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Assessment of heavy metals and ecological risk of pangas (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*) farms in Bangladesh

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ABSTRACT

An investigation was conducted to evaluate the contamination levels of heavy metals (As, Pb, Cd, Cr and Cu) in surface water and sediment of pangas (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*) aquaculture farms in four regions (Mymensingh, Cumilla, Bogura, and Jashore) of Bangladesh. The ecological risk index (ERI) was determined to assess the water quality index. The water contained As concentrations of 3.60–11.14 µg/L; Pb of 1.73–3.83 µg/L; Cd of 1.26–23.12 µg/L; and Cu of 6.08–45.22 µg/L; whereas the sediments contained As concentrations of 2.84–4.57 mg/Kg; Pb of 8.32–16.07 mg/Kg; and Cd of 0.22–12 mg/Kg. Most locations had moderate to low levels of pollution, and the levels of contamination decreased from Cd to Pb to As to Cr. The declining sequence of potential ecological risk factors of toxic metals in sediment was Cd > Pb > As > Cr. Cd's potential ecological risk factor ranged from 2.84 to 21.58, making it one of the elements with the highest potential for ecological danger compared with other metals in this study. The Igeo values for the metals under study showed the following hierarchy Cr > As > Pb > Cd. As had a geo-accumulation index of -1.87 to 0.82, Pb, Cd -0.48 to -1.13, and Cr registered -1.28 to -2.45. Ponds in four different regions were found to be safe and suitable for fish culture, however, it was recommended that continuous monitoring of heavy metal concentrations in the aquaculture sector is necessary to mitigate the threat of contamination.

Introduction

The majority of aquaculture output (1.97 million MT) of Bangladesh comes from ponds, making the country the world's fifth-largest producer of aquaculture (FAO 2020). Ninety-three percent of the country's freshwater fish supply comes from pond-raised stocks of carp, pangas, and tilapia. Mymensingh, Jessore, Bogura, and Comilla are home to the vast majority of Bangladesh's 1,038

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registered hatcheries. In recent years, the aquaculture industry in Bangladesh has flourished, grown, and varied, with an upward tendency in some regions' intensification of production methods (Belton and Azad, 2012; Ali, 2009). High growth rates, disease resistance, and commercial viability have made pangas and tilapia two of the most popular fish species farmed today. Since pangas and tilapia are omnivores, they need artificial feed for accelerated development. In Bangladesh, however, tannery solid waste protein concentrate is put to good use in animal feeds. There is substantial evidence that heavy metals have contaminated the food supply in Bangladesh (Islam *et al*., 2014).

Due to their toxicity, longevity and propensity to accumulate in species including people, fish, and invertebrates. Heavy metals are considered as one of the most dangerous environmental contaminants (Martin *et al*., 2015; Ahmed *et al*., 2015). The fishing industry is a major contributor to the presence of HMs and metal-based contaminants in the aquatic environment, which in turn damages the quality of the water body as a whole (Saha *et al*., 2016; Gu *et al*., 2015). It has been shown that aquatic species are highly susceptible to the hazardous effects of metal contamination in natural water (Rakib *et al*., 2021). HMs can enter the human body through direct skin contact or through the ingestion of water or aquatic animals that contain the metals (Rakib *et al*., 2021). HMs in the aquatic environment and bioaccumulation in aquatic animals like fishes pose health risks to humans (Ahsan *et al*., 2022; Dhanakumar *et al*., 2015; Lawson, 2011). The source of these metals is anthropogenic activities such as industry, urban and residential areas, agriculture, catchment runoff, shipping, and mining. Heavy concentrations of essential metals like copper, zinc, nickel, and iron can be harmful. Mercury, lead, and cadmium are examples of non-essential metals that are often regarded as either useless or dangerous (Gu *et al*., 2015; Ruelas *et al*., 2011).

