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RESEARCH

Assessing Heavy Metal Contamination in Commonly Used Fertilizers for Polyculture Fish Ponds and Its Implications for Human Health: A Comprehensive Investigation

Saira Naz[1] · Madeeha Arshad[2] · Saima Majeed[3] · Sawera Maqaddas[4] · Syed Sikandar Habib[5] · Osman Sabri Kesbiç[6] · Salim S. Al-Rejaie[7] · Mohamed Mohany[7] · Teresa Bottari[8] · Francesca Aragona[9] · Francesco Fazio[9]

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Abstract

Over-fertilizing fish ponds can cause pollution, introducing heavy metals into the food chain and posing health risks. The present study investigated the incidence of heavy metals (Pb, Cu, Cd, and Cr) in commonly applied fertilizers, including nitrogen, phosphorus and potassium (NPK), triple superphosphate (TSP), and di-ammonium phosphate (DAP), and their association with heavy metals in water, sediment, and cultured fish species (*Catla catla*, *Labeo rohita*, and *Cyprinus carpio*) in polyculture fish ponds. The study was conducted over 4 months, with four groups in triplicates: control (no fertilizer), group 1 (NPK), group 2 (TSP), and group 3 (DAP). Heavy metal analysis was carried out using atomic absorption spectrophotometry before and after fertilizer application. Significantly ($p < 0.05$) higher levels of heavy metals were observed in water and sediment after applying fertilizers, with the most pronounced results in group 3 (DAP) followed by group 2 (TSP). The concentration of heavy metals was significantly ($p < 0.05$) higher in group 3 (DAP) fertilizers compared to other groups. Compared to the control, the concentration and bioaccumulation of heavy metals were significantly ($p < 0.05$) higher in the fertilizer-applied groups, with notably higher levels in group 3 (DAP). Cluster analysis and the correlation matrix did not show any significant association between the heavy metals and the fertilizers, indicating a complex interplay between the biotic and abiotic factors of the system. The health index (HI) value was < 1 in fish muscles of all studied groups, indicating the fish are safe for consumption. The study recommends monitoring and regulating fertilizer use, especially DAP, to prevent heavy metal contamination, and exploring sustainable alternatives to minimize environmental and health risks.

Keywords Fertilizer · Heavy metals · Health risk · Environmental pollution · Water · Aquaculture

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✉ Francesco Fazio
ffazio@unime.it

[1] Centre for Research On Fish Nutrition and Environmental Ecology of the Ministry of Agriculture, Shanghai Ocean University, Shanghai 201306, China

[2] Department of Zoology, Division of Science and Technology, University of Education Lahore, Faisalabad Campus, Faisalabad 38040, Punjab, Pakistan

[3] Department of Maritime Sciences, Bahria University Karachi Campus, Karachi 75000, Sindh, Pakistan

[4] Department of Zoology, Government College University Faisalabad, Faisalabad 38000, Punjab, Pakistan

[5] Department of Zoology, University of Sargodha, Sargodha 40100, Punjab, Pakistan

[6] Department of Animal Nutrition and Nutritional Diseases, Kastamonu University Veterinary Faculty, 37150 Kastamonu, Turkey

[7] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 55760, 11451 Riyadh, Saudi Arabia

[8] Institute for Biological Resources and Marine Biotechnology, National Research Council. Spianata S. Raineri, 86 98122 Messina, Italy

[9] Department of Veterinary Sciences, University of Messina, Via Palatucci, N. 13, 98168 Messina, Italy

Introduction

Fertilizers are commonly utilized in both land-based agriculture and fish ponds to enhance productivity [20, 58]. They come from various sources, including natural and industrial sources [49]. In modern agriculture, fertilization typically involves three primary macronutrients: nitrogen, potassium, and phosphorus, along with additional micronutrient supplements [64]. Farmers use different methods to apply fertilizers, such as liquid, pelletized, and dry applications, using manual tools or machinery [26, 48]. The presence of natural food like vegetation and crustaceans in a pond significantly impacts fish growth. However, these natural feeds also rely on available food for their own survival [1]. Therefore, aquaculture utilizes a range of organic and inorganic fertilizers to boost overall production. Nonetheless, excessive fertilizer usage can harm the habitat and lead to groundwater contamination with inorganic substances [40].

Heavy metals can find their way into fertilizers during production, and when these fertilizers are applied to soil and fish ponds, they enter the food chain, potentially impacting human health [53, 56]. This is because some heavy metals are known to be carcinogenic, mutagenic, and toxic to the reproductive system, posing a significant health risk when present in the environment [9]. Studies have investigated the effects of heavy metal pollution on various components of the ecosystem, including vegetables, crops, fish species, and habitats [21, 22, 29]. Both organic and inorganic fertilizers used in fishponds and soil contain heavy metals [56]. Fish, for example, absorb metals from the water and sediment through ingesting suspended particulates. Consuming such contaminated produce can have adverse health effects on both humans and animals [51].

Several factors can contribute to the contamination of food, land, and water with heavy metals, including the use of contaminated water for irrigation, fertilizers containing trace and heavy metals, industrial emissions, metal-based pesticides, transportation, and post-harvesting processes [2, 37, 39]. This contamination at various stages of the food production process can have long-term effects on human health and future generations [30]. Chemical fertilizers are increasingly used globally in aquaculture ponds and agricultural soil to enrich nutrient levels, driven by the growing human population's demand for food [3, 14]. This trend has led to the widespread use of inorganic fertilizers over the past five decades to boost food production [35]. Continuous fertilizer application can lead to the redistribution and accumulation of toxic heavy metals such as lead (Pb), copper (Cu), cadmium (Cd), and chromium (Cr) in water and sediment, resulting in bioaccumulation in food [40]. Over the last few decades, environmental pollutants, particularly heavy metals, have surged worldwide due to population growth, urbanization, and human activities [15, 55]. Despite the critical role of fertilizers in aquaculture, there is limited research focusing specifically on the incidence of heavy metal contamination in commonly used fertilizers applied to fish ponds. Understanding the levels of heavy metal contamination in these fertilizers is essential for assessing potential risks to aquatic ecosystems and human consumers of farmed fish.

Pakistanis traditionally prefer wild-caught fish; farmed fish has become increasingly popular due to its wider availability in the market. Many consumers now enjoy farmed fish as a reliable and accessible alternative, as it offers consistent quality and freshness [44]. The fish species selected for this study, including *Catla catla*, *Labeo rohita*, and *Cyprinus carpio*, are widely consumed due to their economic viability, with farmers often using fertilizers to increase yield. However, this practice poses a risk of heavy metal contamination in the food chain. Therefore, this study aims to fill a critical gap in the literature by systematically investigating the presence of heavy metals (Pb, Cu, Cd, and Cr) in commonly used fertilizers, fish feed, fish species, sediment, and water in polyculture fish ponds. By evaluating the health risks associated with heavy metal pollution from these sources, this research will provide valuable insights into the implications of current aquaculture practices on human health, guiding policy recommendations for safer farming practices and contributing to sustainable aquaculture management.

Materials and Methods

Experimental Design

The current research delved into the prevalent use of various fertilizers in fish ponds across Punjab, Pakistan, prior to conducting experiments. Surveys revealed that fish farmers commonly utilized fertilizers such as NPK (nitrogen, phosphorus, potassium), TSP (triple superphosphate), and DAP (di-ammonium phosphate) without expert assessment of pond requirements. These fertilizers were applied to ponds where multiple fish species were cultured together (polyculture). Fish farming in Pakistan primarily follows extensive and semi-intensive practices, utilizing both organic and inorganic fertilizers to enhance natural pond productivity, similar to global trends where these fertilizers are key to increasing primary productivity in aquaculture systems worldwide [20]. In Pakistan, underground water is typically the main water source for aquaculture, and antibiotics are commonly administered to treat diseases and control pathogens. Popular commercial fish feed manufacturers include Supreme, Aquafeed, and Hitech. However, in some regions, farmers produce their own feed by mixing essential ingredients tailored to the specific species they are culturing [38, 56].

The study comprised four distinct groups: a control group and an experimental group such as group 1 (NPK), group 2 (TSP), and group 3 (DAP). The experimental groups were formed based on fertilizer application. The experiment was conducted in triplicate, with a total of 12 fish ponds (3 fish ponds per group). The size of each pond was 890 and 2226 m^2. Tube well water was used as the water source for all ponds. We opted for tube well water in our fish farming setup to closely replicate the conditions that many farmers rely on, as it provides a familiar and controlled environment essential for the growth and health of the fish. Each pond, except the control, was fertilized for 7 days before stocking. The initial application of fertilizers was 20–25 kg per hectare, and the maintenance application was 8–10 kg per hectare per month. The duration of the study was 4 months. In each pond, a polyculture system was established by stocking three commonly farmed fish species: *Catla catla* (40%), *Labeo rohita* (40%), and *Cyprinus carpio* (20%). The total stocking density was 5000 fish per hectare. The initial average weight of the fish species was 4–5 g. The culture system followed a semi-intensive approach. The feed source for the fishes was commercial feed from Supreme company Pakistan, with a crude protein level of 35%. The fish were fed twice daily, in the morning and evening, with the feed amount adjusted to 4% of their body weight. Water and sediment samples were collected from the control group before fish stocking and after stocking (at the end of study period), while samples from the experimental groups were collected before and after fertilizer application. Fish samples from the study groups were gathered at the end of the study period. Additionally, samples of fertilizers (NPK, TSP, and DAP) and fish feed used during the study period were also collected and analyzed for heavy metals content. Weekly measurements of water quality parameters in the fish ponds (each group) were conducted throughout the study period (Table 1). An HI 9828 multi-parameter probe from YSI Incorporation in Yellow Springs, USA, was used for in situ measurements of temperature, pH, dissolved oxygen, and conductivity. The Freshwater Master test kit (USA) was used to determine the levels of ammonia, nitrite, and nitrate.

