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RESEARCH PAPER

Bioaccumulation of Potentially Toxic Metals by *Anabas testudineus, Heteropneustes microps,* and *Channa punctata* Using Industrial Effluents in Hydroponics

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ABSTRACT

This study evaluates the bioaccumulation of trace metals in fish body parts (*Anabas testudineus, Heteropneustes microps,* and *Channa punctata*) exposed to industrial effluents in hydroponic culture. Physico-chemical and trace metals analyses were performed following standard methods and protocols. Industrial effluent showed declining water quality parameters (substantially higher temperature, pH, electrical conductivity, biochemical oxygen demand, total suspended solids, total dissolved solids, turbidity, and total organic carbon, with markedly low dissolved oxygen). Elevated concentrations of chromium, cadmium, and lead were determined, which are non-compliant with standard limits. Hydroponics showed that metal content in fish exceeded allowable limits (except Zn), leading to significant bioaccumulation (BAF >1) and fish mortality (10–50% within 1–7 days). Metal accumulation in fish tissues increased with effluent strength (100% effluent > 50% effluent > control), with the highest accumulation in the gill. All studied fish tissues accumulated metals in the sequence Zn > Cu > Cd > Pb > Cr > Ni. *A. testudineus* absorbed more Cr (BAF=10.45), Zn (BAF=121.77), Ni (BAF=5.26), and Pb (BAF=21.68), while *H. microps* and *C. punctata* accumulated elevated Cd (BAF=20.63) and Cu (BAF=49.38), respectively. The gills of *A. testudineus* and *C. punctata* significantly bioaccumulated metals ($p<0.05$) when exposed to 100% industrial effluent. Even when effluent metal concentrations are moderate, fish can quickly accumulate harmful tissue levels, posing risks to fish health and higher trophic levels, including carcinogenic and noncarcinogenic risks to human health.

Keywords: Hydroponic culture, Industrial pollution, Metal bioaccumulation, Water quality

Introduction

Pollution is a significant global issue that affects biodiversity, ecosystems, and human health. Untreated industrial effluents are substantial contributors to pollution that severely impacts aquatic ecosystems through bioaccumulation by various species (El-Gendy *et al.* 2025; Hossain *et al.* 2022; Pandey *et al.* 2018). Contamination levels are rising in developing countries like Bangladesh due to population growth and industrial activities (Wahiduzzaman *et al.* 2021; Ahmed *et al.* 2016). Industrial discharges, a major source of potentially toxic metals, pose a significant threat to aquatic ecosystems, presenting serious risks to both fish and humans (Rahman *et al.* 2026; Akter, 2014; Matouke and Abdullahi, 2020).

Potentially toxic metals in the aquatic environment can occur through both natural and anthropogenic actions, which are non-degradable and can be dissolved into water by changes in environmental conditions, thereby creating secondary pollution (Islam *et al.* 2017; Habib *et al.* 2022). Industrial activities, such as textiles, wood and paper processing, refineries, coal burning, petroleum combustion, nuclear power, and metal processing, release toxic metals into the environment. Trace amounts of non-metabolized metals can cause toxicities in biological systems through assimilation, deposition, or incorporation into abiotic elements (Rahman *et al.* 2026; Biswas *et al.* 2021; Islam *et al.* 2017).

Bioaccumulation occurs across the food chain from microorganisms to humans, resulting in higher metal concentrations in organisms than in their surrounding environment. Toxic metals tend to accumulate and persist in living tissues due to the bio-magnification process and impart tremendous harmful effects on biota (Rahman *et al.* 2026; Jena and Hembram, 2024; Biswas *et al.* 2021; Wahiduzzaman *et al.* 2021). Cu, Fe, Mn, and Zn are essential trace elements in biological systems, whereas Pb, Cr, Cd, Hg, Ni, and Sn are noxious, even at low concentrations, and are not required to carry out metabolic activities (Shetty *et al.* 2025; Akindele *et al.* 2020). Bangladesh boasts a diverse array of fish resources, but the quality and safety of fish have become major health concerns due to uncontrolled industrialization and urbanization. Potentially toxic metals accumulate in fish organs through contact with polluted water and sediment. This accumulation primarily occurs in the gills, skin, and muscles (Ali *et al.* 2019; Castro-Gonzalez and Mendez-Armenta 2008; El-Moselhy *et al.* 2014). The concentration of metals in fish tissues and organs thus reflects both water contamination and bioaccumulation within food chains (Rahman *et al.* 2026; Hasan *et al.* 2023; Wahiduzzaman *et al.* 2021).

Hydroponics (growing plants in water without soil) is effective for testing effluent toxicity in plants and aquatic animals (Walsh *et al.* 1991). *Heteropneustes microps* (Shing), *Anabas testudineus* (Koi), and *Channa punctata* (Taki) are the main freshwater fish species consumed in Bangladesh. Despite concerns regarding potentially toxic metals released from industrial sources, there remains limited research on the bioaccumulation of these metals by the native fish species of Bangladesh. With trace metal pollution rising and the growing prominence of native fish species as a major protein source, there is an increasing need for rigorous evidence to evaluate toxic metal accumulation in these fish. This is particularly important under diverse hydroponic conditions involving exposure to fish-industrial effluent. This study aims to: 1) measure physical such as temperature, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), turbidity), chemical (pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total organic carbon (TOC) parameters in industrial effluent; 2) quantify trace metal concentrations (Cr, Cd, Zn, Ni, Pb) in industrial wastewater; 3) evaluate fish mortality rates and concentrations of metals (Cr, Cd, Cu, Zn, Ni, Pb) in muscle, gill, and bone tissues of selected species; and 4) assess metal-specific bioaccumulation factors (BF) by the muscle gill and bone of studied effluent exposed fishes.