HMs contamination in water, sediments, and fish has been the subject of several studies conducted in various regions of the world (Ahsan *et al*., 2022; Rakib *et al*., 2021; Yun-Ru *et al*., 2017; Kumar *et al*., 2011). There have been a number of studies on the topic of HMs concentrations in Bangladeshi fish, water, and sediment, but only a few have gained widespread attention (Ahsan *et al*., 2022; Maruf *et al*.,2021; Shovon *et al*., 2017; Bhuyan *et al*., 2017; Islam *et al*., 2017; Hossain *et al*., 2016; Ahmed *et al*., 2010). While commercial aquaculture systems like pangas and tilapia ponds are increasingly popular but little is known about HM concentrations in these systems. Therefore, the objectives of this research were to quantify the metal contamination in commercially cultured pangas and tilapia in four different regions of Bangladesh, and assess the water quality index for ecological risk index (ERI).

Materials and Methods

Sample collection, preparation, and determination of heavy metals concentration

In Bangladesh, 51 fish ponds (30 pangas and 21 tilapia) were sampled among the cities of Mymensingh, Cumilla, Bogura, and Jashore (Fig. 1). Water samples were collected from the surface of the ponds at the end of each production cycle. Three water samples were taken from each pond using a grab sampler of 1 L capacity (Ben Meadows Company, WI, and USA) and were subsequently mixed and stored in plastic bottles as one composite sample (1 L). After that, 1 ml of 65% HNO_3 acid was added to the water to make it acidic. The samples were taken to the lab in an ice chest, kept at 4 ˚C, and filtered using a membrane filter with a very small pore size (0.45 m). The filtered water was frozen at -20˚C for testing later. Using a sediment corer, we took samples of sediment up to 5 cm deep, which we then sealed in plastic bags. In a 100 ml glass beaker, we heated 130 °C for 5 hours and added 2 g of sediment to 10 ml of concentrated HNO_3 and 5 ml of concentrated $HClO_4$. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the levels of Cr, Cu, Pb, Cd, and As in the water and sediment samples.

