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Assessment of heavy metal concentrations in commercially Farmed Fishes in the South–West region of Bangladesh and associated health risk assessment

M. Aslam[1]*, S. Roy[1], M. S. Ali[2], M. Kamruzzaman[3], M. M. Hasan[4] and M. T. Hassan[4]

[1]*Department of Biochemistry and Molecular Biology, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Gopalganj 8100, Dhaka, Bangladesh*

[2]*Department of Biotechnology and Genetic Engineering, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Gopalganj 8100, Dhaka, Bangladesh*

[3]*Department of Applied Chemistry and Chemical Engineering, Engineering Faculty, Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Gopalganj 8100, Dhaka, Bangladesh*

[4]*Food Toxicology Research Section, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Qudrat-I-Khuda Road, New Elephant Road, Dhanmondi, Dhaka-1205, Bangladesh*

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Abstract

Due to the different hazardous effects of heavy metals on human health, this study investigated the concentrations of the heavy metals lead (Pb) and chromium (Cr) in different commercially farmed fishes cultivated in Bangladesh and in their respective fish feeds (poultry stool from integrated farming or commercial fish feeds) to determine the sources of the contaminants and their associated health risks. Thirty fish samples were collected from ten different farms in three districts (Gopalganj, Madaripur, and Khulna) of Bangladesh. Farms from Gopalganj and Madaripur utilized poultry stool as the sole source of food, whereas commercially available fish feeds were used in Khulna. The concentrations of Pb and Cr in the fish and their respective diets were measured via an atomic absorption spectrophotometer. Although the Pb concentration exceeded the safe concentration in 53% of the fishes, the Cr concentration did not surpass in any of the fish samples. The Pb and Cr concentrations in the fish samples correlated with those in their respective fish feeds, suggesting bioaccumulation of these heavy metals through their diet. Although health risk analyses of the mean Pb and Cr concentrations in fish muscles revealed no noncarcinogenic (THQ and HI) risk, the carcinogenic risk associated with Cr was unacceptable in all three districts. The Pb and Cr concentrations in poultry stool samples were not significantly greater than those in commercial feed samples, indicating that, from a heavy metal perspective, there are no additional health risks associated with integrated farming compared with the usage of commercial fish feeds.

Keywords: Heavy metal contamination in fish; Integrated farming; Atomic absorption spectrophotometer; Toxicity; Poultry stool; Commercial feed

Introduction

In Bangladesh, fish is considered the third most consumed food (62.58 gm/day) after starch and vegetables and accounts for approximately 60% of the total annual protein demand (Waid *et al.* 2019; BBS, 2019). To meet the increasing demand for fishes in both the national and international markets, fish aquaculture and fish farming have experienced dramatic growth over the past decade (DOF, 2006). Bangladesh was placed fifth in the world for farmed fish production in 2018 (FAO, 2020). Due to its socioeconomic importance, the quality and safety of this staple protein source has become a prime interest.

The success of an intensive fish aquaculture system largely depends upon the use of well-balanced feed. In Bangladesh, fish culture has rapidly evolved from farms with no feed to

*Corresponding author's e-mail: aslam_du@yahoo.com, aslam@bsmrstu.edu.bd

commercial feeds and subsequently to those with integrated farming with the poultry industry (Mahmud *et al.* 2012). Compared with no feed, formulated commercial fish feed raises the culture capacity of the system and can boost fish yield by 7.7-fold. However, commercial feed can cost up to 50–60% of the total operating cost (Sarkar *et al.* 2021). A popular and economic method of aquaculture is integrated fish farming, where fishes are cultured beneath a poultry farm and poultry stool is used as fish feed. Integrated farming technology ensures the efficient use of resources and additional sources of feed and extra income for poor farmers and reduces the risk of total production failure (Islam *et al.* 2007). However, contamination with different pollutants, especially heavy metals, in fish feed and poultry feed (source of poultry stool) has recently become a grave concern (Francis *et al.* 2020).