Sampling

Sediment and water samples were collected in triplicate from each pond (9 samples/group) across all four groups, approximately 15 m away from the shoreline. Sediment samples were obtained using cylindrical PVC cores with an internal diameter of 5.2 cm, sunk to a depth of 8 cm, and placed in acid-washed plastic containers. Water samples were collected from approximately 30 cm below the water surface and stored in acid-washed polyethylene bottles with a capacity of 1.5 L.

Fertilizers (NPK, TSP, and DAP) and fish feed samples were gathered in the required quantities and placed in grip-seal polyethylene bags. Each sample was collected in triplicate, then transported to the laboratory and stored in a dry environment.

At the end of the experimental period, fish samples ($n = 5$ of each species per earthen pond, totaling 45 per group) from both control and experimental groups were randomly collected using cast nets. The collected samples were then transported to the laboratory in containers filled with ice and stored in a refrigerator at − 20 °C.

Samples Preparation and Acid Digestion

The sediment samples were air-dried and then digested by following the method of Harikumar et al. [27]. The water samples were digested directly. All collected fertilizer and fish feed samples were subjected to drying in an oven set at 60 °C until a constant weight was achieved, following the method outlined by Tasleem et al. [56] and Habib et al. [21]. Then, the dried samples were finely ground using an electric grinder. The ground samples were then sealed in polyethylene zipper bags, each labeled with a unique code number for identification during subsequent analysis. Following thawing at room temperature, the frozen samples of fish species underwent thorough washing with distilled water. Afterwards, the dorsal muscle, devoid of skin, was extracted and placed into polypropylene vials. Then, all fish samples underwent oven drying at 95 °C for a duration of 8 h. Prior to acid digestion, all dried samples were ground to achieve homogeneity using a porcelain mortar and pestle.

Table 1 Water quality parameters of different groups

Parameters	Control	Group 1 (NPK)	Group 2 (TSP)	Group 3 (DAP)
Temperature (°C)	26.24 ± 0.15	26.54 ± 0.21	26.47 ± 0.25	26.58 ± 0.33
Dissolved oxygen (mg L^{-1})	6.23 ± 0.12	6.19 ± 0.22	6.21 ± 0.25	6.42 ± 0.34
pH	6.54 ± 0.028	6.82 ± 0.08	6.62 ± 0.06	6.77 ± 0.08
Unionized ammonia (mg L^{-1})	0.05 ± 0.001	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.02
Nitrite (mg L^{-1})	0.22 ± 0.003	0.05 ± 0.001	0.06 ± 0.002	0.04 ± 0.003
Nitrate (mg L^{-1})	0.05 ± 0.003	0.06 ± 0.003	0.04 ± 0.002	0.07 ± 0.006
Conductivity (µS/cm)	73.2 ± 13.28	67.26 ± 12.43	69.82 ± 9.15	70.25 ± 7.27

To determine the heavy metal content, 0.5 g of each fertilizer, fish feed, sediment, and fish muscle sample, along with 50 mL of water, underwent digestion with concentrated HNO_3, H_2SO_4, and $HClO_4$ (37%) (Sigma–Aldrich) at a temperature of 105 °C [21, 40]. After digestion, the samples were cooled to room temperature, filtered through Whatman filter paper (< 0.45 µm pore size), and adjusted to 50 mL with de-ionized water before further analysis. The concentration of targeted heavy metals (Pb, Cu, Cd, and Cr) was then estimated. The flame condition for atomic absorption was optimized to ensure better absorption and a linear response. Blank, working standard, and actual biological samples were aspirated directly into the flame, and absorption was recorded using an atomic absorption spectrometer (model: AA240FS, Varian Atomic Absorption Spectrophotometer). Additionally, 100 mL of filtered water sample with HNO_3 was digested at 100 °C. The sample was then cooled to room temperature and diluted before being filtered using Whatman-42 filter paper. A 50 mL portion of the filtrate was prepared with 0.01 N HNO_3, and the samples were then analyzed. Throughout the process, high-quality chemicals and reagents were utilized, and the glassware was meticulously cleaned and de-ionized to ensure the quality of the results.

Bioaccumulation Factor

The bioaccumulation factor (BAF) was determined using the following equation.

$$\mathrm{BAF} = \frac{C\ \text{substance in the organism}}{C\ \text{substance in the matrix}}$$

In this equation, the organism under consideration is the fish, while the matrix refers to the water in the fish pond.

Risk Assessment

Estimated Daily Intake (EDI)

The EDI of metals was calculated by taking into account the concentration of the metal, the daily food intake, and the body weight of the consumer. This calculation was conducted using the formula described by Naz et al. [40].

$$\mathrm{EDI}\left(\frac{\text{mg}}{\text{kg}}\text{body weight of}\frac{\text{consumer}}{\text{day}}\right) = \frac{(\text{concentration of metal}\ \times\ \text{weight of fish consumed}}{\text{body weight of consumer}}$$

Fish muscle metal concentrations were converted into wet weight (ww) equivalents using an average concentration factor of 4.8. It was assumed that, on average, Pakistani men consume 70 g of fish per day, with an average body weight of 60 kg, as reported by Qasim et al. [44].

Target Hazard Quotient (THQ) and Hazard Index (HI)

The THQ is a measure used to assess non-carcinogenic risks related to exposure to contaminants. THQ is calculated using the following equation:

$$\mathrm{THQ} = \frac{\mathrm{MC} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED} \times \mathrm{CF}}{\mathrm{Rfd} \times \mathrm{BW} \times \mathrm{ATn}} \times 10^{-3}$$

MC	Metal concentration in fish muscles (mg/kg dw)
IR	Ingestion rate of fish (70 g/kg dw)
EF	Exposure frequency (365 days of year)
ED	Duration of exposure (average 30 years)
CF	Conversion factor (4.8)
RfD	Reference dose
BW	Body weight (average 60 kg)
ATn	Average exposure time (10,950 days for non-carcinogen)

A THQ value exceeding 1 suggests potential health risks from consuming contaminated food. The HI measures the overall risk associated with exposure to multiple metals in contaminated food and is calculated as follows:

$$HI = \sum THQ$$

Statistical Analysis

A one-way ANOVA was applied to observe if there were any significant differences in the average levels of the heavy metals detected in water, sediment, fertilizers, and various fish species, and means were compared with Duncan's multiple range test. To investigate the source of contamination in the system, cluster analysis using Ward's method was performed using the PAST free statistical software. Furthermore, the correlation of individual heavy metals in the system was assessed using a correlation matrix. An alpha value of 0.05 was set for significance testing. The analysis was done using Prism GraphPad software (version 10.1.1).

Results

Comparative Analysis of Heavy Metals in Water and Sediment

The results of heavy metal concentrations in water from both the control group (where no fertilizers were applied) and the experimental groups before and after fertilizer application are presented in Table 2. Before the application of fertilizers, there were no significant ($p > 0.05$)

differences observed in the levels of the studied heavy metals between the control and experimental groups. However, after the application of fertilizers, the levels of heavy metals (Pb, Cu, and Cd) were significantly higher ($p < 0.05$) in the experimental groups compared to the control. Additionally, the concentration of Cr was below detection limits (BDL) in the control group, while significantly higher levels were recorded in group 2 (TSP) and group 3 (DAP) compared to group 1 (NPK).

Table 3 illustrates the concentrations of heavy metals in pond sediment before and after the application of fertilizers in both the control and experimental groups. Prior to fertilizer use, no significant ($p > 0.05$) difference was noted in all experimental groups and control group. Following fertilizer application, Pb and Cr concentrations exhibited a significant ($p < 0.05$) increase in sediment from group 2 (TSP) and group 3 (DAP) than the control group. Further in all groups where fertilizers were applied, the levels of Cd and Cu were significantly ($p < 0.05$) higher compared to the control group.

Comparative Analysis of Heavy Metals in Fertilizers and Fish Feed

The heavy metal concentrations in fertilizers and fish feed are presented in Table 4. Specifically, the concentrations of Pb and Cr were significantly ($p < 0.05$) higher in DAP compared to TSP and control. Conversely, both Cd and Cu levels were notably ($p < 0.05$) elevated in both DAP and TSP compared to NPK and control.

In the fish feed, the concentration of Pb was measured at 1.92 ± 0.12 mg/kg, while the Cu level was recorded at 1.24 ± 0.09 mg/kg. However, the levels of Cd and Cr were below detection limits (BDL).