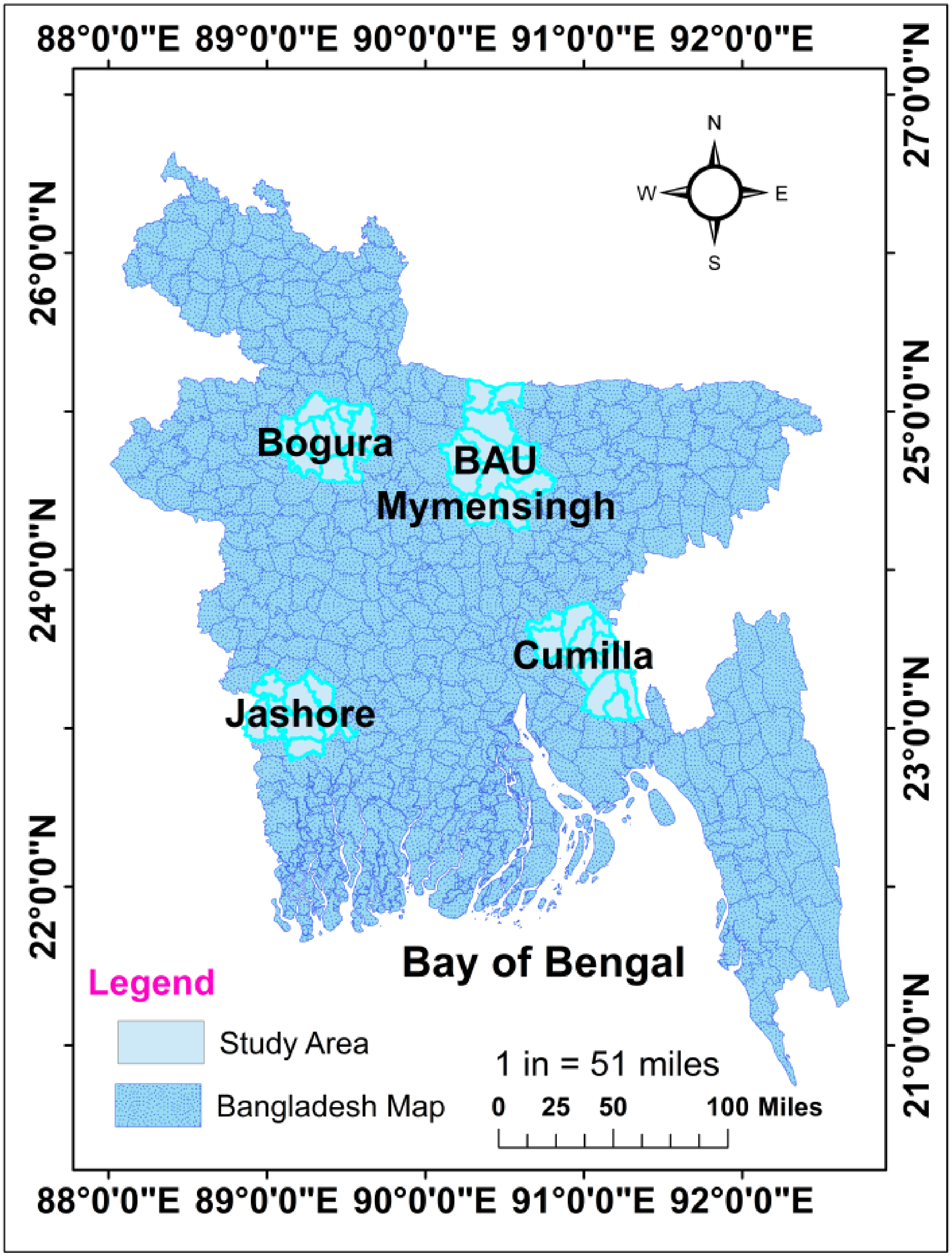

Fig. 1. Study areas from where samples were collected for this study.

Ecological risk assessment
Pollution load index

An integrated approach of the four metals was calculated (Islam *et al*., 2015a).

$$PLI = (CF_1 \times (CF_2 \times CF_3 \times \ldots\ldots (CF_n)^{(1/n)} \ldots\ldots\ldots (1)$$

Where CF_{metals} is the background values of HMs were taken from the pre-industrial samples of the study area in Bangladesh. The background values of Cr, As, Cd, and Pb in sediments were 41, 8.5, 0.92, and 23, respectively.

Contamination factor (C_f^i) and degree of contamination **(**Yi *et al*., 2011 **)**

$$C_f^i = \frac{C^i}{C_n^i}\ ,\ C_d = \sum_{i=1}^{n} C_f^i \ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where C_f^i is the single element pollution factor, C^i is the content of the element in samples, and C_n^i is the reference value of the element. The reference values of Cr, As, Cd, and Pb in sediments were 60, 13, 0.3, and 20 mg/kg (Islam *et al*., 2015b; Yi *et al*., 2011). Contamination factor (C_f^i) classified into 4 grades for monitoring the pollution of one single metal over a period of time (Loska and Wiechula, 2003; Hakanson, 1980): low degree C_f^i <1, moderate degree ($1 \leq C_f^i \leq 3$), considerable degree ($3 \leq C_f^i \leq 6$) and very high degree (C_f^i).

Potential ecological risk (PER)

The equations for calculating the PER are as follows

$$E_r^i = T_r^i \times C_f^i\ ,\ PER = \sum_{i=1}^{m} E_r^i \ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Where E_r^i is the potential ecological risk index. T_r^i is the biological toxic factor. The toxic–response factors for cr, As, Cd, and Pb were 2, 10, 30, and 5, respectively (Guo *et al*., 2010).