Materials and Method

The industrial effluent and fish species sampling point, located in Dhamsona Union, Savar Thana (23°51′ N, 90°15′ E), lies 32 km north of Dhaka, Bangladesh. It features many industrial sites and two export processing zones (Old and New). A national highway connecting northern Bangladesh to Dhaka passes between the DEPZs.

Water sample collection, preservation and analysis

Wastewater samples were collected from the DEPZ discharge point (23056'53.01" N, 90015'38.12" E) during the winter season (November-December) and were transported in thick Polyvinyl Chloride (PVC) drums (50 L). Temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), and turbidity were measured on-site using a multiparameter meter (HI 9829, HANNA) and a DO meter (YSI PRO 20i) in accordance with standard protocols. Total suspended solids (TSS) and total dissolved solids (TDS) were determined gravimetrically (APHA 2023). Biochemical oxygen demand (BOD) was assessed via the Winkler method (De 2000), and total organic carbon (TOC) was analyzed using a TOC analyzer (TOC-V CPN, Shimadzu). Portions of the water samples were acidified with nitric acid and refrigerated for metal analysis.

Collection of test fish species and overview of methodologies

Three native fish species of Bangladesh, namely *Anabas testudineus* (common name Climbing perch and Bangla name Koi fish), *Heteropneustes microps* (common name Stinging catfish and Bangla name Shing fish), and *Channa punctata* (common name Spotted snakehead and Bangla name Taki fish), were collected from the wetlands of Dhamsona, Ashulia, Dhaka, with the assistance of local fishermen. The fish were collected from the pond in fully natural conditions and were first washed with site water and then under running water to ensure thorough cleaning. They were then transported to the culture site using polythene bags and preserved in an aquarium. Before being placed in hydroponic culture, the fish were acclimated for 13 days. A total of 165 fish were collected; among them, 57 were *A. testudineus*, 55 were *H. microps*, and 53 were *C. punctata.* Individuals of each fish species with comparable size and weight were chosen for the experiment. **Figure 2** illustrates the schematic overview of the methodologies employed.

Hydroponic culture for metal bioaccumulation by the fish species

Hydroponic experiments were conducted in half-spherical earthen containers, cleaned with KMnO4 (20 ppm), with a capacity of 50 L of water. The tests were conducted using 50% and 100% of the effluents, and effluent-free water. The experiments were conducted at the Water Research Center of Jahangirnagar University. *A. testudineus, H. microps,* and *C. punctata* were directly taken from a preserved aquarium for the experiment. Each experiment was conducted for 90 days with three repetitions, and the same water levels were maintained by adding the same percentage of water. Active and healthy fish species of almost the same size were used in the experiment. In the experiment, aquatic plants were used to provide aeration and a natural environment. The death of the fish species was recorded every day to assess the toxicity of the collected effluent.

Preparation of fish samples

After the experiments, fish samples were frozen in ice boxes and promptly taken to the laboratory. After descaling with a steam-cleaned stainless-steel knife, the fish were thoroughly rinsed with deionized water. The muscle, gill, and bone were then dissected, cut into small pieces, and dried in an electric oven at 70 °C for 48 hours. Fish samples were repeatedly washed with acetone to remove oil, and the dried samples were ground into a powder using a clean grinder and then homogenized. All glassware was rinsed with diluted HNO_3, then washed with distilled water before use.

Digestion of water and fish samples

The digestions of water and fish samples were carried out according to the AOAC (Association of Analytical