Comparative Analysis of Heavy Metals in Fish Species

The levels of heavy metals in the muscle tissues of various fish species cultured in polyculture ponds were examined, with results summarized in Table 5. For *C. catla*, it was observed that the concentrations of Pb, Cu, and Cr were significantly

Table 2 Concentration of heavy metals (mg/L) in water from different groups before and after the application of fertilizers

Heavy metals	Control	Group 1 (NPK)	Group 2 (TSP)	Group 3 (DAP)
Before				
Pb	2.10 ± 0.15	2.08 ± 0.10	2.14 ± 0.20	2.21 ± 0.18
Cu	1.16 ± 0.06	1.14 ± 0.05	1.15 ± 0.04	1.17 ± 0.04
Cd	1.48 ± 0.25	1.50 ± 0.27	1.42 ± 0.26	1.45 ± 0.17
Cr	BDL	BDL	BDL	0.21 ± 0.05
After				
Pb	2.22 ± 0.20^{b}	2.98 ± 0.10^{a}	3.01 ± 0.09^{a}	3.06 ± 0.15^{a}
Cu	1.24 ± 0.05^{b}	1.72 ± 0.26^{a}	1.77 ± 0.12^{a}	1.80 ± 0.19^{a}
Cd	1.54 ± 0.25^{b}	2.19 ± 0.11^{a}	2.21 ± 0.07^{a}	2.23 ± 0.10^{a}
Cr	BDL	0.26 ± 0.13^{b}	0.31 ± 0.05^{a}	0.38 ± 0.07^{a}

Means in the same row with different superscripts are considered to be significantly different

BDL below detection limit

Table 3 Concentration of heavy metals (mg/kg) in sediment from different groups before and after the application of fertilizers

Heavy metals	Control	Group 1 (NPK)	Group 2 (TSP)	Group 3 (DAP)
Before				
Pb	3.01 ± 0.52	2.99 ± 0.42	3.01 ± 0.32	3.03 ± 0.28
Cu	2.11 ± 0.22	2.09 ± 0.22	2.13 ± 0.18	2.13 ± 0.23
Cd	2.18 ± 0.15	2.17 ± 0.14	2.15 ± 0.11	2.17 ± 0.13
Cr	0.51 ± 0.03	0.53 ± 0.08	0.50 ± 0.07	0.51 ± 0.04
After				
Pb	2.93 ± 0.04^{c}	3.53 ± 0.29^{b}	3.60 ± 0.26^{ab}	3.66 ± 0.24^{a}
Cu	2.22 ± 0.20^{b}	2.65 ± 0.38^{a}	2.68 ± 0.22^{a}	2.71 ± 0.19^{a}
Cd	2.13 ± 0.15^{b}	2.80 ± 0.12^{a}	2.82 ± 0.10^{a}	2.84 ± 0.22^{a}
Cr	0.59 ± 0.09^{c}	0.91 ± 0.06^{b}	0.95 ± 0.09^{ab}	1.02 ± 0.11^{a}

Means in the same row with different superscripts are considered to be significantly different

BDL below detection limit

Table 4 Comparative analysis of heavy metals (mg/kg) in fertilizers groups and fish feed

Heavy metals	Group 1 (NPK)	Group 2 (TSP)	Group 3 (DAP)
Pb	3.80 ± 0.41^{c}	7.82 ± 0.55^{b}	10.96 ± 0.60^{a}
Cu	3.56 ± 0.27^{b}	6.01 ± 0.58^{a}	7.10 ± 0.56^{a}
Cd	0.79 ± 0.07^{b}	2.47 ± 0.28^{a}	1.95 ± 0.45^{a}
Cr	1.46 ± 0.12^{c}	1.87 ± 0.08^{b}	2.54 ± 0.33^{a}
Fish feed			
Pb	1.92 ± 0.12		
Cu	1.24 ± 0.09		
Cd	BDL		
Cr	BDL		

Means in the same row with different superscripts are considered to be significantly different

BDL below detection limit

($p < 0.05$) higher in group 3 (DAP) and group 2 (TSP) compared to both the control group and other experimental groups. Additionally, the Cd levels were significantly ($p < 0.05$) elevated in group 3 (DAP), followed by group 2 (TSP).

In the case of *L. rohita*, notably ($p < 0.05$) higher concentrations of Cu and Cr were detected in group 3 (DAP) compared to all other experimental groups and the control. Similarly, significantly ($p < 0.05$) elevated levels of Cd were found in group 3 (DAP) and group 2 (TSP) compared to the remaining groups. In the muscle tissues of *C. carpio*, it was noted that the concentrations of the examined heavy metals were significantly ($p < 0.05$) higher in group 3 (DAP) when compared to both the control group and the other experimental groups. Compared to the standard limit, the Cd levels in all fish species were higher than those in the control group. However, the Pb level was only above the tolerance limit in the muscle tissue of *C. carpio*.

Bioaccumulation of Heavy Metals in Fish Species

Table 6 presents a detailed overview of the bioaccumulation factor in the muscle tissue of various fish species. Across all studied species, including *C. catla*, *L. rohita*, and *C. carpio*, it was observed that the bioaccumulation of heavy metals such as Pb, Cu, Cd, and Cr was higher in groups subjected to fertilizer application compared to the control group.

Specifically, in *C. catla*, the bioaccumulation factor for Pb, Cu, and Cd was found to be highest in group 3 (DAP), while Cd accumulation was particularly elevated in group 2 (TSP). In *L. rohita*, Pb accumulation was higher in both group 1 (NPK) and group 2 (TSP) compared to other groups, while Cu and Cd accumulation peaked in group 3 (DAP). Conversely, in *C. carpio* muscle tissue, Pb and Cu bioaccumulation exhibited the highest levels in group 3 (DAP), whereas Cu and Cr accumulation were elevated in group 1 (NPK) when compared to all other groups.

Cluster Analysis

Figures 1 and 2 illustrate the cluster analysis of group 1 (NPK) and group 2 (TSP). In both figures, the results

Table 5 Comparative analysis of heavy metals (mg/kg) in muscle of fish species from different groups after the experimental period

Heavy metals	Control	Group 1 (NPK)	Group 2 (TSP)	Group 3 (DAP)	WHO/FAO [61, 63]
C. catla					
Pb	0.60 ± 0.04^{c}	1.40 ± 0.08^{b}	1.43 ± 0.09^{ab}	1.46 ± 0.06^{a}	2.00
Cu	0.32 ± 0.01^{c}	0.91 ± 0.06^{b}	0.95 ± 0.04^{ab}	1.01 ± 0.09^{a}	30
Cd	0.43 ± 0.02^{d}	1.17 ± 0.09^{c}	1.21 ± 0.07^{b}	1.25 ± 0.10^{a}	1.00
Cr	BDL	0.26 ± 0.02^{b}	0.29 ± 0.08^{ab}	0.32 ± 0.01^{a}	1.00
L. rohita					
Pb	0.84 ± 0.07^{b}	1.73 ± 0.08^{a}	1.75 ± 0.10^{a}	1.76 ± 0.11^{a}	2.00
Cu	0.43 ± 0.03^{d}	1.08 ± 0.04^{c}	1.11 ± 0.18^{b}	1.16 ± 0.09^{a}	30
Cd	0.52 ± 0.05^{c}	1.25 ± 0.07^{b}	1.29 ± 0.08^{a}	1.32 ± 0.07^{a}	1.00
Cr	BDL	0.31 ± 0.06^{c}	0.36 ± 0.09^{b}	0.40 ± 0.02^{a}	1.00
C. carpio					
Pb	1.00 ± 0.01^{c}	2.25 ± 0.12^{b}	2.33 ± 0.12^{b}	2.41 ± 0.13^{a}	2.00
Cu	0.57 ± 0.04^{c}	1.80 ± 0.14^{b}	1.83 ± 0.14^{ab}	1.86 ± 0.10^{a}	30
Cd	0.65 ± 0.02^{d}	1.32 ± 0.09^{c}	1.36 ± 0.09^{b}	1.42 ± 0.11^{a}	1.00
Cr	0.20 ± 0.02^{c}	0.36 ± 0.07^{b}	0.42 ± 0.03^{a}	0.46 ± 0.04^{a}	1.00

Means in the same row with different superscripts are considered to be significantly different

BDL below detection limit

Table 6 Bioaccumulation of heavy metals (mg/kg) in muscle of fish species from different groups

Heavy metals	Control	Group 1 (NPK)	Group 2 (TSP)	Group 3 (DAP)
C. catla				
Pb	0.22	0.46	0.47	0.47
Cu	0.25	0.52	0.53	0.56
Cd	0.27	0.53	0.54	0.56
Cr	-	1.00	0.93	0.84
L. rohita				
Pb	0.37	0.58	0.58	0.57
Cu	0.34	0.62	0.62	0.64
Cd	0.33	0.57	0.58	0.59
Cr	-	1.19	1.16	1.05
C. carpio				
Pb	0.45	0.75	0.007	0.78
Cu	0.45	1.04	1.03	1.03
Cd	0.42	0.60	0.61	0.63
Cr	-	1.38	1.35	1.21

indicate that for *C. catla* and *L. rohita*, fertilizers show a stronger correlation with feed, forming a small cluster. In contrast, sediment and water cluster together, with fertilizers being less correlated with them, contributing to the overall contamination of the system. For *C. carpio*, muscle shows a stronger relationship with water and sediment, while feed is less correlated with fish muscle and water. Heavy metals from the fertilizers exhibit similar correlations as before. Additionally, in this system, Pb and Cu are strongly correlated, while Cd and Cr are more positively correlated with each other.

In Fig. 3A and C for *C. catla* and *L. rohita*, it can be observed that sediment and water are closely related to each other, further correlating with the fertilizer used in the system. However, fertilizer is distantly related to fish muscles, which are more closely associated with the fish feed. Conversely, in the case of *C. carpio*, the feed, fish muscle, and water are correlated with each other, while sediment is more closely related to group 3 (DAP).