Geo-accumulation index (Igeo)

The degree of contamination from the HMs could be assessed by measuring the Igeo (Saleem *et al*., 2015; Santos *et al*., 2003).

$$I_{geo} = \mathrm{Log}_2[C_n\ /\ 1.5\ B_n] \ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Where C_n is the measured concentration of metal in the sediment and B_n is the geochemical background value of element n in the background sample (Rahman and Ishiga, 2012; Yu *et al*., 2011). Igeo ≤ 0: practically uncontaminated; 0 ≤ Igeo ≤ 1: uncontaminated to moderately contaminated; 1 ≤ Igeo ≤ 2: moderately contaminated; 2 ≤ Igeo ≤ 3: moderately to heavily contaminated; 3 ≤ Igeo ≤ 4: heavily contaminated; 4 ≤ Igeo ≤ 5: heavily to extremely contaminated; and 5 < Igeo: extremely contaminated.

Statistical analysis

All values are expressed as means ± standard deviation (SD) by descriptive statistics. Significant differences for mean metal concentrations across different water and sediment in the regions were tested applying the one-way analysis of variance (ANOVA) with 95% confidence. Tukey's HSD test was applied for post hoc detection of significant pair-wise comparisons between the regions. All the statistical tests were carried out using the statistical software SPSS (version 23.0, SPSS, Chicago, IL, USA).

Results and Discussion

Concentration of HMs in water samples

Water samples from 30 pangas and 21 tilapia farms were analyzed for HMs, and the average levels are presented in Table 1. Cd ranged 1.26 to 23.12 µg/L in different samples. Bogura had the greatest mean Cd concentration for pangas and tilapia farms (23.12±12.89 µg/L), whereas Cumilla had the lowest (1.26±0.07 µg/L). Bogura had the highest Pb values (3.83±2.03 µg/L), whereas Jashore had the lowest (1.73±0.71 µg/L). Table 1 displays the average As concentration found in the water samples taken from Jashore (11.14±1.12 µg/L) and Bogura (3.60±1.01 µg/L), respectively. Cumilla had the greatest mean Cu concentration among the pangas and tilapia farms, at 45.22±15.44 µg/L, while Jashore had the lowest, at 6.08±1.32 µg/L. Mymensingh had the greatest Cr concentration (11.19±2.11 µg/L), while BAU (Bangladesh Agricultural University) had the lowest (3.27±0.74 µg/L). The average concentration of the HMs tested decreased from Cu to Cd to As to Cr to Pb in the water samples. There was a significant difference ($p < 0.05$) in As concentrations between Bogura, BAU, and Cumilla, Jashore, and Mymensingh, but no such difference ($p > 0.05$) existed between the latter three locations. There was no significant difference ($p > 0.05$) in the Pb concentrations found in the water samples from each location. For both pangas and tilapia ponds, there was a statistically significant ($p < 0.05$) regional difference in the concentrations of Cd, Cr, and Cu in water samples.

Table 1. Heavy metal concentrations in water (µg/L) of tilapia-pangas ponds

Region	As	Pb	Cd	Cr	Cu
Bogura	3.60±1.01[a]	3.83± 2.03[a,b]	23.12± 12.89[c]	4.31± 1.03[a]	20.16±15.66[c]
Cumilla	5.26± 0.56[b]	2.22± 1.04[a]	1.26± 0.07[a]	3.73± 1.16[a]	45.22± 15.44[d]
Jashore	11.14±1.12[b]	1.73± 0.71[a]	3.03± 1.31[a]	3.74± 1.04[a]	6.08± 1.32[a]
Mymensingh	5.79± 0.94[b]	2.32± 1.19[a]	14.92± 11.19[b]	11.19± 2.11[b]	34.96± 3.44[c,d]
BAU	4.37± 1.45[a]	1.91± 0.49[a]	13.51± 9.33[b]	3.27± 0.74[a]	20.28± 2.46[b]
WHO, 1993	50	20	5	50	100