A broad category of metals, or combinations of metals and metalloids, that are present in biological systems at very low concentrations and have a density of more than 4 g/cm3 are referred to as heavy metals (Duruibe *et al.* 2007). Some of these heavy metals (e.g., Cu, Cr, Mg, Ni, and Zn) are required in minute amounts for various biochemical and physiological functions (WHO/FAO/IAEA, 1996) but might be toxic when taken at elevated concentrations. Some metals (e.g., As and Pb) are toxic even when taken at low concentrations. (Wang and Shi, 2001). Consuming food tainted with these heavy metals can cause major health issues in people. Lead (Pb), for instance, has been shown to have a negative impact on young children's brain and nervous system development. Pb can also cause nephrotoxicity and hypertension and above all is characterized as a possible carcinogen in humans. On the other hand, Cr can cause ulcers, immunological defects, kidney and liver damage, lung cancer and even death (Garcia-Leston *et al.* 2010, Shekhawat *et al.* 2015).

Both fish feed and poultry feeds can be contaminated with heavy metals in several ways. Studies have shown that with rapid industrialization, the production and dumping of waste containing heavy metals have increased in Bangladesh. Among those, the textile and tannery industries are considered as the major contributors to heavy metal pollution in adjacent water sources, including rivers and canals (Rahman *et al.* 2012, Islam *et al.* 2018). Cereals and grains grown with these waters can be direct sources of these pollutants when they are used for feed production (Ullah *et al.* 2022). Animal proteins such as meat and milk can also be contaminated when raised on those crops. Moreover, as a less expensive protein source, heavy metal-contaminated animal residue and tannery waste may also be used to prepare fish feeds and poultry feeds (Anhwange *et al.* 2012, Nazmul Haque et al., 2019, Hossain *et al.* 2007). Poultry stools are also polluted by the ingestion of heavy metals in poultry feeds (Rahman *et al.* 2022, Nnaji *et al.* 2011). Thus, both commercial feeds and poultry stools can transfer heavy metals to cultivated fishes and ultimately to human consumers after ingestion.

Gopalganj, Madaripur and Khulna are three major cities in the southwestern region of Bangladesh. However, unfortunately, studies concerning heavy metal contamination levels in commercially farmed fishes and their feed in this region are lacking. Due to the hazardous impact of heavy metals, this research investigated the heavy metal concentrations (Pb and Cr) in different economically important fish species cultivated in these three districts and their respective fish feeds (poultry stool in the case of integrated farming or commercial fish feeds) to determine the level and source of heavy metal contaminants.

We also wanted to evaluate the possible harmful health effects of these heavy metals when they are included in the diet of the human population. Therefore, we assessed the potential noncarcinogenic and carcinogenic health hazards associated with the ingestion of these heavy metal contaminated fishes. We believe that these data will provide valuable information for monitoring authorities and policymakers to make sophisticated plans that can ensure food safety in Bangladesh.

Despite the different economic and ecological benefits of integrated farming in Bangladesh, there are some demoralizing reports on this topic due to heavy metal contamination (Nnaji *et al.* 2011). Poultry litter has been shown to contain high levels of trace element contamination (Jackson *et al.* 2003). When poultry stool is used as feed for fish, these hazardous substances can be incorporated into humans after ingestion. Therefore, in this research, we also compared the heavy metal contamination levels in poultry stool samples with those in commercial feed samples. These data may be useful for ensuring the safety of integrated poultry–fish farming in Bangladesh from the perspective of heavy metal toxicity.

