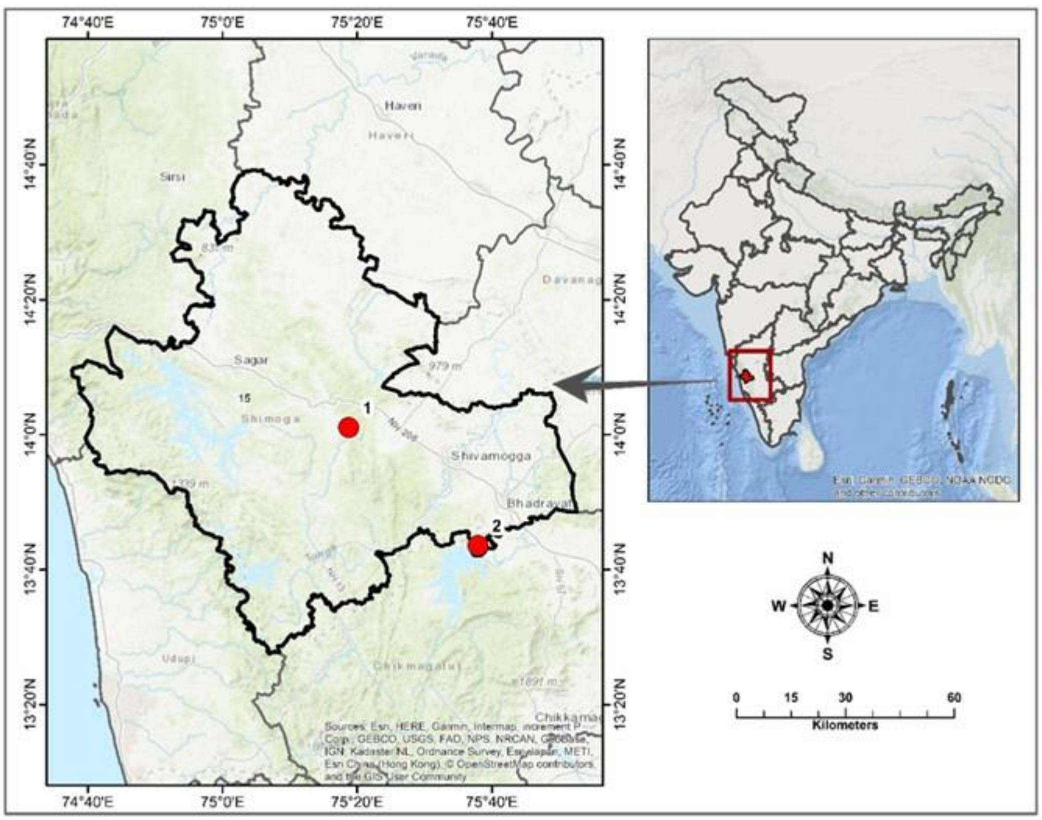

Fig. 1. Map of the study area. The red dots numbered 1 and 2 represent sampling sites 1 and 2, respectively

2. Water, sediment, and fish sampling

Fish samples were collected from S1 and S2 using a gill net with the help of fishermen during 2021 and 2022. A subsample of *C. carpio* was taken from the gillnet catch. The total length (mm) and body mass (g) of each fish in the subsample were recorded on the spot. Then, samples were transported to the laboratory in an ice-cold box and samples were kept under -20°C for further analysis.

The total length of fish in sub-samples of S1 was 23.5 ± 4.90cm and in S2 it was 23.7 ± 5.23cm, and the differences in the total length of samples taken from the two sites were insignificant ($F_{1,38}$ =0.01, P= 0.928). Similarly, the body mass of the fish used in the experiment was 249.3 ± 153.85g at S1 and 233.4 ± 13.46g at S2, and the differences were insignificant ($F_{1,38}$ =0.11, P= 0.742).

The body condition factors are an important tool in the fishery since they provide information about the growth of the fish, its general well-being, and fitness **(Jisr *et al.*, 2018)**. Based on the length and body mass of the fish, the body condition factor (BCF) was calculated using the following equation of **Fulton (2011)**:

$$BCF = 100 \times W / L^3$$

Where, W is the total weight of the fish and L is its total length. The BCF was used as standard criteria for analyzing the heavy metal accumulation in fish tissue.