Correlation Matrix

After applying the correlation matrix, it was found that Pb from group 1 (NPK) (Fig. 4) in sediment is positively correlated to the applied fertilizer with a correlation coefficient of 0.14. However, this correlation was not as strong as the correlation between feed and sediment (0.34). A stronger correlation was found between *L. rohita* and sediment, with a negative correlation (− 0.66) observed between sediment and *C. catla*, while no correlation was

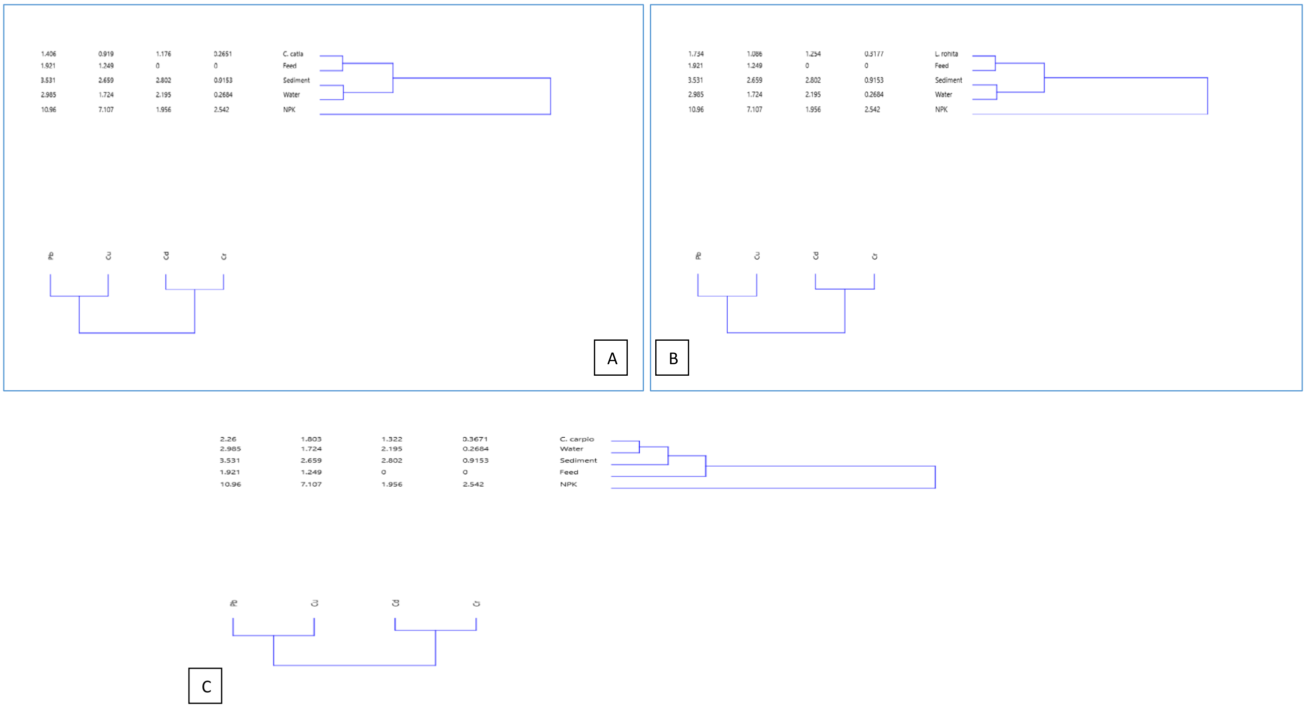


Fig. 1 Cluster analysis for group 1 (NPK), focusing on **A** *C. catla*, **B** *L. rohita*, and **C** *C. carpio*

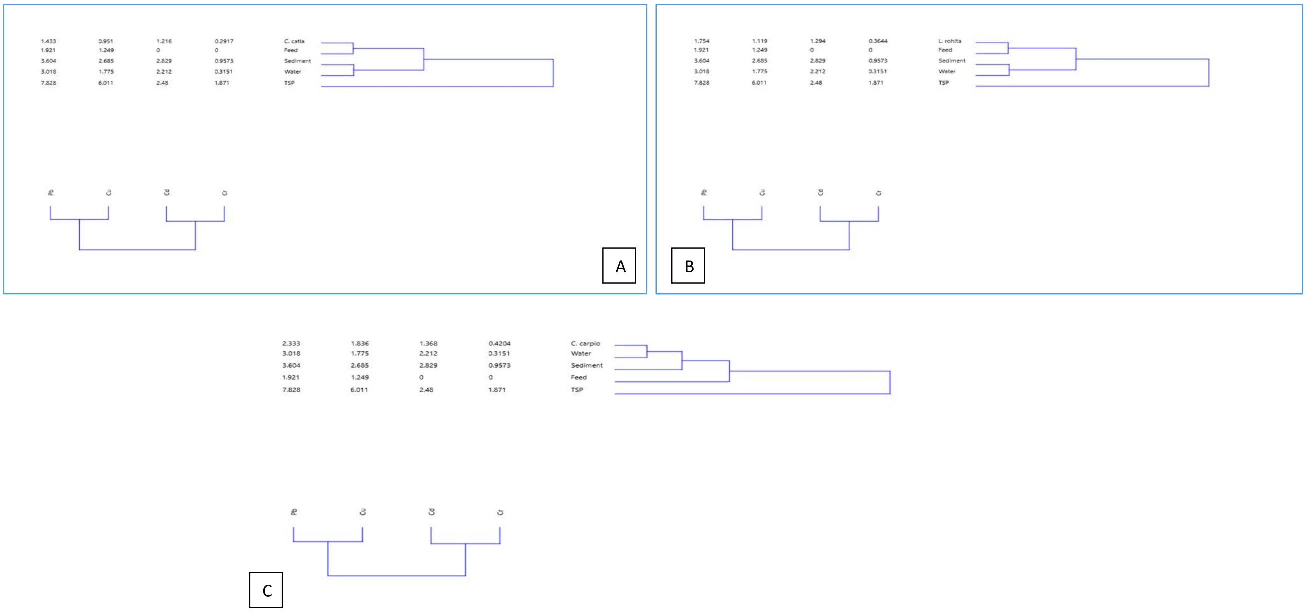


Fig. 2 Cluster analysis for group 2 (TSP), focusing on **A** *C. catla*, **B** *L. rohita*, and **C** *C. carpio*

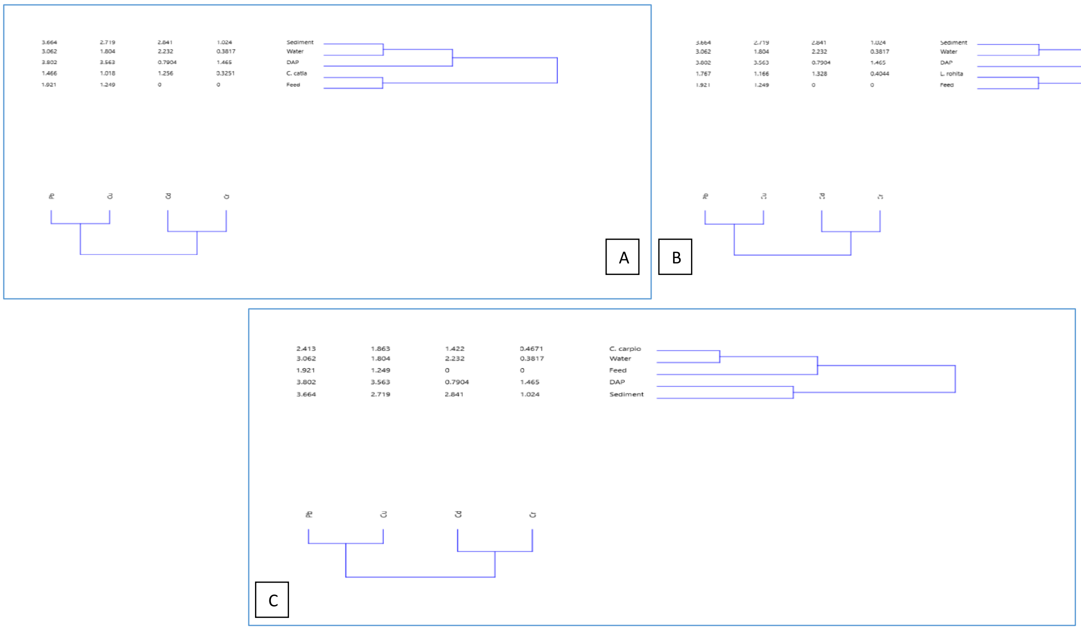
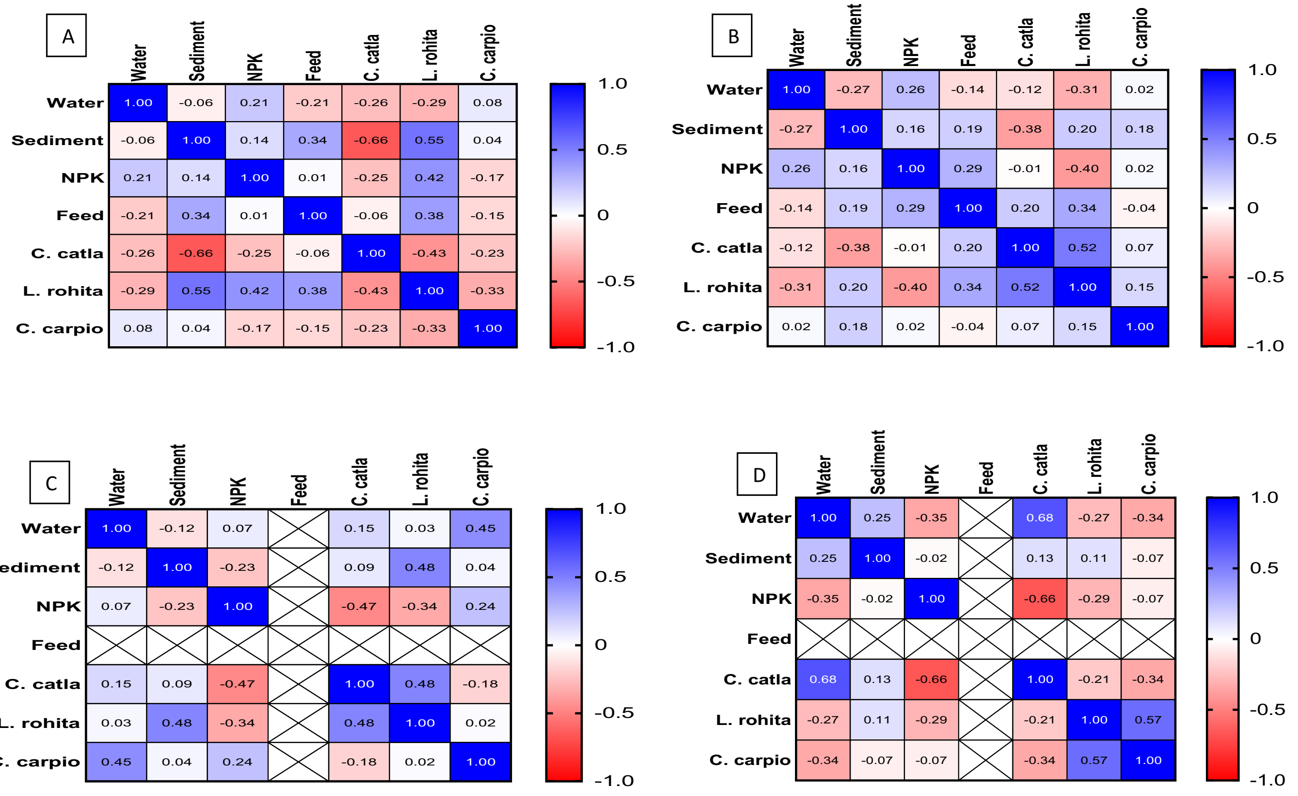