Materials and methods

Collection of fish, poultry waste, and feed samples

In this study, ten farms from each of the districts of Gopalganj, Madaripur, and Khulna located in the southwest region of Bangladesh (Figure 1) were selected, where *Oreochromis niloticus* (Tilapia), *Anabas testudineus* (Koi), *Pangasius hypophthalmus* (Pangas), or *Clarias batrachus* (Magur) fish were commercially farmed. Among these, farms from Gopalganj and Madaripur used poultry stool as their feed source, whereas farms from Khulna utilized commercial fish feeds available on the local market. Therefore, a total of thirty fish samples were collected from thirty different firms from May 2022 to June 2022. All the fish samples were obtained from the commercial farm owners during harvesting. All samples were packed in iceboxes and delivered on the same day to the laboratory.

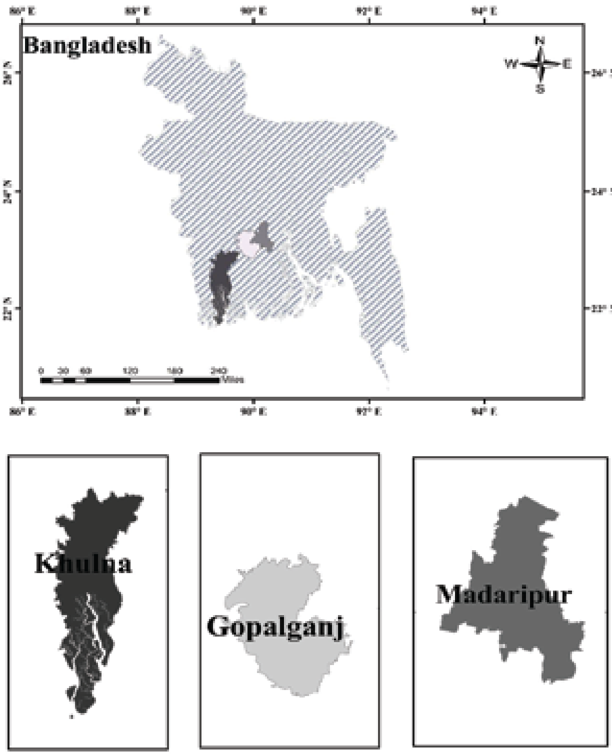

Fig. 1. Three South-West districts (Gopalganj, Madaripur, and Khulna) in Bangladesh selected for the study (ArcMap 10.8.2)

The fish samples were labeled (ID) G01-10 for Gopalganj, M01-10 for Madaripur, and K01-10 for the Khulna samples. In addition to the 30 fish samples from those farms, respective poultry stool feed (labeled ID+fw) and commercial fish feed samples (labeled ID+fc) were also collected.

Preparation of samples

All of the samples were brought to room temperature upon arrival at the laboratory. Using a stainless steel knife that had been steam-cleaned, the edible portions (fleshes) of the fish samples were removed following a thorough washing in distilled water. The flesh samples were subsequently chopped into little bits, and 5 gm of each sample was blended to make a paste. Similarly, 5 gm of collected fish feed or poultry waste samples were taken in a blender and homogenized to a powder or paste. All the samples were either immediately acid digested or stored in a freezer at -20°C until digestion.

Digestion of samples and heavy metal extraction

All the samples were brought to room temperature, placed in separate ceramic crucibles and acid digested to determine their heavy metal concentrations following the methods of Hadiani *et al.* (2014). After adding five milliliters of concentrated HNO_3 (MERCK 70% v/v), the samples were digested on a hot air plate within a fume hood. Following digestion, the samples were heated to 600°C for five or six hours in a muffle furnace to produce ash. After adding 10 milliliters of HCl (MERCK 70% v/v) to the ash, the sample was once more digested inside the fume hood by boiling it on a hot plate. The samples turned translucent or colorless after boiling. The extracts were then passed through a Whatman No. 1 filter paper, volume up to 100 ml with deionized water inside the fume hood and stored in a refrigerator for analysis via an atomic absorption spectrophotometer (AAS).