The water and sediment samples were collected simultaneously by sampling fish. The water and sediment sampling were done following the standard method of **APHA (2017)**. A Mercury bulb thermometer (GH Zeal Ltd, London; precision 0.1 °C) and a portable pH pen Hanna (model HI 98107, precision 0.1 pH) were used to measure water and sediment temperature and pH on the spot. **De Vos *et al.* (2005)** described the loss of ignition method to estimate the total organic carbon in sediment samples. The same method was followed to estimate the total organic carbon of sediment samples.

3. Heavy metals quantification in water, sediment, and fish tissue samples

Water, sediment, and fish tissue samples were analyzed for Pb and Cr content following the 3050B **US-EPA (1996)** and **APHA (2017)** methods. To analyze Pb and Cr content in fish, 20 specimens were randomly selected from the sample. Since local people typically remove the gut, gills, and scales before consuming fish, only muscle tissue was analyzed to estimate heavy metal levels. White muscle tissue samples were taken from the fish, oven-dried at 60°C, and ground into a fine powder. One gram of the finely ground tissue was treated with a 4:1 ratio of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$), and heated on a hotplate at 50 °C until digestion was complete, as indicated by the appearance of white fumes. The digest was then diluted to 50 mL using double-distilled water **(Javed *et al.,* 2015)**. Acid-extracted samples were analyzed for lead and chromium content using an atomic absorption spectrophotometer (AAS) (PinAAcle 900F).

4. Data analysis

SPSS (version 20) was used for all statistical analyses. Karl Pearson's correlation was applied to determine significant relationships between heavy metal concentrations in water, sediment, and fish tissue. Analysis of variance (ANOVA) was performed to assess significant differences in various parameters between the two sites. In both statistical tests, a significance level of $P < 0.05$ was considered statistically significant.

RESULTS

The water pH of S1 ranged between 5.6 – 7.6 and that of S2 was 7.5 – 8.3, showing a significant difference between the sites over the study period ($F_{1,30}$ =7.81, P= 0.023). The water temperature of both water bodies fluctuated between 20 and 29°C. At S1 and S2, the mean water temperature was 25 ± 3.67 °C and 24.0 ± 3.52°C with an insignificant difference between sites ($F_{1,30}$ =0.47, P= 0.51). The dissolved oxygen content of the water between the sites fluctuated in a narrow range (S1: 6.7 ± 0.92, and S2: 7.1 ± 0.82) and differences were insignificant ($F_{1,30}$ =1.47, P= 0.234). The total organic content of the sediment was higher at S1 (17.3± 1.97 %) than S2 (6.4 ± 0.65 %) and showed clear differences ($F_{1,30}$ =522.69, P= 0.001).

At both sites, Pb and Cr content in water remained low compared to sediment. Pb content in water at S1 and S2 was 0.13 ± 0.001mg/L and 0.09 ± 0.003 mg/L respectively, showing a significant difference between sites ($F_{1,37}$ =39.63, P= 0.001). The corresponding sediment Pb content was 29.2 ± 1.77 and 16.1 ± 3.19 mg/kg respectively. Between the S1 and S2, there were significant differences in the Pb content of the sediment ($F_{1,37}$ =241.58, P= 0.001). Like Pb content in water, the Cr was represented with a low concentration of 0.4 ± 0.09mg/ L at S1 and 0.03 ± 0.01mg/ L for S2. Even at low concentrations, we found a significant difference in the Cr content of water between S1 and S2 ($F_{1,37}$ =41.06, P = 0.001). However, like Pb of the sediment, the Cr content too showed a high concentration in sediment (S1: 114.6 ± 15.94 mg/ kg sediment; S2: 337.4 ± 9.86mg/ kg sediment) with considerable significant differences between the two sites ($F_{1,37}$ =2961.9, P= 0.001).