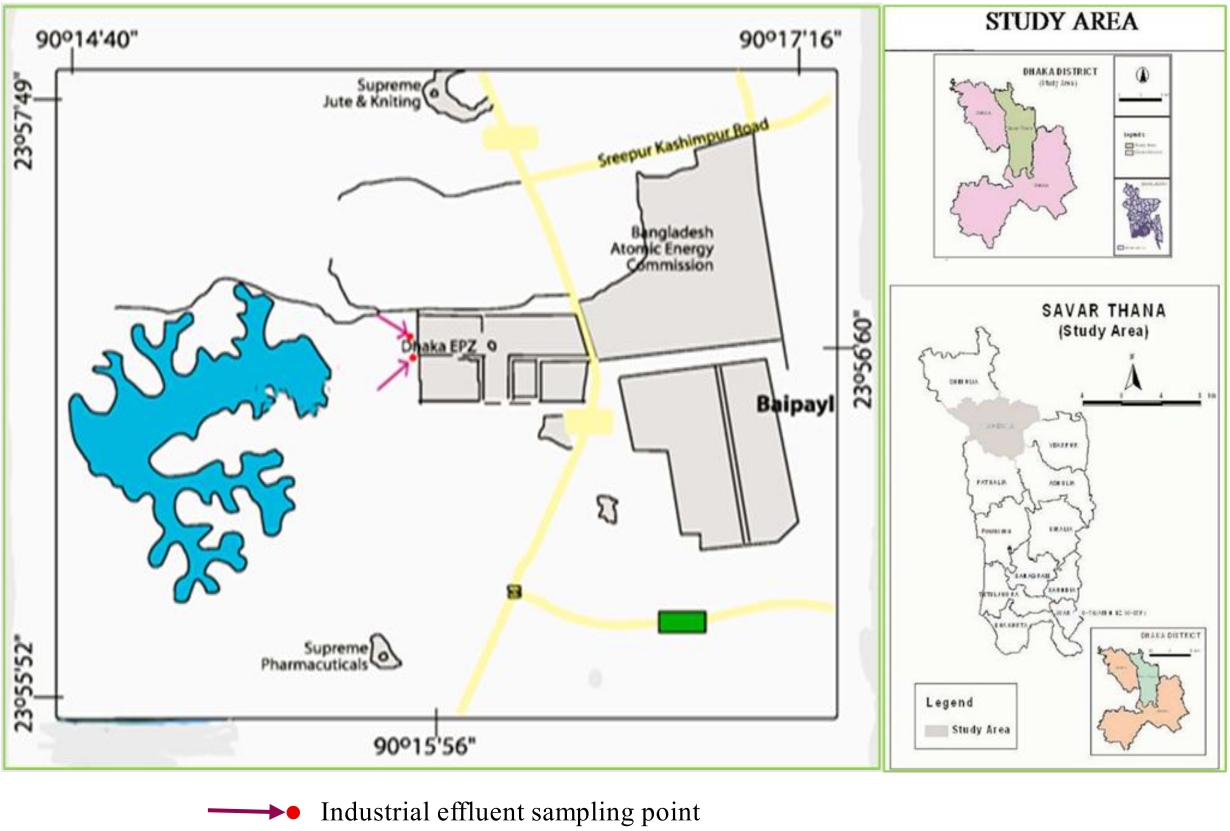


Figure 1: Location of the study area and sampling point

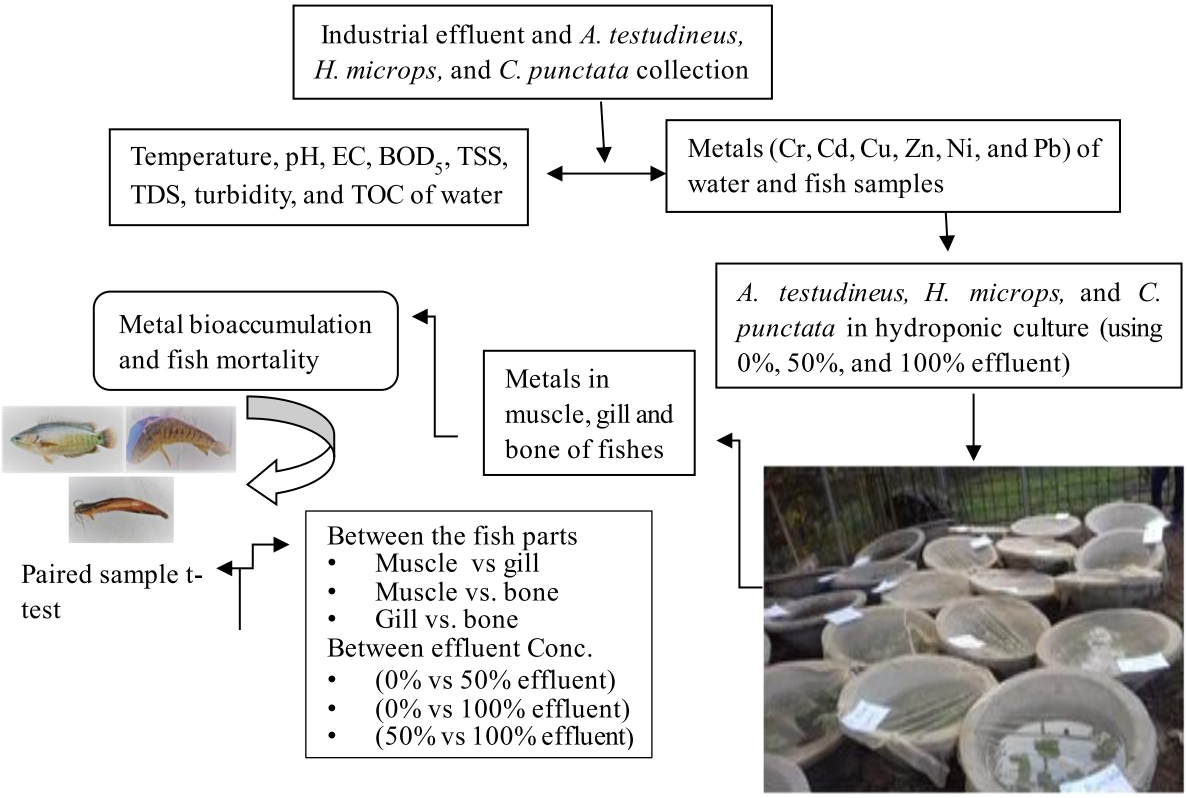


Figure 2: Schematic showing methodologies

Communities) Official Method (APHA 2023; Murtala *et al.* 2012; Mehnaz *et al.* 2022). Water samples were digested with Ultrapure HNO_3 (65% HNO_3), and for the fish specimens, precisely 0.5 g of each was digested in a Teflon vessel using 10 mL of 65% HNO_3 and 5 mL of 60% $HClO_4$ (Merck, Germany). The digested solution underwent a three-step program using a microwave digestion apparatus (Berghof, Germany) with up to 35 bar pressure. Firstly, the temperature and power were maintained at 175 °C and 80%, respectively, for 15 minutes; secondly, the temperature was subsequently held at 195°C for an additional 15 minutes with the power increased to 85%; and thirdly, the vessels were cooled by reducing the temperature to 95°C and the power to 35% for 10 minutes. The samples were allowed to cool and then transferred into clean volumetric flasks. Each solution was adjusted to a final volume of 50 mL by adding double-distilled water. The solutions were filtered with filter paper (Whatman No. 42) and stored in labelled screw-cap plastic bottles. Three-times digestion was employed for each sample. Three blank samples, containing all digestion inputs except the sample itself, were processed identically as controls to verify instrument accuracy.

Analysis of trace metals

Trace element contents in water and fish samples were analyzed using a SHIMADZU AA-7000 Atomic Absorption Spectrophotometer at the Bangladesh Council for Scientific and Industrial Research (BCSIR) laboratory in Dhaka. The AAS was initially calibrated by consecutive dilution with standard solutions according to the manufacturer's protocol, and all procedures were validated in-house in accordance with EC567/2002. Trace metal concentrations were determined with calibration curves based on Beer-Lambert's law (Skoog *et al.* 2005).