Fig. 3 Cluster analysis for group 3 (DAP), focusing on **A** *C. catla*, **B** *L. rohita*, and **C** *C. carpio*

found between sediment and *C. carpio*. Furthermore, Pb from the fertilizer is positively correlated with *L. rohita*, sediment, and water and negatively correlated with feed, *C. catla*, and *C. carpio*.

On the other hand, Cu from feed is positively correlated with all studied fish species except *C. carpio* and water. Cu from the fertilizer is negatively correlated with *L. rohita*. Additionally, sediment Cu is positively correlated with fertilizer, feed, *L. rohita*, and *C. carpio*.

Regarding Cd, it was below the detection limit in feed, so no correlation with feed was found. However, Cd from fertilizer is negatively correlated with most factors, with a weak correlation found with water and *C. carpio*. Moreover, sediment is negatively correlated with Cd in water.



A

	Water	Sediment	NPK	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.06	0.21	-0.21	-0.26	-0.29	0.08
Sediment	-0.06	1.00	0.14	0.34	-0.66	0.55	0.04
NPK	0.21	0.14	1.00	0.01	-0.25	0.42	-0.17
Feed	-0.21	0.34	0.01	1.00	-0.06	0.38	-0.15
C. catla	-0.26	-0.66	-0.25	-0.06	1.00	-0.43	-0.23
L. rohita	-0.29	0.55	0.42	0.38	-0.43	1.00	-0.33
C. carpio	0.08	0.04	-0.17	-0.15	-0.23	-0.33	1.00

B

	Water	Sediment	NPK	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.27	0.26	-0.14	-0.12	-0.31	0.02
Sediment	-0.27	1.00	0.16	0.19	-0.38	0.20	0.18
NPK	0.26	0.16	1.00	0.29	-0.01	-0.40	0.02
Feed	-0.14	0.19	0.29	1.00	0.20	0.34	-0.04
C. catla	-0.12	-0.38	-0.01	0.20	1.00	0.52	0.07
L. rohita	-0.31	0.20	-0.40	0.34	0.52	1.00	0.15
C. carpio	0.02	0.18	0.02	-0.04	0.07	0.15	1.00

C

	Water	Sediment	NPK	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.12	0.07	☒	0.15	0.03	0.45
Sediment	-0.12	1.00	-0.23	☒	0.09	0.48	0.04
NPK	0.07	-0.23	1.00	☒	-0.47	-0.34	0.24
Feed	☒	☒	☒	☒	☒	☒	☒
C. catla	0.15	0.09	-0.47	☒	1.00	0.48	-0.18
L. rohita	0.03	0.48	-0.34	☒	0.48	1.00	0.02
C. carpio	0.45	0.04	0.24	☒	-0.18	0.02	1.00

D

	Water	Sediment	NPK	Feed	C. catla	L. rohita	C. carpio
Water	1.00	0.25	-0.35	☒	0.68	-0.27	-0.34
Sediment	0.25	1.00	-0.02	☒	0.13	0.11	-0.07
NPK	-0.35	-0.02	1.00	☒	-0.66	-0.29	-0.07
Feed	☒	☒	☒	☒	☒	☒	☒
C. catla	0.68	0.13	-0.66	☒	1.00	-0.21	-0.34
L. rohita	-0.27	0.11	-0.29	☒	-0.21	1.00	0.57
C. carpio	-0.34	-0.07	-0.07	☒	-0.34	0.57	1.00

Fig. 4 Correlation matrix for group 1 (NPK), **A** Pb, **B** Cu, **C** Cd, and **D** Cr

As for Cr, it was found to be strongly correlated with *C. catla* from the water, but negatively correlated with other fish species. Similarly, the same heavy metals from the fertilizer are negatively correlated with all factors, indicating that these heavy metals are entering the system through other means such as contaminated water.

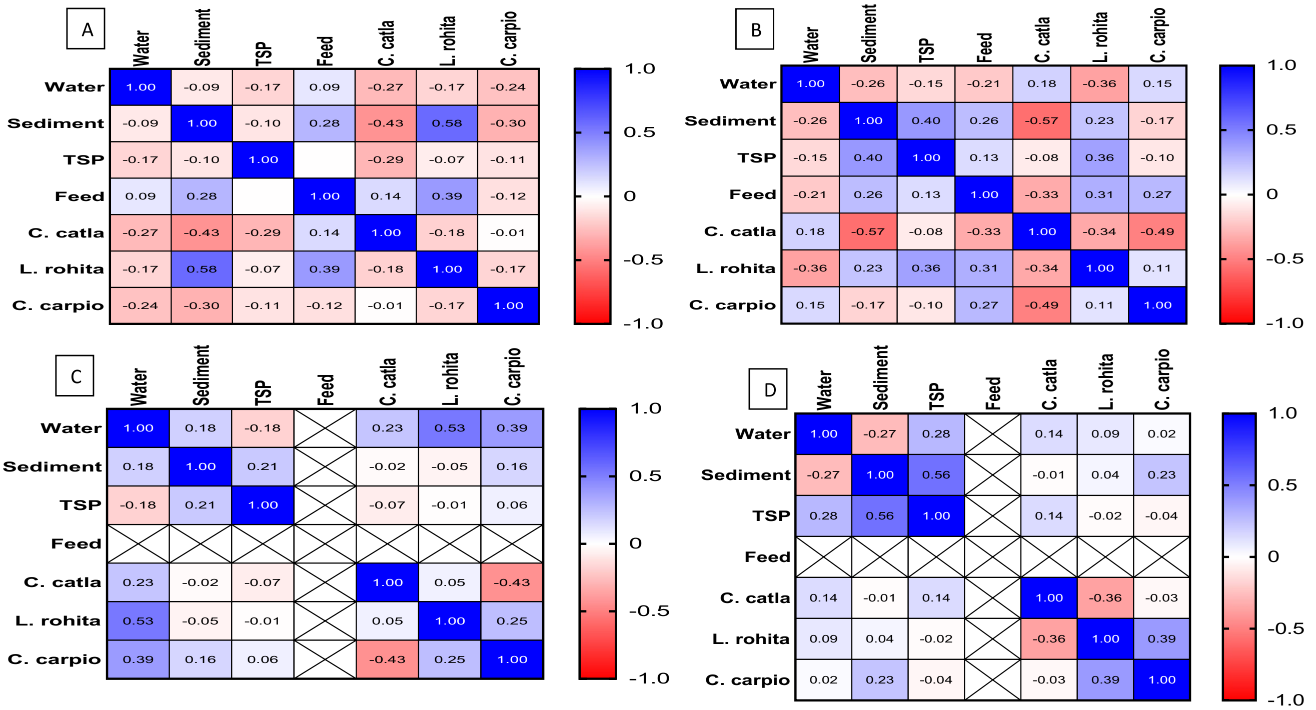
In the case of group 2 (TSP), it was surprising to find that Pb (Fig. 5A) was negatively correlated in almost all cases. However, weak positive correlations were found between feed and other factors, indicating that feed is the major source of contamination in this case. Cu (Fig. 5B) from the fertilizer showed a slight correlation with sediment and *L. rohita*. Additionally, Cd (Fig. 5C) was found to be positively correlated in most cases except for water and fertilizer. Similarly, Cr showed positive correlations with most factors except for water and sediment (Fig. 5D).