The Pb and Cr content in the fish was high in S1 (Pb = 12.4 ± 4.85 mg/kg dry wt; Cr = 14.0 ±3.10 mg/kg dry wt) compared to S2 (Pb = 7.8 ± 1.81 mg/kg dry wt; Cr = 11.1 ± 2.40 mg/kg dry wt) and showed a significant difference between the sites (Pb: $F_{1,37}$ =18.82, P= 0.0001; Cr: $F_{1,37}$ =7.39, P= 0.010). In both sites, the water pH, dissolved oxygen, water temperature, and total organic matter did not show a significant correlation ($P < 0.05$) with the Pb and Cr content of water, sediment, and in the fish tissue. However, the Pb content of water decreased with decreasing Pb content of the sediment (r = 0.59, P= 0.005) indicating the role of sediment Pb in determining the

Pb content in the water. The Pb in fish tissue did not show a correlation with the Pb content of water (r = -0.08, P= 0.748) and sediment (r = -0.26, P= 0.264). However, the Cr content of the fish tissue showed a strong significant correlation with sediment Cr (r = -0.67, P= 0.001) and a feeble correlation with water Cr (r = -0.43, P= 0.05). Fig. (2) illustrates the accumulation of Pb and Cr in fish tissue, plotted against the body condition factor of fish. Both Pb and Cr showed a significant increase in the BCF of fish at S1 (Pb: r= 0.96, P= 0.0001, Cr: r = 0.87, P= 0.001) and S2 (Pb: r = 0.82, P= 0.0001, Cr: r= 0.58, P= 0.002) indicating that the Pb and Cr accumulation in tissue increased with the growth of fish.

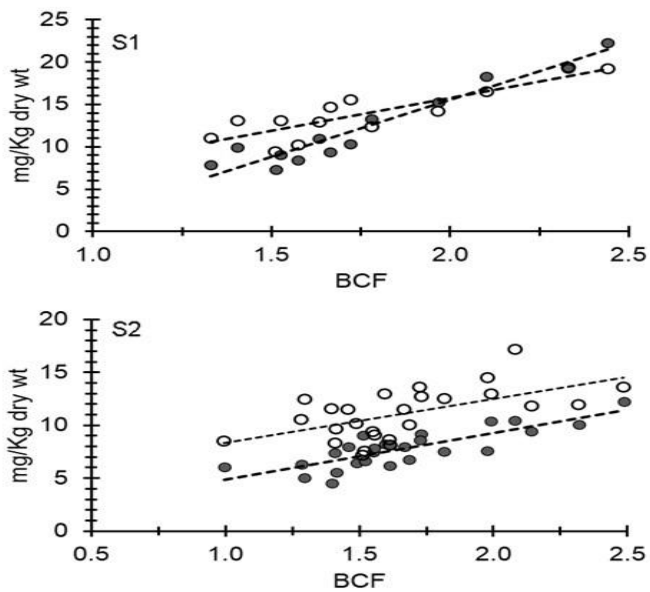

Fig. 2. Pb and Cr content in fish tissue at S1 and S2 plotted against the body condition factor. The closed circle represents the fish's Pb content (mg/kg dry wt) and the open circle represents the Cr content (mg/kg dry wt). BCF is the Fulton body condition factor

DISCUSSION

Sediment provides a long-term record of the effects of anthropogenic discharges in areas where impacts often occur before being manifested in the water column **(Ramessur & Ramjeawon, 2002)**. Sediments in water bodies are the major zones for metal deposition, with studies revealing that sediments hold more than 99% of the total metal content in aquatic systems **(Odiete, 1999)**.

In the present study, the concentration of Pb followed the order: Water < Fish tissue < Sediment (S1: 0.13 ± 0.01mg/ L < 9.8 ± 1.6mg/ kg dwt < 29.2 ± 1.77mg/ kg sed; S2: 0.09 ± 0.003mg/ L < 7.4 ± 2.12mg/ kg dwt < 16.1 ± 3.19mg/ kg sed), representing approximately 0.4% of Pb in water, 28.3% in fish tissue, and 71.4% in sediment. The LC50 value of Pb for *C. carpio* is reported as 5.417 mg/L at 96 hours **(Mathew *et al.*, 2023)**. In this study, water at both sites contained Pb levels significantly lower than the LC50 value. However, fish tissue showed approximately 30–40% of the LC50 concentration, indicating substantial bioaccumulation.