One source of Cr in surface waters is naturally occurring as a result of a concentration of Cr-bearing minerals; another is the release of Cr-based oxidants (chromates, dichromates, etc.) from industrial processes such those used in the tanning and textile industries (Rakib *et al*., 2021). Thus, the elevated Cr level in the surrounding waters can be traced back to the wastewater discharged from these factories. Since $K_2Cr_2O_7$ is a critical molecule in the purification process, the greater Cr concentration seen in this study may be attributable to the fish diet, which may contain poultry and/or tanner waste (Sultana *et al*., 2017). HMs can also be absorbed by the sediment from uneaten pelleted feed in aquaculture ponds (Sarkar *et al*., 2022). HMs can accumulate in water and sediments for a number of reasons, including a lack of water exchange during farming and the failure to remove bottom sediments between production cycles (Sarker *et al*., 2016). Cr (0.20 µg/L) and Cu (1.97 µg/L) were also identified in high amounts in fish farms on Taiwan's southwestern coast. USEPA (United States Environmental Protection Agency) set the Pb limit for household water systems at 50 µg/L, while ISI set it at 10 µg/L. Pb limits for drinking, fishing, service, irrigation, and raising water were all set at 50 µg/L. According to the World Health Organization (2004), 20 µg/L is the maximum allowable level of Pb in drinking water.

Concentration of HMs in sediment samples

HMs concentrations and analytical results from sediment samples collected from 51 pangas and tilapia farms are shown in Table 2. Average As level 3.57±0.37 mg/kg in the sediment of Bogura, 3.18± 1.40 mg/kg in that of Cumilla, 4.57±0.93 mg/kg in that of Jashore, 2.84±1.42 mg/kg in that of Mymensingh, and 4.57±0.47 mg/kg in that of BAU. In Jashore area, the As concentration was 4.57 mg/kg, while in the Mymensingh area, it was only 2.84 mg/kg. The average amount of Pb found in the Cumilla area was 8.32 mg/kg, whereas the amount found in the Bogura area was 16.07 mg/Kg. Sediment Pb values ranged from 16.07-8.32 to 12.47 to 8.50 to 9.86 mg/Kg in the locations of Bogura, Cumilla, Jashore, Mymensingh, and BAU, respectively. Cd levels measured throughout the research period were 0.22 mg/Kg in Bogura, 0.26 mg/Kg in Cumilla, 7.87 mg/Kg in Jashore, 12.47 mg/Kg in Mymensingh, and 0.08 mg/Kg in BAU. Cd concentrations ranged from 0.08 mg/Kg in the BAU region to 7.87 mg/Kg in the Jashore region. Sediment samples from Bogura, Cumilla, Jashore, Mymensingh, and BAU all have similar mean Cr values of 16.94, 9.21, 8.33, 14, and 18.51 mg/Kg, respectively. The Cr concentration was lowest in the Jashore region (8.33 mg/Kg) and greatest in the BAU region (18.51 mg/Kg). Sediment samples from Bogura, Cumilla, Jashore, Mymensingh, and BAU had mean Cu values of 23.13, 10.69, 10.82, 19.85, and 18.51 mg/Kg, respectively. Sediment samples from both pangas and tilapia ponds showed no statistically significant changes in As concentrations between locations ($p > 0.05$). In both pangas and tilapia ponds, regional differences in sediment Cd, Cr, Cu, and Pb concentrations were significant ($p < 0.05$; Table 2).

Table 2. Heavy metal concentrations (mg/Kg) in sediment in tilapia-pangas ponds

Region	As	Pb	Cd	Cr	Cu
Bogura	3.57± 0.37[a]	16.07± 3.26[b,c]	0.22± 0.14[a]	16.94± 8.96[b]	23.13± 2.43[b]
Cumilla	3.18± 1.40[a]	8.32± 4.14[a]	0.26± 0.16[a]	9.21± 3.77[a]	10.69± 6.22[a]
Jashore	4.57± 0.93[a]	12.47± 2.7[b]	7.87± 2.44[b]	8.33± 4.47[a]	10.82± 4.99[a]
Mymensingh	2.84± 1.42[a]	8.50± 4.28[a]	12.47±2.77[c]	14.00± 2.75[b]	19.85 ± 4.65[b]
BAU	4.57± 0.47[a]	9.86± 1.79[a]	0.08± 0.01[a]	18.51±6.45[b,c]	18.51± 6.45[b]
TRV (Toxicity Reference Value)	8.2	…..	16	81	50
TEL (Threshold Effect Level)	5.9	35	0.596	37.3	35.7