Analysis of heavy metal concentrations

The digested samples were examined using a flame AAS (HITACHI-JR262IPSA) with air serving as the oxidant and argon gas serving as the fuel. The calibration curves generated from standard solutions were used to calculate the amounts of heavy metals.

Analysis of the health risk assessment

Estimated daily intake (EDI) of heavy metals

The EDI was calculated as follows (Maurya *et al.* 2019):

$$EDI=\sum FIR \times C/BW \times 10^{-3}$$

Here FIR is the daily fish ingestion rate for local fish-consuming people (assumed to be 60.58 g), C is the estimated heavy metal concentration in the fish samples (mg/kg wet weight), and BW (60 kg) is the average body weight of individuals (Lipy *et al.* 2021; BBS, 2016).

Target hazard quotient (THQ)

In our study, the health hazards associated with the ingestion of fish by consumers were estimated as follows (USEPA, 2011, Javed and Usmani, 2016):

$$THQ = (EFr \times ED \;\; FIR \times C) \times 10^{-3}/(RfD \times BW \times AT)$$

Here, THQ stands for target hazard quotient for a particular heavy metal; EFr stands for exposure frequency (365 times per year); ED for exposure duration during the average human lifetime (considering 70 years); FIR for food intake rate (62.58 g/person/day); RfD for oral reference dose (mg/kg wet weight) of that metal (RfD = 0.003 for Cr and 0.004 for Pb); BW for average healthy person body weight (about 60 kg); and AT for average time for heavy metals (taking into account 365 days per year for 70 years) (Islam *et al.* 2014; Islam *et al.* 2015) (Lipy *et al.* 2021).

Hazard Index (HI)

The hazard index reflects the overall THQ value when an individual is exposed to more than one or occasionally a variety of toxicants. It is calculated by adding up the individual target hazard quotients (THQ) for each toxicant as follows (USEPA, 2011; Lipy *et al.* 2021):

$$HI = \Sigma THQ = THQ\ (Pb) + THQ\ (Cr)$$

Carcinogenic risk (CR)

This study estimated the carcinogenic risk (CR), which is the incremental probability of an individual developing cancer over a lifetime (70 years) due to daily exposure to various potential carcinogens due to consumption of heavy metal-contaminated fishes. The CR was estimated using the following equation (Saher and Kanwal, 2019; USEPA, 2019):

$$CR = \Sigma\ (CSF \times EDI)$$

Here CSF (cancer slope factor) for Cr and Pb were estimated to be 0.5 and 0.0085 $mgkg^{-1}d^{-1}$, respectively (Atique Ullah *et al.* 2019; Saher and Kanwal, 2019; WHO/FAO/IAEA, 1996). Generally, CR levels between 10^{-6} (the chance of getting cancer in a person's lifetime is 1 in 1,000,000) and 10^{-4} (the chance of getting cancer in a person's lifetime is 1 in 10,000) are acceptable, whereas CR levels more than 10^{-4} are deemed unacceptable (Atique Ullah *et al.* 2019).

Statistical analysis

The data were processed via IBM SPSS Statistics 25.0, and graphs were constructed by using GraphPad Prism 6. Due to the small sample size, Spearman's correlation analysis was used to analyze the correlation between the contamination levels of heavy metals in fish feeds and fishes. The Mann-Whitney test was used to assess significant differences

Table I. Heavy metal concentration, Estimated daily intake (EDI), and noncarcinogenic risk assessment (THQ and HI) of metals from consumption of fish species collected from Gopalganj