Figure 6 illustrates the correlation matrix results from group 3 (DAP). It was found that Pb (Fig. 6A) from water is negatively correlated with all factors, while from sediment, it is positively correlated with feed and highly correlated with *L. rohita*. Additionally, Pb from fertilizer is positively correlated with *C. catla*. Cu (Fig. 6B) from fertilizer and feed is positively correlated with all factors except water and *C. catla*. Cd (Fig. 6C) is highly positively correlated with *C. catla*, followed by water and *L. rohita*. Furthermore, Cd from water is also correlated with *L. rohita*, followed by fertilizer.

Regarding Cr (Fig. 6D), Cr in fertilizer is positively correlated with almost all factors except sediment. Furthermore, Cr from sediment is negatively correlated with water and fertilizer, with a strong negative correlation found between water and sediment.

Risk Assessment

Table 7 presents the implications of heavy metals on human health risks, focusing on fish muscle in different groups. Results revealed that all groups are considered safe for consumption, despite differing levels of heavy metals. In the control group, *C. catla*, *L. rohita*, and *C. carpio* showed EDI values for Pb of 0.702, 0.987, and 1.174, respectively, with corresponding THQ and HI values indicating low health risks. In group 1 (NPK), EDI values for Pb increased to 1.640 for *C. catla*, 2.022 for *L. rohita*, and 2.636 for *C. carpio*, yet all remained categorized as safe to consume. Group 2 (TSP) presented similar findings, with *C. catla* at 1.671, *L. rohita* at an unusually high 2299.5 for Pb, yet still safe for consumption, and *C. carpio* at 2.721. Finally, group 3 (DAP) maintained the safety status with EDI values for Pb at 1.709 for *C. catla*, 2.061 for *L. rohita*, and 2.815



A

	Water	Sediment	TSP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.09	-0.17	0.09	-0.27	-0.17	-0.24
Sediment	-0.09	1.00	-0.10	0.28	-0.43	0.58	-0.30
TSP	-0.17	-0.10	1.00		-0.29	-0.07	-0.11
Feed	0.09	0.28		1.00	0.14	0.39	-0.12
C. catla	-0.27	-0.43	-0.29	0.14	1.00	-0.18	-0.01
L. rohita	-0.17	0.58	-0.07	0.39	-0.18	1.00	-0.17
C. carpio	-0.24	-0.30	-0.11	-0.12	-0.01	-0.17	1.00

B

	Water	Sediment	TSP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.26	-0.15	-0.21	0.18	-0.36	0.15
Sediment	-0.26	1.00	0.40	0.26	-0.57	0.23	-0.17
TSP	-0.15	0.40	1.00	0.13	-0.08	0.36	-0.10
Feed	-0.21	0.26	0.13	1.00	-0.33	0.31	0.27
C. catla	0.18	-0.57	-0.08	-0.33	1.00	-0.34	-0.49
L. rohita	-0.36	0.23	0.36	0.31	-0.34	1.00	0.11
C. carpio	0.15	-0.17	-0.10	0.27	-0.49	0.11	1.00

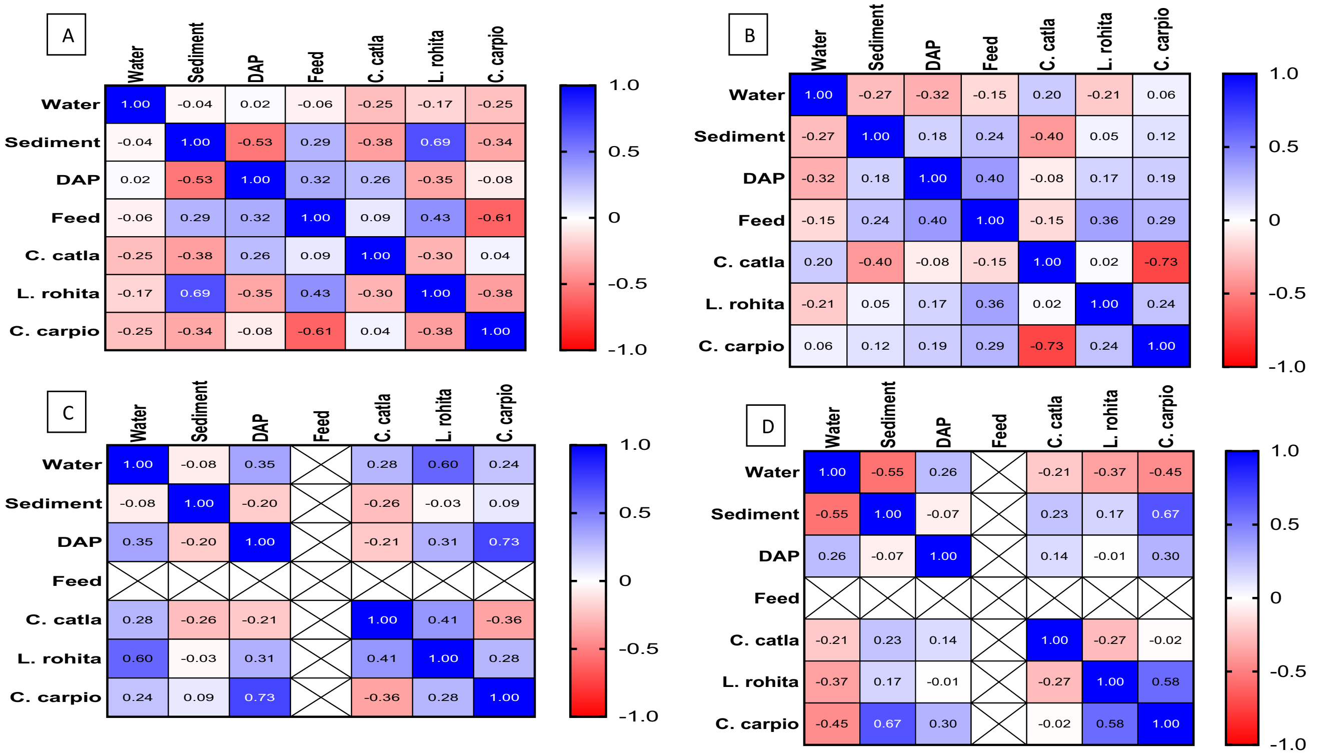
C

	Water	Sediment	TSP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	0.18	-0.18	☒	0.23	0.53	0.39
Sediment	0.18	1.00	0.21	☒	-0.02	-0.05	0.16
TSP	-0.18	0.21	1.00	☒	-0.07	-0.01	0.06
Feed	☒	☒	☒	☒	☒	☒	☒
C. catla	0.23	-0.02	-0.07	☒	1.00	0.05	-0.43
L. rohita	0.53	-0.05	-0.01	☒	0.05	1.00	0.25
C. carpio	0.39	0.16	0.06	☒	-0.43	0.25	1.00

D

	Water	Sediment	TSP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.27	0.28	☒	0.14	0.09	0.02
Sediment	-0.27	1.00	0.56	☒	-0.01	0.04	0.23
TSP	0.28	0.56	1.00	☒	0.14	-0.02	-0.04
Feed	☒	☒	☒	☒	☒	☒	☒
C. catla	0.14	-0.01	0.14	☒	1.00	-0.36	-0.03
L. rohita	0.09	0.04	-0.02	☒	-0.36	1.00	0.39
C. carpio	0.02	0.23	-0.04	☒	-0.03	0.39	1.00

Fig. 5 Correlation matrix for group 2 (TSP), **A** Pb, **B** Cu, **C** Cd, and **D** Cr



A

	Water	Sediment	DAP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.04	0.02	-0.06	-0.25	-0.17	-0.25
Sediment	-0.04	1.00	-0.53	0.29	-0.38	0.69	-0.34
DAP	0.02	-0.53	1.00	0.32	0.26	-0.35	-0.08
Feed	-0.06	0.29	0.32	1.00	0.09	0.43	-0.61
C. catla	-0.25	-0.38	0.26	0.09	1.00	-0.30	0.04
L. rohita	-0.17	0.69	-0.35	0.43	-0.30	1.00	-0.38
C. carpio	-0.25	-0.34	-0.08	-0.61	0.04	-0.38	1.00

B

	Water	Sediment	DAP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.27	-0.32	-0.15	0.20	-0.21	0.06
Sediment	-0.27	1.00	0.18	0.24	-0.40	0.05	0.12
DAP	-0.32	0.18	1.00	0.40	-0.08	0.17	0.19
Feed	-0.15	0.24	0.40	1.00	-0.15	0.36	0.29
C. catla	0.20	-0.40	-0.08	-0.15	1.00	0.02	-0.73
L. rohita	-0.21	0.05	0.17	0.36	0.02	1.00	0.24
C. carpio	0.06	0.12	0.19	0.29	-0.73	0.24	1.00

C

	Water	Sediment	DAP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.08	0.35	☒	0.28	0.60	0.24
Sediment	-0.08	1.00	-0.20	☒	-0.26	-0.03	0.09
DAP	0.35	-0.20	1.00	☒	-0.21	0.31	0.73
Feed	☒	☒	☒	☒	☒	☒	☒
C. catla	0.28	-0.26	-0.21	☒	1.00	0.41	-0.36
L. rohita	0.60	-0.03	0.31	☒	0.41	1.00	0.28
C. carpio	0.24	0.09	0.73	☒	-0.36	0.28	1.00