*Guidelines for metal contamination in sediment TEL (Threshold Effect Level) (Macdonald *et al*., 2000; USEPA, 1998)

The physicochemical features of As in water at different valences are reflected in the large variety of inorganic and organic compounds that AS creates (Rakib *et al*., 2021). Cr is a versatile metal that can be found in a wide variety of deposits, from plants to minerals. Both natural and anthropogenic causes contribute to its continued existence (Maurya and Kumari, 2021). Mining, metallurgy, agriculture, and the chemical sector all release Cu into the environment, and this metal is also widely employed in industry and agriculture (Yunus *et al*., 2020). Cu, like zinc (Zn), is necessary for human health, aids in haemoglobin formation, and participates in enzymatic processes; nevertheless, excessive levels can be hazardous. Stable Pb is toxic to humans and aquatic animals, especially the kidneys and nervous system (Fasae and Abolaji, 2022). In most cases, atmospheric deposition and by-products of carbon combustion enter it into aquatic environments. The highest levels of Pb are found in mud due to the movement of lead-contaminated materials (Anbuselvan and Sridharan, 2018).

Assessment of metal pollution

Pollution load index (PLI)

Metal PLI values for sediments that are calculated shown in Fig. 2. During the study period, As PLI values varied from 0.11 to 0.41, Pb PLI values ranged from 0.11 to 0.49, Cd PLI values ranged from 0.02 to 0.05, and Cr PLI values ranged from 0.07 to 0.23, all of which were estimates (PLI 1). The Jashore area had a higher PLI for As and Pb than the rest of the country. A PLI of 0 means that the site is in perfect condition, while a PLI of 1 means that there is only a minimal amount of pollutants present. The main causes of elevated metal concentrations in surface sediments include domestic wastewater discharge, municipal wastewater, industrial effluent, and atmospheric deposition. PLI can provide people an idea of the mud's quality. It also supplies policymakers with crucial data on pollution levels in the area under investigation (Fig. 2).

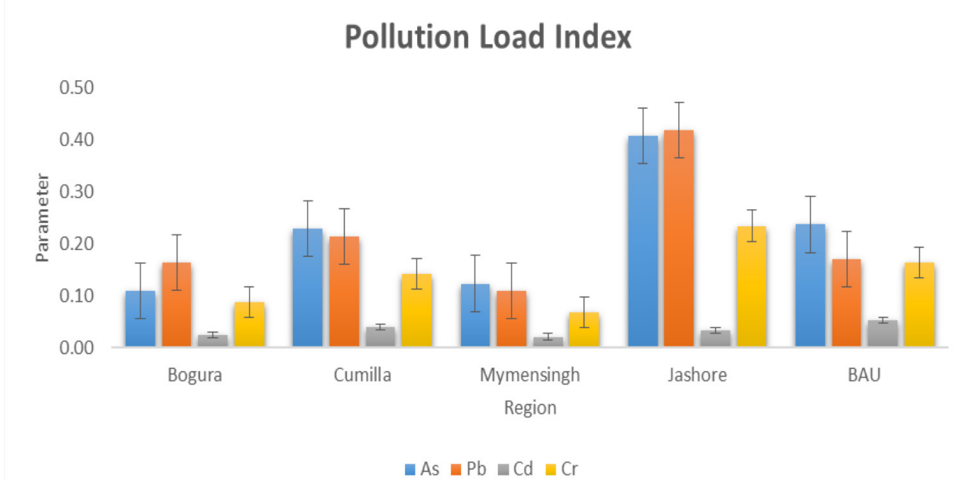

Fig. 2. Pollution load index of different sampling regions.