Fish	Pb conc. (mg/kg)	Cr conc. (mg/kg)	EDI_{Pb}	EDI_{Cr}	THQ_{Pb}	THQ_{Cr}	$HI = \Sigma THQ = THQ_{Pb} + THQ_{Cr}$
G01	0	0.34	0	3.55E-	0.00	0.12	0.12
G02	0.75	0.41	7.82E-04	4.28E-04	0.20	0.14	0.34
G03	0	0.69	0	7.20E-04	0.00	0.24	0.24
G04	0.88	0.24	9.18E-04	2.50E-04	0.23	0.08	0.31
G05	0	0.10	0	1.04E-04	0.00	0.03	0.03
G06	0	0.17	0	1.77E-04	0.00	0.06	0.06
G07	1.8	0.38	1.88E-03	3.96E-04	0.47	0.13	0.60
G08	0.8	0.30	8.34E-04	3.13E-04	0.21	0.10	0.31
G09	0	0.27	0	2.82E-04	0.00	0.09	0.09
G10	0	0.50	0	5.22E-04	0.00	0.17	0.17
Mean	0.42	0.34	4.41E-04	3.55E-04	0.11	0.12	0.23

in the concentrations of heavy metals between poultry stool samples and commercial fish feed samples.

Results and discussion

According to the FAO and EC guidelines, the safety limit for Pb in fish is 0.2 mg/kg, whereas the safety limit for Cr in fish is 1.0 mg/kg (EC, 2001; FAO, 1983; Saha *et al.* 2021; Islam *et al.* 2016).

Lead (Pb) in fishes

In Gopalganj, four out of the ten fish muscle samples (40%) crossed the safe limit of Pb. The highest concentration of Pb (1.8 mg/kg wet weight) was found in G07 (Table I). For eight (80%) of the fish samples collected from Madaripur, the Pb concentration exceeded the safety limit. The highest level of Pb contamination (3.80 mg/kg wet weight) was detected in M09 (Table II). On the other hand, four out of the ten fish samples (40%) collected from the Khulna district that were fed commercial fish feed exceeded the safety limit of Pb. The highest level of Pb contamination was observed in K02 (6.79 mg/kg) (Table III).

Lead (Pb) in fish feeds

In Gopalganj and Madaripur, where poultry stools were fed as feed, the Pb concentration in the poultry stools ranged from 0.0-9.78 mg/kg, with a mean of 2.424 mg/kg. In Khulna, where commercial fish feeds were used, the Pb concentration in the feeds ranged from 0.0-37.98 mg/kg, with a mean of 7.095 mg/kg (Figure 2).

Chromium (Cr) in fishes

None of the thirty (0%) fish samples collected from Gopalganj, Madaripur or Khulna surpassed the safety limit of Cr (1.00 mg/kg). In all three districts, the Cr concentration ranged from 0.10 mg/kg to 0.69 mg/kg (Figure 3).

Chromium (Cr) in fish feeds

In Gopalganj and Madaripur, where poultry stools were fed as feed, the Cr concentration in poultry waste ranged from 0.34-10.61 mg/kg, with a mean of 1.739 mg/kg. In Khulna, where commercial fish feeds were used, the Cr concentration in the feeds ranged from 0.52-52.5 mg/kg, with a mean of 9.084 mg/kg (Figure 3).

Table II. Heavy metal concentration, Estimated daily intake (EDI), and noncarcinogenic risk assessment (THQ and HI) of metals from consumption of fish species collected from Madaripur

Fish	Pb conc. (mg/kg)	Cr conc. (mg/kg)	EDI_{Pb}	EDI_{Cr}	THQ_{Pb}	THQ_{Cr}	$HI = \Sigma THQ = THQ_{Pb} + THQ_{Cr}$
M01	0.31	0.34	3.23E-04	3.55E-04	0.08	0.12	0.20
M02	0.77	0.10	8.03E-04	1.04E-04	0.20	0.03	0.24
M03	0	0.24	0	2.50E-04	0.00	0.08	0.08
M04	0.85	0.69	8.87E-04	7.20E-04	0.22	0.24	0.46
M05	0	0.26	0	2.71E-04	0.00	0.09	0.09
M06	1.8	0.34	1.88E-03	3.55E-04	0.47	0.12	0.59
M07	0.91	0.52	9.49E-04	5.42E-04	0.24	0.18	0.42
M08	0.71	0.38	7.41E-04	3.96E-04	0.19	0.13	0.32
M09	3.8	0.17	3.96E-03	1.77E-04	0.99	0.06	1.05
M10	1.8	0.30	1.88E-03	3.13E-04	0.47	0.10	0.57
Mean	1.10	0.33	1.14E-03	3.48E-04	0.29	0.12	0.40