Quality control and quality assurance

Powder-free latex gloves and lab coats were used to minimize contamination while handling samples. Before use, all glass and plasticware were soaked in a 14% HNO_3 solution for 24 hours and then rinsed with distilled water. Analytical grade reagents and chemicals (Merck, Germany) and double-distilled water were employed for analysis. All methods underwent in-house validation according to EC567/2002. Each experiment run included blanks, CRM320 (certified reference materials) as an internal standard, and triplicate sample analyses to minimize batch errors. Certified and observed values matched closely. RSD (relative standard deviation) ranged from 0.75% to 9%, with metal recoveries between 87% and 103%. For quality control, a blank extract containing all reagents except the sample was prepared to check for contamination from other chemicals. The blank value obtained by AAS analysis was subtracted from each sample result to determine the original value.

Bioaccumulation of metals by fish species

Bioaccumulation factor (BAF) is a quantitative term used to indicate the degree of metal accumulation in an organism relative to its environment. It is calculated on a dry weight basis of the fish sample by the following formula (Rahman *et al.* 2026; Shetty *et al.* 2025):

$BAF = C_{\text{fish tissue}}/C_{\text{water}}$

Where $C_{\text{fish tissue}}$ and C_{water} represent metal contents in fish extracts and water, respectively.

Statistical analysis

The physicochemical parameters and concentration of heavy metals were calculated as mean ± standard deviation. The significant differences in heavy metal concentrations between the fish parts and different concentrations of effluent were analyzed by t-test (paired two-sample for means) using the data analysis tool packaged SPSS software for Windows 10.

Results and discussion

The mean concentrations of physicochemical parameters and potentially toxic metals in water samples collected from both industrial and control sites, along with their comparison to EPA and DOE standards, are presented in **Tables 1** and **2.**

Table 1: Comparison of control water and wastewater quality parameters with EPA and DOE limits for wastewater (Mean±SD).

Water	Physical parameters					Chemical parameters			
	Temp. (^0C)	EC (µS/cm)	TSS (mg/L)	TDS (mg/L)	Turbidity (NTU)	pH	DO (mg/L)	BOD_5 (mg/L)	TOC (mg/L)
Effluent	42 ±0.5	13107 ±2	2087 ±2.5	7132 ±3.4	98.47 ±2.3	11.81 ±0.01	0.14 ±0.01	856 ±3.5	397.21± 1.4
Control	24 ±1.5	1134 ±3	87.6 ±1.8	1328 ±1.6	11.09 ±0.3	6.13 ±0.02	5.8 ±0.3	7.9 ±0.5	15.87 ±0.5
DOE (2009)	40	1200	50	2100	10	6-9	4.5-8	20	-----
EPA (1981)	<30	750	50	1500	75	6-9	1	50	-----

EC: Electrical Conductivity, TSS: Total Suspended Solids, TDS: Total Dissolved Solids, DO: Dissolved Oxygen, BOD_5: Biochemical Oxygen Demand at 5-d test, TOC: Total Organic Carbon, DOE: Department of Environment, EPA: Environmental Protection Agency

Table 2: Comparison of metal contents of industrial effluents with control and standard limits for industrial wastewater and water

Water	Cr (mg/L)	Cd (mg/L)	Cu (mg/L)	Zn (mg/L)	Ni (mg/L)	Pb (mg/L)
Effluent	0.71±0.02	0.19±0.01	0.78±0.01	0.82±0.02	0.89±0.03	0.31±0.02
Control	<0.01	<0.01	0.04±0.001	0.25±0.001	0.02±0.001	<0.01
DOE (2009)	0.05	0.01	0.20	2.0	0.20	0.10
WHO (2019)	---	0.003	2	0.01-3	---	0.01

DOE: Department of Environment, WHO: World Health Organization.

Analysis of the physicochemical parameters indicates that the values for temperature (42°C), pH (11.81), EC (13,105 µS/cm), BOD_5 (856 mg/L), TSS (2,087 mg/L), TDS (7,132 mg/L), turbidity (98.47 NTU), and TOC (397.21 mg/L) in industrial effluents were substantially higher than those observed at control sites and exceeded the permissible limits set by the DOE and EPA.

Specifically, the pH recorded in industrial wastewater was 11.81, which is above the DOE and EPA accepted range (6–9). The elevated biochemical oxygen demand (BOD_5) value of 856 mg/L in industrial effluents is likely responsible for the markedly low dissolved oxygen (DO) concentration of 0.14 mg/L, indicating considerable organic pollution. Furthermore, excessive levels of total suspended solids (TSS), total dissolved solids (TDS), and turbidity in industrial effluents could lead to anaerobic conditions and increased microbial contamination, as noted by the US EPA (2012). The high TOC concentration (397.21 mg/L) observed in the industrial effluents may also contribute to elevated BOD_5 levels and a rapid decline in dissolved oxygen, which is consistent with Momtaz *et al.* (2013) (**Table 1**).

Table 2 shows chromium (Cr) in effluent was 0.71 mg/L, exceeding both the control and DOE limits (0.05 mg/L). Cadmium (Cd) was 0.19 mg/L in industrial effluents, much higher than in effluent-free water (<0.01 mg/L), surpassing DOE and WHO standards. Industrial effluent also had elevated levels of Cu (0.78 mg/L), Ni (0.89 mg/L), and Pb (0.31 mg/L), all above DOE limits. The content of Zn (0.82 mg/L) was higher in effluents (0.82 mg/L) than in the control, but remained within DOE and WHO standards. The mean metal concentration in industrial effluent was moderate (Cr, Cd, Cu, and Pb exceeded recommended values except Zn) (**Table 2**), but fish can hyperaccumulate these metals, which could pose risks to higher trophic level organisms via the food chain (El-Moselhy *et al.* 2014; Mehnaz *et al.* 2022; Shetty *et al.* 2025).