Contamination factor (C_f^i) and degree of contamination (C_d)

Results from this investigation demonstrated that the contamination factor (C_f^i) for sediments increased from Cd to Pb to As to Cr. The degree of contamination (C_d) was used as a metric for evaluating integrated metal pollution in sediment. Most locations had moderate to low levels of contamination, with the levels of contamination decreasing from Cd, Pb, As, and Cr. Sediment tested had a considerable C_d across all elements, with values ranging from 4.77 to 13.55 (Table 3). Cd was found to be considerable in Bogura, Cumilla, and Mymensingh, while Cr was found to be very low across all study areas (Table 3). C_f^i <1, 1≤ C_f^i < 3, 3≤ C_f^i <6, and C_f^i >6 indicate low, moderate, considerable, and high contamination of individual metals. C_d< 5, 5≤ C_d<10, 10 ≤ C_d< 20 C_d 20 indicates low, moderate, considerable, and high contamination degree of environment according to Indices and grades of the degree of contamination of HMs (Luo *et al*., 2007).

Table 3. Contamination factor, degree of contamination, and contamination level of toxic metals in sediment collected from different regions of Bangladesh.

Regions	Month	As	Pb	Cd	Cr	Degree of Contamination	Contamination Level
Bogura	May	0.09	2.60	3.30	0.82	6.81	Moderate
	July	0.15	3.70	0.76	1.75	6.36	Moderate
	Sep.	0.13	2.32	2.02	0.52	4.99	Low
Cumilla	May	1.42	2.53	5.75	0.87	10.57	Considerable
	July	1.21	1.96	1.07	0.75	4.99	Low
	Sep.	1.11	1.77	2.09	0.74	5.71	Moderate
Mymensingh	May	1.17	2.25	4.13	0.76	8.31	Moderate
	July	1.53	2.67	1.42	0.95	7.57	Moderate
	Sep.	1.01	2.17	0.98	0.61	4.77	Low
Jashore	May	3.32	5.26	1.57	1.89	12.04	Considerable
	July	2.92	5.93	2.48	2.22	13.55	Considerable
	Sep.	1.92	3.30	2.22	1.30	8.74	Moderate
BAU	May	2.09	4.43	2.49	1.52	10.53	Considerable
	July	1.95	3.21	1.54	1.50	8.2	Moderate
	Sep.	1.31	1.95	2.77	0.87	6.9	Moderate

Potential ecological risk (PER)

A synopsis of the prospective PER and the ecological risk factor for specific elements (E_r^i) were found. The potential ecological risk factor of toxic metals in sediment was Cd > Pb > As > Cr in descending order. With an E_r^i value between 2.84 and 21.58, Cd exhibited a very high potential ecological risk when compared to the other elements. The PERs for the sampling regions are often less than 65, indicating a low risk. E_r^i< 40, 40≤E_r^i< 80, 80≤E_r^i<160, 160≤E_r^i< 320, E_r^i ≥ 320 indicates low, moderate, considerable, high, and very high ecological risk of individual metal (Table 4). PER < 65, 65 ≤ PER <130, 130 ≤ PER <260, PER ≥ 260 indicates low, moderate, considerable, and very high potential ecological risk according to Indices and grades of the degree of contamination of heavy metals (Luo *et al*., 2007).

Table 4. Potential ecological risk factors (E_r^i) and potential ecological risk indices (PER) of heavy metals in sediments of different distinct regions of Bangladesh.

Regions	Month	As	Pb	Cd	Cr	Risk Index	Pollution Level
Bogura	May	0.93	1.63	12.37	0.20	15.13	Low
	July	7.12	2.31	2.84	0.44	12.71	Low
	Sep.	1.26	1.45	7.58	0.13	10.42	Low
Cumilla	May	0.88	1.58	21.58	0.22	24.26	Low
	July	4.83	1.23	4.00	0.19	10.25	Low
	Sep.	0.55	1.11	7.85	0.19	8.7	Low
Mymensingh	May	0.68	1.41	15.48	0.19	17.76	Low
	July	5.96	1.67	5.33	0.24	13.2	Low
	Sep	0.57	1.36	3.69	0.15	5.77	Low
Jashore	May	2.47	3.29	5.90	0.47	12.13	Low
	July	21.11	3.70	9.30	0.56	34.67	Low
	Sep	1.02	2.06	8.32	0.33	11.73	Low
BAU	May	1.20	2.77	9.35	0.38	13.7	Low
	July	7.34	2.01	5.77	0.38	15.5	Low
	Sep	0.59	1.22	10.41	0.22	11.44	Low