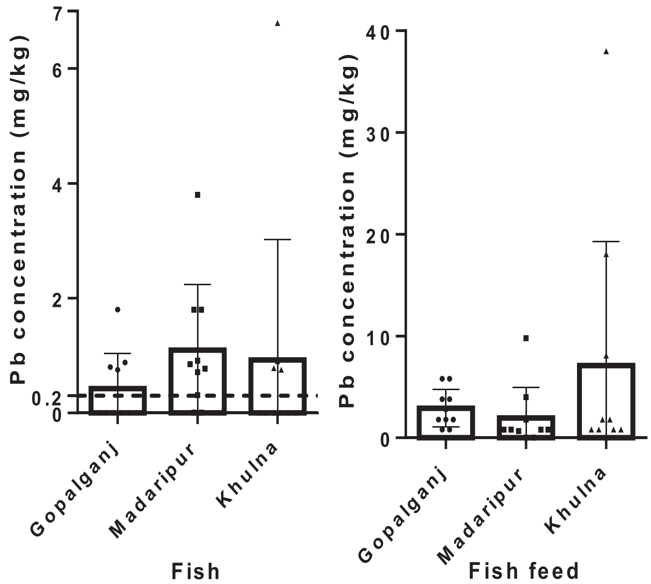

Fig. 2. Mean concentration ± SD of Pb in fishes and their respective feeds collected from the three districts (Gopalganj, Madaripur, and Khulna); the safety level of Pb in fishes is denoted by a dotted line

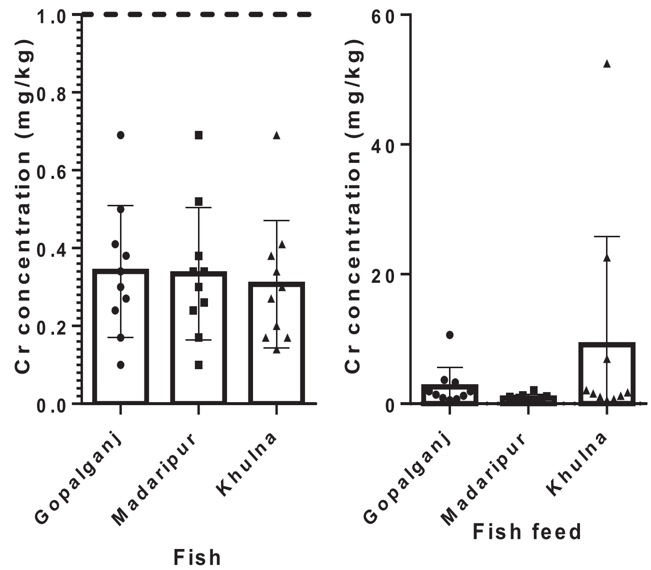

Fig. 3. Mean concentration ± SD of Cr in fishes and their respective feeds collected from the three districts (Gopalganj, Madaripur, and Khulna); the safety level of Cr in fishes is denoted by a dotted line

Correlations between heavy metal concentrations in fish muscles and in their respective feed samples

To determine whether the fish bioaccumulated heavy metals from their feed samples, Spearman's correlation analysis was performed. We found a significant correlation between the Pb concentrations analyzed in 30 fish muscle samples and those in their respective feed samples ($r = 0.583^{***}$, $p < 0.001$). Like for Pb, there was also a significant correlation ($r = 0.6113^{***}$, $p < 0.001$) between the Cr concentrations found in the fish samples and their respective feeds analyzed in the three districts.