Mortality rate of fish along with the exposure times to varying concentrations of industrial effluents is shown in **Table 3.**

Table 3. Mortality rate of *A. testudineus, H. microps,* and *C. punctata* with time frame, exposing different percentages of industrial effluent.

Effluent (%)	Fish species						Total cultured of each fish.
	A. testudineus		*H. microps*		*C. punctata*		
	Mortality rate (%)	Time frame (days)	Mortality rate (%)	Time frame (days)	Mortality rate (%)	Time frame (days)	
100	20	2-7	40	1-5	50	1-3	10
50	10	3-8	30	2-6	40	2-5	10
Control	0	23-30	0	19-27	10	19-30	10

During hydroponic experiments, fish exhibited weakness, stunted growth, reduced movement, and skin injuries. Mortality increased with higher effluent concentration: at 100%, *C. punctata* had the highest mortality (50% in 1-3 days), followed by *H. microps* (40% in 1-5 days) and *A. testudineus* (20% in 2-7 days). 10%, 30%, and 40% mortality of *A. testudineus, H. microps,* and *C. punctata* occurred within 3-8, 2-6, and 2-5 days, respectively, after exposure to 50% effluent. No mortality was observed in the control group except for *C. punctata*. The mortality rate of tested fish species increased with effluent concentration, which is consistent with Mehnaz *et al.*(2022). In 100% industrial effluent, fish probably lose resistance to toxicants, resulting in the death of 20 to 50% individuals (**Table 3**). This may be due to environmental stress, the impact of metals, and other pollutants of industrial effluent.

The average metals (Cr, Cd, Cu, Zn, Ni, and Pb) content in different parts of *A. testudineus, H. microps,* and *C. punctata* cultured in effluent and their permissible limits are presented in **Table 4.**

Cr in muscle, gill, and bone of *A. testudineus* were found to be 3.86, 7.42, and 4.13 mg/kg, respectively, when cultured using 100% effluent, which were higher than those in 50% effluent-exposed and control fishes and exceeded the recommended level. At 50% and 100% effluents, Cr in *H. microps* was 2.03 and 3.81 mg/kg, respectively, in muscle; 5.56 and 6.27 mg/kg, respectively, in gill; while 4.33 and 6.94 mg/kg, respectively, in bone; which crossed the suggested value. The levels of Cr in the muscle, gill, and bone of *C. punctata* the Cr was 5.19, 4.97, and 3.16 mg/kg, respectively, at 100% cultured effluents, which was higher than the other cultures (50% effluent, control) (**Table 4**).

When *A. testudineus, H. microps,* and *C. punctata* were cultured in 100% effluents, Cd was 2.04, 2.45, and 2.19 mg/kg, respectively, in muscle; 3.81, 3.92, and 3.48 mg/kg, respectively, in gill; 2.78, 3.36, and 2.27 mg/kg, respectively, in bone, which crossed the permissible levels. In 50% effluent, Cd uptake was lower compared to 100% effluent, following the order: gill>bone > muscle with *H. microps* accumulating the most Cd (**Table 4**).

In 100% effluents, Cu levels in *C. punctata* muscle, gill, and bone reached 36.25, 38.52, and 32.89 mg/kg, respectively, exceeding allowable limits and surpassing those in other cultures. In 50% effluents, Cu concentrations were higher than the control but remained within recommended levels. Across all treatments, gills absorbed the most Cu, followed by bone and muscle (**Table 4**).

Table 4. Mean concentrations of Cr, Cd, Cu, Zn, Ni, and Pb (mg/kg, Mean±SD) in the parts of *A. testudineus, H. microps,* and *C. punctata* cultured in various industrial effluents and comparison with the maximum permissible limit.