D

	Water	Sediment	DAP	Feed	C. catla	L. rohita	C. carpio
Water	1.00	-0.55	0.26	☒	-0.21	-0.37	-0.45
Sediment	-0.55	1.00	-0.07	☒	0.23	0.17	0.67
DAP	0.26	-0.07	1.00	☒	0.14	-0.01	0.30
Feed	☒	☒	☒	☒	☒	☒	☒
C. catla	-0.21	0.23	0.14	☒	1.00	-0.27	-0.02
L. rohita	-0.37	0.17	-0.01	☒	-0.27	1.00	0.58
C. carpio	-0.45	0.67	0.30	☒	-0.02	0.58	1.00

Fig. 6 Correlation matrix for group 3 (DAP), **A** Pb, **B** Cu, **C** Cd, and **D** Cr

Table 7 Human health risk assessment

Groups	Fish species	Heavy metal	EDI	THQ	HI	Remarks
Control	*C. catla*	Pb	0.702	0.041	0.14	Safe to consume
		Cu	0.380	0.0021		
		Cd	0.508	0.105		
		Cr	0	0		
	L. rohita	Pb	0.987	0.0586	0.188	Safe to consume
		Cu	0.513	0.002		
		Cd	0.609	0.126		
		Cr	0	0		
	C. carpio	Pb	1.174	0.0697	0.249	Safe to consume
		Cu	0.668	0.003		
		Cd	0.765	0.159		
		Cr	0.240	0.016		
Group 1 (NPK)	*C. catla*	Pb	1.640	0.097	0.410	Safe to consume
		Cu	1.072	0.006		
		Cd	1.371	0.285		
		Cr	0.309	0.021		
	L. rohita	Pb	2.022	0.120	0.457	Safe to consume
		Cu	1.266	0.0071		
		Cd	1.463	0.304		
		Cr	0.370	0.025		
	C. carpio	Pb	2.636	0.156	0.518	Safe to consume
		Cu	2.103	0.011		
		Cd	1.542	0.320		
		Cr	0.428	0.029		
Group 2 (TSP)	*C. catla*	Pb	1.671	0.099	0.424	Safe to consume
		Cu	1.109	0.006		
		Cd	1.418	0.295		
		Cr	0.340	0.023		
	L. rohita	Pb	2299.5	0.121	0.472	Safe to consume
		Cu	24,374.7	0.007		
		Cd	657	0.314		
		Cr	1971	0.0294		
	C. carpio	Pb	2.721	0.161	0.539	Safe to consume
		Cu	2.142	0.012		
		Cd	1.596	0.332		
		Cr	0.490	0.034		
Group 3 (DAP)	*C. catla*	Pb	1.709	0.101	0.439	Safe to consume
		Cu	1.187	0.006		
		Cd	1.465	0.304		
		Cr	0.379	0.026		
	L. rohita	Pb	2.061	0.122	0.485	Safe to consume
		Cu	1.36	0.007		
		Cd	1.549	0.322		
		Cr	0.471	0.032		
	C. carpio	Pb	2.815	0.167	0.562	Safe to consume
		Cu	2.173	0.012		
		Cd	1.65	0.34		
		Cr	0.544	0.037		

for *C. carpio*. Overall, while heavy metal levels varied, all assessed fish species across the groups were deemed safe for consumption.

Discussion

Fertilizers play a crucial role in maintaining the health and productivity of fish ponds, as they provide essential nutrients for the growth of phytoplankton, zooplankton, and other organisms that form the base of the aquatic food chain [20]. Excessive use of fertilizers can result in significant environmental issues [56]. One of the primary concerns associated with fertilizers is the contamination of water bodies with heavy metals. These metals can infiltrate the food chain, posing serious health hazards to humans [53]. According to the World Health Organization (WHO), more than a quarter of global diseases stem from prolonged exposure to environmental pollutants like heavy metals [62]. The present research delves into the potential risk of heavy metal contamination in commonly used fertilizers applied to fish ponds. The findings indicate that DAP exhibited the highest concentration of heavy metals, followed by TSP, in comparison to NPK and the control group. This corroborates the results of previous studies conducted by Kinaichu et al. [18] in Kenya, which also noted elevated levels of heavy metals, namely Cu, Cd, and Pb, in DAP followed by TSP. However, this study is not consistent with Tasleem et al. [56] who observed higher concentrations of heavy metals in TSP and NPK, while Sarker et al. [47] found increased levels of Cd and Cr in TSP, followed by DAP. Wang and Li [59] conducted a comprehensive review focusing on heavy metal contents in chemical fertilizers. Their analysis revealed that fertilizer, containing phosphate fertilizers, micronutrients, and liming materials contained elevated levels of Cd, Pb, and As compared to other fertilizers. However, the difference in the results can be explained by the fact that DAP and TSP are derived from phosphate rock, which often contains naturally occurring heavy metals. Cd is one of the most common contaminants in TSP. Other heavy metals such as Pb, Hg, and Cr may also be present depending on the source of the phosphate rock [19, 60].

In the current study, the heavy metal concentration after fertilization was significantly higher in water and sediment of fish ponds compared to control. After fertilizer application, there is a possibility of excess nutrients and heavy metals leaching into the water or being washed off the land into the pond through runoff [40, 54]. Heavy metals present in fertilizers can easily dissolve in water and be transported along with the runoff into the pond environment [46]. Over fertilization stimulates the growth of algae and unwanted aquatic plants in ponds. This overgrowth on the surface of the pond can block sunlight from penetrating deeper into the water, reducing photosynthesis in the lower layers. As these plants die, they sink to the bottom and accumulate as sediment [20]. Additionally, the decomposition of organic matter increases oxygen consumption by bacteria, leading to a depletion of dissolved oxygen (DO) in the water. This oxygen reduction can stress aquatic life, particularly fish, and create unfavorable conditions for the pond ecosystem [25].

Further heavy metals from the fertilizer can accumulate in this sediment over time, either directly from the fertilizers or through absorption by aquatic organisms [16]. In our current research, we found that heavy metal concentrations were higher in the sediment compared to the water, and they increased further after the application of fertilizer. This finding is consistent with the study by Zhang and Shan [67], which reported that heavy metals in sediment increase following agricultural intensification. Various studies conducted worldwide have reported different concentrations of heavy metals in both water and sediment, attributed to a range of human activities. For instance, Lipy et al. [34] investigated heavy metal pollution in the Dhaleshwari River in Bangladesh, primarily due to discharge from tanneries and other industries. Their findings indicated that the average concentrations of metals in the water followed the order Cr > Cd > Pb > Cu > As, while in sediment, the order was Cr > Pb > Cu > As > Cd. Similarly, a study by Malik and Maurya [36] in India focused on heavy metal contamination in the River Kali, a significant recipient of industrial effluents in the Muzaffarnagar district of western Uttar Pradesh. Their research revealed that in river water, the occurrence of heavy metals was highest (Pb > Zn > Mn > Cr > Cd), and in sediment, the order was Mn > Zn > Pb > Cr > Cd. It is commonly known that heavy metals accumulate in the sediment at the bottom of bodies of water, alongside various inorganic and organic substances like petroleum products and pesticides [40, 50, 66]. Sediment often contains higher concentrations of heavy metals compared to water due to its role as a sink for various pollutants. This phenomenon occurs through several processes. First, heavy metals from agricultural runoff, industrial discharges, and atmospheric deposition settle to the bottom of water bodies and become trapped in the sediment. Additionally, sediment particles have a high surface area that allows for adsorption, where heavy metals bond to the particles and accumulate over time. This accumulation is further enhanced by sedimentation, where suspended particles settle out of the water column during calm conditions. As a result, sediments act as reservoirs for heavy metals, reflecting a history of pollution in the surrounding environment, while the water column remains relatively less concentrated [23, 65].

Heavy metal concentrations in water reveal alarming levels of contamination that significantly exceed permissible limits set for public health. The permissible limits are notably low, with Pb at 0.010 mg/L, Cu at 1.3 mg/L, Cr at 0.1 mg/L, and Cd at 0.003 mg/L [57, 61, 63]. Before fertilizer application, Pb concentrations were already concerning, ranging from 2.08 ± 0.10 to 2.14 ± 0.20 mg/L across the groups, well above the permissible limit. This issue intensified post-application, with Pb levels increasing significantly to 3.06 ± 0.15 mg/L in group 3 (DAP), underscoring the detrimental impact of agricultural runoff on water quality. Similarly, while Cu levels were initially close to the permissible limit, with values around 1.16 ± 0.06 to 1.17 ± 0.04 mg/L, they surged after fertilizer application, reaching 1.80 ± 0.19 mg/L in group 3 (DAP), indicating an upward trend that necessitates vigilant monitoring. Cd concentrations were particularly troubling, starting at 1.48 ± 0.25 mg/L and escalating to 2.23 ± 0.10 mg/L after fertilizer application, far surpassing the permissible limit and highlighting its toxic potential, which can lead to severe health issues, including kidney damage and carcinogenic effects [52]. Interestingly, Cr levels were initially below detection limits across all groups but emerged post-application, with concentrations of 0.31 ± 0.05 mg/L and 0.38 ± 0.07 mg/L in groups 2 and 3, respectively. Although these values are still below the permissible limit, they signal a concerning trend of contamination linked to fertilizer use. While in sediment, heavy metal concentrations reveal a troubling picture of contamination relative to permissible limits. The permissible limits for heavy metals are set at Pb at 5 mg/kg, Cu at 0.2 mg/kg, Cr at 0.1 mg/kg, and Cd at 0.6 mg/kg [57, 61, 63]. Before fertilizer application, Pb levels were recorded between 2.99 ± 0.42 and 3.03 ± 0.28 mg/kg, indicating a concerning proximity to the limit but remaining within a safe range. However, post-application, Pb concentrations increased significantly, particularly in group 3 (DAP), reaching 3.66 ± 0.24 mg/kg, highlighting an upward trend that could pose risks if not addressed. Cu levels were alarmingly high from the start, ranging from 2.09 ± 0.22 to 2.13 ± 0.18 mg/kg before fertilization, which far exceeds the permissible limit of 0.2 mg/kg,after fertilization, these levels increased to 2.71 ± 0.19 mg/kg in group 3 (DAP). Similarly, Cd concentrations began at 2.18 ± 0.15 mg/kg and rose to 2.84 ± 0.22 mg/kg after fertilizer application, significantly surpassing the safe limit of 0.6 mg/kg and indicating serious contamination issues. Cr, initially lower, was found at 0.51 ± 0.03 mg/kg and increased to 1.02 ± 0.11 mg/kg post-application, also exceeding the permissible limit of 0.1 mg/kg.