Comparison of heavy metal contamination levels between poultry stool samples and commercial feed samples

To determine whether the heavy metal contamination levels in the poultry stool (feed) samples were different from those in the commercial feed samples, the Mann–Whitney test was performed between the 20 poultry stool samples collected from Gopalgonj and Madaripur, with the 10 commercial feed samples collected from Khulna. We found no significant differences in the concentrations of Pb or Cr between poultry stool and commercial feed samples for either Pb ($p=0.7993$) or Cr ($p=0.1389$).

Dietary Intake of Fishes and Risk of Heavy Metal Toxicity

Noncarcinogenic health risks (THQ and HI)

The target hazard quotient (THQ) is defined as the ratio of the possible exposure to a chemical to the threshold at which no negative effects are anticipated. Exposure may not have any potentially harmful noncarcinogenic health consequences if the estimated THQ is less than 1. Unfavorable non-carcinogenic health consequences are anticipated if the HI and THQ values are higher than 1 (USEPA, 2011).

Although the noncarcinogenic risks (THQ and HI) calculated from the average Pb and Cr concentrations in all of those districts were lower than the safety limit (1.0) set by the USEPA (USEPA, 2011), for two individual samples (M09 and K02), the THQ and HI values were greater than 1 (Tables I, II and III).

Carcinogenic health risk (CR)

We calculated the CR values from the average concentrations of Pb and Cr found in the fish samples from Gopalganj, Madaripur and Khulna, the values of which are listed in Table IV. Although the carcinogenic risk of Pb in all

Table III. Heavy metal concentration, Estimated daily intake (EDI), and noncarcinogenic risk assessment (THQ and HI) of metals from consumption of fish species collected from Khulna

Fish ID	Pb conc. (mg/kg)	Cr conc. (mg/kg)	EDI_{Pb}	EDI_{Cr}	THQ_{Pb}	THQ_{Cr}	$HI = \Sigma THQ = THQ_{Pb} + THQ_{Cr}$
K01	0	0.30	0	3.13E-04	0.00	0.10	0.10
K02	6.79	0.34	7.08E-03	3.55E-04	1.77	0.12	1.89
K03	0.75	0.17	7.82E-04	1.77E-04	0.20	0.06	0.25
K04	0	0.38	0	3.96E-04	0.00	0.13	0.13
K05	0	0.41	0	4.28E-04	0.00	0.14	0.14
K06	0.9	0.17	9.39E-04	1.77E-04	0.23	0.06	0.29
K07	0	0.69	0	7.20E-04	0.00	0.24	0.24
K08	0	0.20	0	2.09E-04	0.00	0.07	0.07
K09	0	0.27	0	2.82E-04	0.00	0.09	0.09
K10	0.78	0.14	8.14E-04	1.46E-04	0.20	0.05	0.25
Mean	0.92	0.31	9.62E-04	3.20E-04	0.24	0.11	0.35

Table IV. Average concentrations of heavy metals, their Estimated daily intake (EDI) and respective carcinogenic risk due to consumption of fishes from three districts

Location	Average Pb conc. (mg/kg)	Average Cr conc. (mg/kg)	EDI_{Pb}	EDI_{Cr}	CR_{Pb}	CR_{Cr}	CR
Gopalganj	0.42	0.34	4.41E-04	3.55E-04	3.75E-06	1.77E-04	1.81E-04
Madaripur	1.1	0.33	1.14E-03	3.48E-04	9.71E-06	1.74E-04	1.84E-04
Khulna	0.92	0.31	9.62E-04	3.20E-04	8.17E-06	1.60E-04	1.68E-04

three districts was within the acceptable range ($10^{-6} < CR < 10^{-4}$), that of Cr was found to be at an unacceptable level ($CR > 10^{-4}$) in all three districts.