Metal	Effluent (%)	*A. testudineus*			*H. microps*			*C. punctata*			Permissible limits (mg/kg)
		Muscle	Gill	bone	Muscle	Gill	Bone	Muscle	Gill	Bone	
Cr	Control	0.63 ± 0.04	1.32 ± 0.19	0.84 ± 0.02	0.87 ± 0.03	2.01 ± 0.12	1.35 ± 0.10	<0.49	1.03 ± 0.07	0.86 ± 0.02	1.0 (WHO 1995, FAO 1983, EU 2001)
	50% eff.	2.34 ± 0.042	5.21 ± 0.08	2.29 ± 0.06	2.03 ± 0.031	5.56 ± 0.11	4.33 ± 0.08	3.52 ± 0.025	3.78 ± 0.013	2.73 ± 0.05	
	100% eff.	3.86 ± 0.06	7.42 ± 0.09	4.13 ± 0.015	3.81 ± 0.04	6.27 ± 0.14	6.94 ± 0.08	5.19 ± 0.04	4.97 ± 0.03	3.16 ± 0.01	
Cd	Control	1.21 ± 0.09	2.23 ± 0.13	1.54 ± 0.08	1.67 ± 0.12	2.49 ± 0.07	2.18 ± 0.09	1.08 ± 0.02	1.56 ± 0.14	1.33 ± 0.05	1.0 (WHO 1995) 2.0 (FAO 1983, EU 2001)
	50% eff.	1.63 ± 0.11	2.46 ± 0.15	2.01 ± 0.06	1.89 ± 0.19	3.08 ± 0.12	2.87 ± 0.23	1.35 ± 0.06	2.13 ± 0.15	1.58 ± 0.04	
	100% eff.	2.04 ± 0.07	3.81 ± 0.17	2.78 ± 0.16	2.45± 0.08	3.92± 0.23	3.36± 0.17	2.19± 0.08	3.48± 0.21	2.27± 0.18	
Cu	Control	13.36 ± 0.21	16.08 ± 0.13	14.85 ± 0.05	9.53 ± 0.11	11.76 ± 0.21	9.05 ± 0.07	15.62 ± 0.17	17.08 ± 0.12	14.86 ± 0.09	30 (WHO 1995, FAO 1983, EU 2001)
	50% eff.	24.21 ± 0.1	28.47 ± 0.25	22.78 ± 0.12	14.69 ± 0.06	18.1 ± 0.16	13.49 ± 0.2	27.82 ± 0.32	29.2 ± 0.18	23.65 ± 0.15	
	100% eff.	29.5 ± 0.34	31.23 ± 0.45	27.14 ± 0.17	21.45 ± 0.07	23.75 ± 0.18	16.31 ± 0.05	36.25 ± 0.3	38.52 ± 0.23	32.89 ± 0.41	
Zn	Control	37.48 ± 0.11	71.56 ± 0.26	59.07 ± 0.19	42.36 ± 0.32	52.75 ± 0.41	47.2 ± 0.08	27.18 ± 0.5	46.9 ± 0.32	34.74 ± 0.14	100 (WHO 1995, FAO 1983) 120 (USEPA 2000)
	50% eff.	59.24 ± 0.3	93.52 ± 0.51	72.43 ± 0.21	64.15 ± 0.13	71.32 ± 1.2	69.06 ± 0.24	44.68 ± 0.06	65.89 ± 0.13	47.72 ± 0.09	
	100% eff.	96.65 ± 0.31	99.85 ± 0.27	97.08 ± 0.19	87.7 ± 0.12	91.56 ± 0.24	85.93 ± 1.12	61.8 ± 0.46	72.35 ± 0.54	59.42 ± 0.67	
Ni	Control	0.33 ± 0.01	0.65 ± 0.03	0.42 ± 0.007	<0.24	0.35 ± 0.02	0.31 ± 0.008	<0.29	0.67 ± 0.02	0.45 ± 0.005	1.0 (WHO 1995, FAO 1983, EU 2001)
	50% eff.	1.62 ± 0.08	3.56 ± 0.12	2.75 ± 0.16	1.22 ± 0.05	1.46 ± 0.01	1.19 ± 0.03	1.48 ± 0.11	2.54 ± 0.07	1.89 ± 0.09	
	100% eff.	2.87 ± 0.19	4.68 ± 0.21	3.21 ± 0.16	2.31 ± 0.13	2.43 ± 0.2	2.37 ± 0.14	2.09 ± 0.07	3.82 ± 0.22	3.02 ± 0.11	
Pb	Control	1.07 ± 0.04	2.61 0.03	0.97 ± 0.01	0.56 ± 0.008	1.15 ± 0.06	0.82 ± 0.09	0.59 ± 0.04	1.41 ± 0.003	1.13 ± 0.05	2.0 (WHO 1995) 1-5 (FAO 1983) 5.0 (EU 2001)
	50% eff.	1.92 ± 0.1	4.11 ± 0.02	1.23 ± 0.06	1.17 ± 0.1	2.15 ± 0.04	1.9 ± 0.03	1.57 ± 0.02	2.69 ± 0.13	2.08 ± 0.09	
	100% eff.	2.44 ± 0.06	6.72 ± 0.12	2.04 ± 0.02	1.84 ± 0.11	3.07 ± 0.05	2.51 ± 0.12	1.98 ± 0.07	5.48 ± 0.2	3.27 ± 0.14	

Eff: effluent, WHO: World Health Organization, FAO: Food and Agricultural Organization, EU: European Union, USEPA: United States Environmental Protection Agency

In 100% effluents, Cu levels in *C. punctata* muscle, gill, and bone reached 36.25, 38.52, and 32.89 mg/kg, respectively, exceeding allowable limits and surpassing those in other cultures. In 50% effluents, Cu concentrations were higher than the control but remained

within recommended levels. Across all treatments, gills absorbed the most Cu, followed by bone and muscle (**Table 4**).

At 100% effluents, Zn in muscles, gills, and bone was 96.65, 99.85 and 97.08 mg/kg, respectively for *A. testudineus*; 87.7, 91.56 and 85.93 mg/kg, respectively for *H. microps,* and 61.8, 72.35, and 59.42 mg/kg, respectively for *C. punctata* which were higher than the 50% effluent exposed and control fishes but within the permissible limits. For *H. microps* and *C. punctata,* Zn content was as gill> muscle> bone, while for *A. testudineus* it was as gills> bone > muscles (**Table 4**).

For *A. testudineus*, *H. microps*, and *C. punctata*, the concentrations of Ni in muscle were 2.87, 2.31, and 2.09 mg/kg, respectively; in gill, 4.68, 2.43, and 3.82 mg/kg; and in bone, 3.21, 2.37, and 3.02 mg/kg. These values were recorded after exposure to 100% effluent, which resulted in higher levels than those found under other culture conditions and exceeded recommended limits. In the control cultures, Ni levels remained within permissible limits. Across all fish species, Ni accumulation was highest in the gill, followed by bone, and lowest in muscle (**Table 4**).