Fish feeds can contain various pollutants, with heavy metals being particularly concerning due to their ability to accumulate in fish tissues. This poses a risk to human health when these contaminated fish enter the food chain [10]. The data in Table 6 revealed a concerning trend in the bioaccumulation of heavy metals including Pb, Cu, Cd, and Cr in the muscle tissues of three fish species such as *C. catla*, *L. rohita*, and *C. carpio* in response to different fertilizer treatments. The significant increases in bioaccumulation levels indicate that fertilizers, particularly those containing nitrogen, phosphorus, and potassium, may enhance the uptake of these toxic metals in aquatic organisms. Heavy metals persist in the environment, and their accumulation in sediments can increase bioavailability, especially after fertilizer application, which may disrupt the sediment–water interface and promote metal release [11]. For instance, the bioaccumulation of Pb in *C. catla* increased from 0.22 mg/kg in the control group to 0.46–0.47 mg/kg in the fertilized groups, suggesting that fertilizers facilitate lead mobilization from sediments, making it more readily absorbed by fish. Similarly, Cu bioaccumulation rose from 0.25 to 1.04 mg/kg in *C. carpio*, highlighting how this essential trace element can become toxic at higher levels, adversely affecting fish growth and neurological functions [33, 42]. The increase in Cd bioaccumulation, from 0.27 to 0.56 mg/kg in *C. catla*, further emphasizes its risks, as Cd can cause renal damage and reproductive issues [31]. Additionally, the detection of Cr, which was undetectable in control groups but rose to 0.84–1.00 mg/kg in treated fish, points to its mobilization through fertilizers. Cr poses genotoxic and endocrine disruption risks to aquatic life [7].

In this study, we found that the concentrations of Pb and Cu in fish feed were within the acceptable limits set by the WHO [61]. This finding is consistent with the findings of Habib et al. [21], who conducted a study in Punjab, Pakistan, revealing that Pb Cr, Zn, and Cd were present in permissible limits in commonly used commercial fish feeds. Additionally, other research has also highlighted the presence of toxic heavy metals like Pb, Cr, Zn, Cu, and Cd in synthetic fish feeds, along with their accumulation in fish tissues [5, 45].

The results of the current investigation showed that mostly the heavy metal concentration in fish muscles was found higher in group 3 (DAP) followed by group 2 (TSP) compared to group 1 (NPK) and control. Tasleem et al. [56] also found similar outcomes, demonstrating elevated levels of heavy metals (Cd, Pb, Cr, Cu, and Zn) in the muscles of *Hypophthalmichthys molitrix* and *C. carpio* collected from ponds treated with fertilizers, in comparison to the control group. Similarly, Naz et al. [40] documented elevated levels of Cr in both *C. carpio* muscle and pond water, with Pb following closely behind, attributed to the irregular use of fertilizers by farmers in Sargodha, Pakistan. Additionally, they observed higher bioaccumulation of Cd in fish muscle. However, in the present study, the bioaccumulation factor of all the heavy metals examined was higher in the muscle tissue of all three fish species collected from the fertilizer-applied groups compared to the control group. Numerous studies have investigated the analysis of heavy metals in fish bodies and pond water [10, 13, 32, 41]. However, there has been a lack of comprehensive research on the occurrence of heavy metals specifically due to the application of fertilizers in fish ponds.

In this study, the Pb and Cd level in the muscle of *C. carpio* was found to be higher than the WHO [61] standard limit. An EDI value in each group and fish indicates the amount of Pb and Cd that could be ingested daily, which is notably above the acceptable threshold. This raises concerns about the bioaccumulation of Pb and Cd in fish, as higher Pb levels in feed can lead to increased concentrations in fish tissues. Prolonged exposure to elevated Pb and Cd levels can have serious health implications [24]. For instance, both are neurotoxins, particularly the Pb, which can cross the blood–brain barrier and interfere with normal neurological function. This can lead to cognitive impairments, developmental delays, and behavioral problems, particularly in children whose brains are still developing [6, 28]. Pb and Cd exposure can also affect other systems in the body. It can cause damage to the kidneys, liver, and reproductive organs [12]. In pregnant women, Pb exposure can result in miscarriage, stillbirth, or developmental abnormalities in the fetus [8]. Furthermore, Pb has been linked to cardiovascular problems such as high blood pressure and an increased risk of heart disease [4, 17, 43]. Despite very high EDI, the THQ value was very low,however, it suggests that there may still be a risk, especially if fish consumption is frequent or if individuals are exposed to other sources of these heavy metals. The THQ is a tool used to assess the potential health risk posed by contaminants, and values greater than 1 indicate a potential health risk. While a THQ lower than this suggests that the immediate risk may be low, it is crucial to consider cumulative exposure from multiple sources, including water, air, and other dietary sources.

Further collective results from the human health risk assessment of heavy metals in fish underscore significant concerns regarding the safety of consuming species such as *C. catla*, *L. rohita*, and *C. carpio* across different treatment groups. In the control group, the EDI for Pb in *C. catla* was recorded at 0.702 mg/kg, yielding a THQ of 0.041, indicating a safe level for consumption, as it remains well below the risk threshold of 1. This pattern of safety was consistent across *L. rohita* and *C. carpio*, which displayed EDIs of 0.987 mg/kg and 1.174 mg/kg, respectively. However, when examining group 1 (NPK), EDI values for Pb significantly increased to 1.640 mg/kg in *C. catla* and 2.022 mg/kg in *L. rohita*, yet all species were still categorized as safe for consumption, suggesting that while the levels of heavy metals were rising, they had not yet reached critical thresholds of concern. The low THQ values in this group indicate that health risks, although increasing, are still manageable. The situation escalates dramatically in group 2 (TSP), particularly for *L. rohita*, which displayed an alarming EDI of 2299.5 mg/kg, raising immediate concerns about potential contamination, as this figure far exceeds safe limits and may point to serious environmental issues or data inaccuracies. The THQ for this species remained at 0.121, suggesting that immediate health risks may not be evident, but the extraordinary EDI highlights the urgent need for investigation. In group 3 (DAP), the trend of elevated Pb levels persisted, with *C. catla*, *L. rohita*, and *C. carpio* exhibiting EDIs of 1.709 mg/kg, 2.061 mg/kg, and 2.815 mg/kg, respectively, along with low THQs indicating safety for consumption; however, the consistent rise in Pb concentrations across treatment groups raises significant alarm regarding long-term health risks associated with cumulative exposure. The observed trends may be attributed to the influence of agricultural practices, particularly fertilizer application, which can lead to increased heavy metal bioaccumulation in aquatic organisms through sediment and water column contamination.

There are few limitations of the study such as the duration of the study, set at 4 months, may not be sufficient to fully assess the long-term impacts of fertilizer application on heavy metal bioaccumulation and water quality. Seasonal variations and their influence on water quality parameters and metal concentrations were not considered, which could affect the study's outcomes. Furthermore, while the study involved regular monitoring of water quality, the lack of comprehensive analysis for additional contaminants (e.g., pesticides, herbicides) limits the understanding of the broader environmental impacts.

Conclusion

There are growing concerns surrounding the use of commonly used commercial fertilizers, stemming from their potential impacts on human health, the environment, and the sustainability of farming practices. The current findings indicate a gradual increase in the concentration of metals found within fish muscles commonly consumed by people. This alarming trend is dangerous to the general population. The HI values derived from all groups where fertilizers were applied were lower. The accumulation of heavy metals in fish raises the threat of multiple diseases associated with these contaminants. To address this issue effectively, it is imperative to emphasize the importance of utilizing metal-free fertilizers in aquaculture practices. By opting for metal-free alternatives, farmers can help ensure that the food produced is free from harmful heavy metals, thereby safeguarding public health and promoting safe consumption. In addition to advocating for the use of metal-free fertilizers, it is essential to provide farmers with proper training and education on responsible fertilizer application practices. By equipping farmers with the knowledge and skills needed to make informed decisions regarding fertilizer use, they can take proactive measures to prevent heavy metal pollution and maintain the quality of their produce.

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Data Availability The datasets from this study are accessible through the corresponding author upon reasonable request.

Declarations

Ethics Approval The study was conducted after ethical committee approval of the Department of Zoology, University of Sargodha Punjab Pakistan.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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