Similarly, Cr concentrations followed the order: Water < Fish tissue < Sediment (S1: 0.4 ± 0.29mg/ L < 12.8 ± 2.31mg/ kg dwt < 114.6 ± 15.94mg/ kg sed; S2: 0.02 ± 0.01mg/ L < 11.9 ± 2.99mg/ kg dwt < 337.4 ± 9.86mg/ kg sed), representing about 0.2%, 6.7%, and 93.1% of total Cr in water, fish tissue, and sediment, respectively. An LC50 value of 87.9 mg Cr/L at 96 hours was reported for Cr **(Bakshi & Panigrahi, 2018)**. The Cr concentrations observed in both water and fish tissue were well below the LC50 value. The findings indicate that the sediment of these small water bodies contains more than 70% of the total Pb and over 90% of the total Cr content.

A study conducted by **(Nayaka *et al*., 2009)** in an uncontaminated pond reported Pb concentrations of 1.0mg/ L in water, 5mg/ g in sediment, and 0.5mg/ g dry weight in *C. carpio* muscle; Cr was present at 0.2mg/ L in water, 18 mg/g in sediment, and 0.8 mg/g dry weight in fish. That study indicated higher Pb levels in sediment, followed by water and then fish tissue, while Cr was highest in sediment, followed by fish tissue, and then water. In comparison, the current study recorded significantly higher concentrations, maintaining the trend of Water < Fish tissue < Sediment. This clearly demonstrates the bioaccumulation of both Pb and Cr in fish tissue.

Given that these small water bodies are perennial and closely linked to irrigation systems surrounding rice paddy fields, there is a high likelihood of Pb and Cr entering the water through agricultural runoff and accumulating in the sediment over time. Aquatic organisms have been shown to absorb Pb and Cr from such contaminated environments **(WHO, 1989; Bakshi & Panigrahi, 2018)**. Sediment plays a key role in the bioaccumulation of heavy metals. As top-level organisms in the aquatic food chain, food fish accumulate heavy metals from water, sediment, and food sources **(Rajeshkumar & Li, 2018; Maurya *et al.*, 2019)**. *C. carpio*, an omnivorous species that feeds on bottom debris and aquatic vegetation, may be exposed more intensively to sediment-bound metals, explaining the high Pb and Cr concentrations in its tissue, despite statistically significant differences in sediment concentrations between S1 and S2.

The levels of heavy metals in aquatic environments have been increasing rapidly. Due to their non-biodegradable nature, heavy metals tend to accumulate in fish and other aquatic organisms **(Zamora-Ledezma *et al.,* 2021)**. Consequently, human consumption of contaminated fish poses serious health risks **(Latif *et al.,* 2024)**. Numerous studies have confirmed that Pb and Cr are highly toxic even at low concentrations **(Hossain *et al.*, 2022)**. According to **(WHO, 1989)**, the maximum permissible limits for Pb and Cr in edible fish are 0.5 mg/kg and 0.05–0.15mg/ kg, respectively **(Bakshi & Panigrahi, 2018)**.

In the current study, Pb concentrations were observed at 12.4 ± 4.85mg/ kg dry weight at Site 1 and 7.8 ± 1.81mg/ kg dry weight at Site 2. Cr concentrations were 14.0 ± 3.10mg/ kg dry weight at Site 1 and 11.1 ± 2.40mg/ kg dry weight at Site 2. These levels significantly exceed the permissible limits set by the WHO. Since these fish are a major nutritional source for local populations, elevated Pb and Cr levels could pose a serious threat to human health.

CONCLUSION

Fish grown in small water bodies located amidst the agriculture fields have a high content of Pb and Cr in their tissue. The sediment of these water bodies has a high content of Pb and Cr compared to water. The high sediment Pb and Cr could be the source of the major factors for high bioaccumulation in fish tissue of omnivorous bottom-feeding *C. carpio.* Both Pb and Cr accumulation increased with the growth of the fish. The Pb and Cr levels recorded in the fish tissue are beyond the permissible level recommended by WHO. Since Pb and Cr are noxious heavy metals toxic to human health, the presence of high contents of these metals in fish grown in these water bodies could pose health issues.

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AUTHOR CONTRIBUTIONS

Both authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Chetanakumara M V and S V Krishnamurthy. The first draft of the manuscript was written by Chetanakumara M V and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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