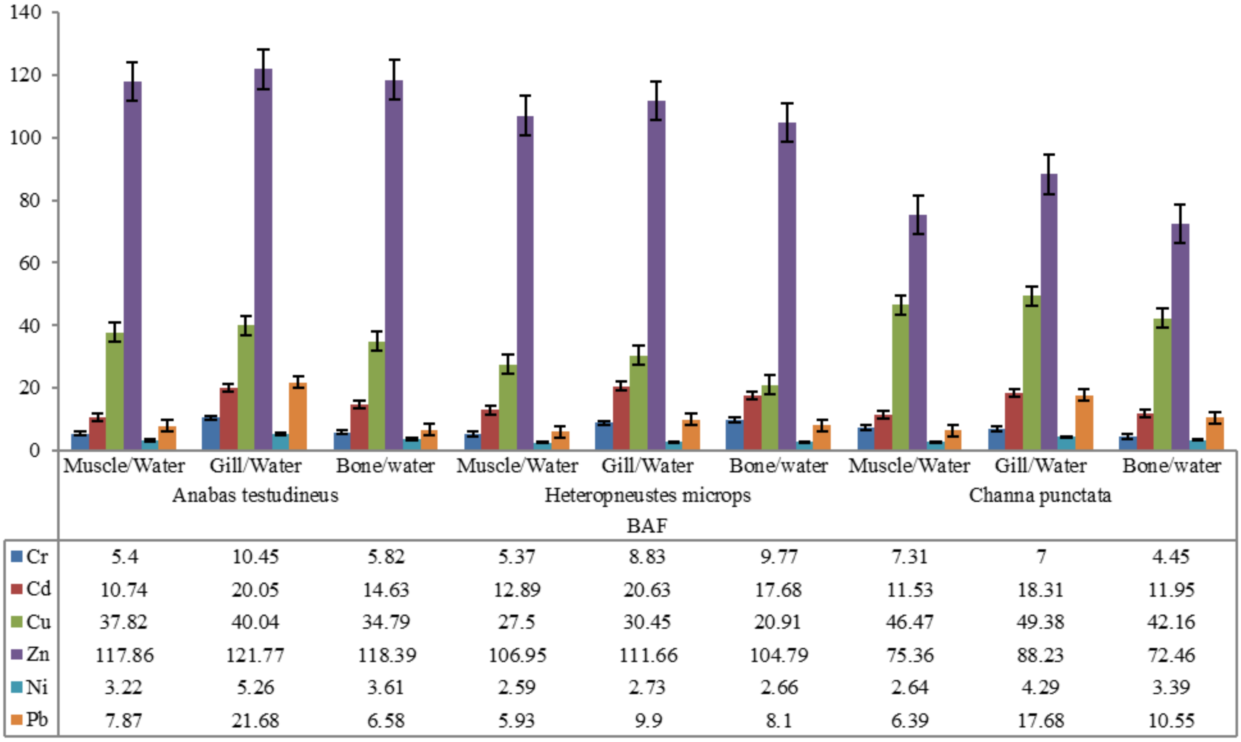
At 100% effluent, Pb levels (mg/kg) for *A. testudineus*, *H. microps,* and *C. punctata* were 2.44, 1.84, and 1.98, respectively, in muscle; 6.72, 3.07, and 5.48, respectively, in gill; 2.04, 2.51, and 3.27, respectively, in bone. These values exceeded those of other cultures but remained within suggested limits, except for the gills of *A. testudineus* and *C. punctata*. For *H. microps* and *C. punctata*, Pb was highest in gills, followed by bone and muscle; in *A. testudineus*, the order was gills > muscles > bone (**Table 4**).

After culture, the mean concentrations of potentially toxic metals Cr, Cd, Cu, Zn, Ni, and Pb in the body parts of *A. testudineus, H. microps,* and *C. punctata* increased with effluent strength (100% > 50% > control), with higher absorption in the gills (Chen *et al.* 2023; Mehnaz *et al.* 2022; El-Moselhy *et al.* 2014). At 100% effluent exposure, Cr, Cd, and Ni were detected above recommended levels (WHO 1995; FAO 1983; EU 2001) in all the studied fish body parts (**Table 4**). This may be due to bioaccumulation of toxic metals in fish tissues due to the formation of metal-binding proteins in metabolic organs (Hasan *et al.* 2023; El-Moselhy *et al.* 2014). The gills, muscle, and bone of the tested fishes concentrated varying amounts of toxic metals, which bear similarity with Hasan *et al.* (2023), El-Moselhy *et al.* (2014), and Siscar *et al.* (2014).

Metal accumulation by fish parts

Figure 3 shows biological metal accumulation in *A. testudineus*, *H. microps,* and *C. punctata* cultured in effluent.

The bioaccumulation factor (BAF) distinguishes hyperaccumulators (BAF > 1) from excluders (BAF < 1) (Rahman *et al.* 2026; Shetty *et al.* 2025; Qadir and Malik, 2011). All examined fish parts exhibited strong bioaccumulation (BAF much greater than 1) from the effluent (**Figure 3**).



	Anabas testudineus			Heteropneustes microps			Channa punctata		
BAF	Muscle/Water	Gill/Water	Bone/water	Muscle/Water	Gill/Water	Bone/water	Muscle/Water	Gill/Water	Bone/water
Cr	5.4	10.45	5.82	5.37	8.83	9.77	7.31	7	4.45
Cd	10.74	20.05	14.63	12.89	20.63	17.68	11.53	18.31	11.95
Cu	37.82	40.04	34.79	27.5	30.45	20.91	46.47	49.38	42.16
Zn	117.86	121.77	118.39	106.95	111.66	104.79	75.36	88.23	72.46
Ni	3.22	5.26	3.61	2.59	2.73	2.66	2.64	4.29	3.39
Pb	7.87	21.68	6.58	5.93	9.9	8.1	6.39	17.68	10.55

Figure 3. Metal-specific Bioaccumulation Factor (BAF) in body parts of *A. testudineus*, *H. microps,* and *C. punctata* cultured with industrial effluent.