Geo-accumulation index (Igeo)

The Igeo values for the investigated HMs are presented in Fig. 3. The Igeo values for the metals under study showed the following hierarchy: Cr > As > Pb > Cd. The Igeo values for the analyzed elements ranged from completely clean to highly polluted. For As, Igeo falls between -1.87 and 0.82, for Pb, between -0.48 and -1.13, for Cd, between -0.78 and 0.01, and for Cr, between -1.28 and -2.45. The universal criterion to evaluate the metal pollution in sediment is the geo-accumulation Index (Igeo) which has been widely used since the late 1960s. Calculated geo-accumulation index (Igeo) values showed that the sediments of the tilapia and pangas pond were unpolluted (Igeo < 0) for all studied metals except As for Cd. As and Cd showed partially polluted. The Igeo values for Pb and Cr showed that they were practically uncontaminated (Fig. 3).

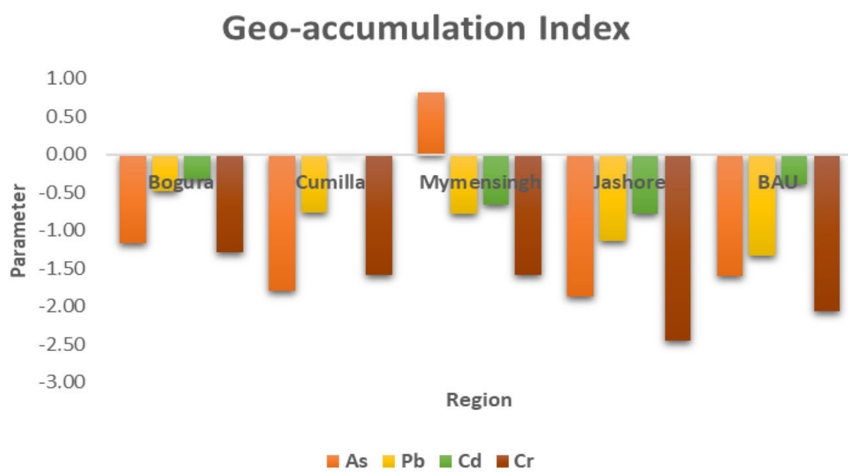

Fig. 3. Geo accumulation index (Igeo) in the sampling regions.

Conclusion

An in-depth analysis of heavy metal concentrations in water and sediment samples from pangas and tilapia farming is provided here, allowing evidence-based policy decisions to be made on these fisheries' impact on the environment. Pb and Cu were found in the lowest concentrations in the Cumilla and Mymensingh regions, whereas As was most concentrated in the Jashore region and least concentrated in the Bogura region. As concentrations in sediments were highest in the Jashore and Mymensingh regions, while Pb and Cd concentrations were lowest in the Jashore and BAU regions. Cd indicated moderate pollution in the Bogura, Cumilla, and Mymensingh regions, while Cr indicated very low pollution in all regions. The metals examined by Igeo were rated as "pristine" except for As in Mymensingh region. There is no danger of contamination in any of the four pond locations, but constant monitoring of HMs levels in the aquaculture industry is necessary. To keep confined inland ecosystems healthy, the study concludes that constant monitoring and adaptive management are essential.

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Author contribution: RAK, JH, AA, AR, MAR, DCS, ZR, and MEA were responsible for the planning and design of the experimental work; RAK with assistance from AA and AR conducted fieldwork and laboratory analysis in Bangladesh; all authors contributed to interpretation of data; RAK and JH drafted the MS with input from MAR, DSC, ZR and MEA.

Data availability statement The datasets used and analyzed during the current study will be provided on request to the corresponding author.

Conflicts of interest All the data were collected according to legal provisions only. The authors declare that they have no conflict of interest.

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