Table 5. Paired sample t-test (2-tailed) result between a) effluent concentrations and b) fish parts in terms of heavy metals.

	p-value								
a) Between effluent	*A. testudineus*			*H. microps*			*C. punctata*		
	Mucl	Gill	Bone	Mucl	Gill	Bone	Mucl	Gill	Bone
(0% vs 50%) effluent	0.1405	0.0924	0.1015	0.2068	0.124	0.1742	0.1034	0.0872	0.090
(0% vs 100%) effluent	0.1979	0.043*	0.1580	0.1927	0.151	0.1739	0.1175	0.037*	0.0993
(50% vs 100%) effluent	0.252	0.016*	0.216	0.181	0.183	0.174	0.138	0.031*	0.113
b) Between fish parts	Mucl vs Gill	Mucl Vs Bone	Gill vs Bone	Mucl vs Gill	Mucl vs Bone	Gill Vs Bone	Mucl vs Gill	Mucl vs Bone	Gill vs Bone
Control	0.2742	0.3166	0.2115	0.1712	0.285	0.1299	0.2730	0.3301	0.2484
50% Effluent	0.2067	0.3957	0.1366	0.045*	0.198	0.0923	0.2361	0.9548	0.1686
100% Effluent	0.0017*	0.7397	0.004*	0.014*	0.769	0.0835	0.0956	0.2995	0.0835

* = p is significant at the <0.05 level (2-tailed); Mucl: Muscle

Research indicates that metal accumulation follows the order Zn>Cu>Cd>Pb>Cr>Ni for all the examined fish species. For metal-specific bioaccumulation, the studied fish follow the pattern as Zn and Cr: *A. testudineus>H. microps> C. punctata*, Pb and Ni: A. *testudineus>C. punctata>H. microps, Cu: C. punctata>* A. *testudineus>H. microps* and Cd: *H. microps>*A. *testudineus>C. punctata.* Specifically, the highest accumulation of Cr (BAF=10.45), Zn (BAF=121.77), Ni (BAF=5.26), and Pb (BAF=21.68) was found in the gills of *A. testudineus* after exposure to full-strength industrial effluent. In contrast, *H. microps* and *C. punctata* accumulated the highest concentration of Cd (BAF=20.63) and Cu (BAF=49.38), while exposed to the same concentrated effluent.

Table 5 presents p-values between effluent percentages and toxic metal accumulation among fish organs. All metals showed significant positive linear correlations with accumulation in the tested species. Metal buildup in the gills of *A. testudineus* and *C. punctata* was significantly higher in 100% effluents than in 50% or control water ($p<0.05$). Gill of *H. microps* significantly concentrated metals compared to muscle at 50% effluent (p=0.045*). In *A. testudineus*, gills absorbed more metals than bone and muscle ($p<0.05$), while in *H. microps* cultured in 100% effluents, gills also showed significantly greater uptake versus muscle (p=0.0145*) (**Table 5**).

Across nearly all species, the gills contained the highest metal concentrations, whereas the muscles showed the lowest (see **Figure 3**), which aligns with Hasan *et al.* (2023) and El-Moselhy *et al.* (2014). The pronounced accumulation in the gills is attributed to their extensive surface area and role as the primary site of metal ion transfer from water, supporting findings reported by Chen *et al.* (2023), Kargin (1998), and Qadir and Malik (2011). Metal accumulation was significantly higher in the gills of all studied fish samples while exposed to 100% industrial effluent, so non-carcinogenic and carcinogenic health effects would be expected to occur due to consumption of these fish (Rahman et al. 2026; Chen et al. 2023; Mehnaz et al. 2022) (**Table 4 & 5**).

Conclusion

Contamination by trace elements in aquatic ecosystems presents significant ecological challenges, with far-reaching implications for public health and economic stability. This study revealed the bioaccumulation of potential toxic metals from industrial effluents using hydroponic cultures of *A. testudineus*, *H. microps*, and *C. punctata*. The findings showed that temperature, pH, electrical conductivity (EC), biochemical oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), turbidity, and total organic carbon (TOC) were significantly elevated, while dissolved oxygen (DO) was markedly reduced. Elevated concentrations of chromium, cadmium, and lead were also observed, signifying industrial wastewater pollution and non-compliance with standard limits. Elevated concentrations of chromium, cadmium, and lead were also observed, signifying industrial wastewater pollution and non-compliance with standard limits.

The measured metal concentrations in fish body parts surpassed permissible limits (except Zn) and were found to be 100% effluent > 50% effluent > control, with substantially higher accumulation in the gill. All studied fish tissues demonstrated strong bioaccumulation (BAF > 1), with metals accumulating in the order Zn > Cu > Cd > Pb > Cr > Ni. Species-specific bioaccumulation patterns emerged: *A. testudineus* showed greater accumulation of Cr, Zn, Ni, and Pb, whereas *H. microps* and *C. punctata* preferentially accumulated Cd and Cu, respectively. At 100% effluent concentration, the gills of *A. testudineus* and *C. punctata* exhibited significantly elevated metal levels ($p<0.05$) compared to bone and muscle and relative to other treatment groups. Gill of *H. microps* significantly accumulated metal uptake compared to muscle only (p=0.0145*) at exposure to 100% industrial effluent. Overall, findings indicate that although metal levels in effluent may not be exceptionally elevated, fish can rapidly accumulate hazardous concentrations in their tissues, which may pose a substantial health risk (both carcinogenic and noncarcinogenic) for human consumers. Continued long-term exposure to industrial effluents is likely to be detrimental to fish health and to organisms at higher trophic levels.

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Author Contributions

HM: Conceptualization, Graphical abstract preparation, Sample collection and analysis, Experimental design, Main manuscript writing and editing, All tables and figures preparation, and Statistical analysis. AKMRA: Conceptualization, Validation, Supervision, Review, and editing. MM: Validation, Sample analysis, and Supervision. All authors reviewed the manuscript.

Declaration of Interest Statement

No potential conflict of interest was reported by the author(s).

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