Heavy metals (e.g., Pb, As, Cd, and Cr) are generally trace elements that have widespread environmental distributions and are also common environmental pollutants. The consumption of these heavy metals through the diet (such as fish) has several acute and chronic effects on human health. Dysfunction of the gastrointestinal and excretory systems, nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects, and cancer are examples of complications resulting from the toxicity of heavy metals (Balali-Mood *et al.* 2021).

In this study, we determined the level of heavy metal (Pb and Cr) contamination in the muscles of several commercially farmed fishes collected from three districts (Gopalganj, Madaripur, and Khulna) of Bangladesh and their respective consumed feeds to determine the source of heavy metal contamination. For this purpose, we collected fish samples from ten different farms in each of the districts of Gopalganj, Madaripur and Khulna. Among these, farms from Gopalganj and Madaripur utilized poultry waste (stool) as the sole source of food, whereas in Khulna, fishes were cultured with commercially available fish feeds. The concentrations of heavy metals, Pb and Cr in fishes (edible muscle portion) and their respective feeds were analyzed by using a flame AAS.

For fish muscle samples, out of thirty, in sixteen samples (53%), the Pb concentration exceeded the safe concentration set by both the FAO and EC directives, whereas none of the fish samples surpassed the safe concentration (1.0 mg/kg) of Cr. A comparison of the Pb and Cr concentrations in the fish muscle samples with those in their respective feed samples revealed a positive correlation (Spearman correlation coefficient= 0.583 for Pb and 0.6113 for Cr), suggesting that the fishes may have bioaccumulated these heavy metals from their diet.

Although health risk analyses of the mean Pb and Cr concentrations in fish muscles revealed no noncarcinogenic (THQ and HI) risk, health risk analyses of individual samples indicated potential noncarcinogenic (THQ and HI) health risks (e.g., kidney disease) due to exposure to two fish samples, K02 and M09 (HI > 1). Interestingly, the feed samples for these two samples also showed high levels of Pb contamination (37.98 mg/kg for K02 and 9.78 mg/kg for M09), indicating possible bioaccumulation of Pb from the feed.

The carcinogenic risk calculated from the average Pb concentration found in fishes from these three districts ranged from 3.72E-06 to 9.75E-06, which was within the acceptable range. However, the CR for Cr ranged from 1.62E-04 to 1.77E-04, which is unacceptable and indicates that vulnerable consumers are at risk of developing cancer throughout their lifetime. A similar result was reported by Lipy *et al.* (2021), where although the CR for Pb was within the acceptable range, that for Cr was within the unacceptable range. (4.35E-04). On the other hand, the CR values for both Pb and Cr exceeded the acceptable limits reported in various other studies. (Islam *et al.* 2016; Rahman *et al.* 2022).

There are some discouraging reports against integrated farming in Bangladesh because of various factors, such as the presence of heavy metals, infectious bacteria, antibiotics, and drug-resistant bacteria in poultry stool (Islam *et al.* 2007; Nnaji *et al.* 2011; Tian *et al.* 2021). When poultry stool is used as feed for fish, these hazardous substances can be transferred to humans after ingestion. In addition, poultry stool can increase the biochemical oxygen demand (BOD) in fish farms, which creates serious conditions for fish to survive in water (Mahmud, 2023; Tian *et al.* 2021). Considering all the reports and factors that demotivate integrated farming in Bangladesh, we were interested in investigating whether the heavy metal contamination load was greater in poultry stools than in commercial feeds.

Mann–Whitney test analysis revealed that the heavy metal concentrations (Pb and Cr) in the poultry stool samples from Gopalganj and Madaripur were not significantly greater than those in the commercial feed samples from Khulna (p=0.7993 for Pb and p=0.1389 for Cr). Taken together, these findings indicate that despite different discouraging reports against integrated farming, at least from a heavy metal perspective, we did not find any additional health risks associated with integrated farming compared with the usage of commercially available fish feeds. In contrast, we found that some of the commercial feeds used in Khulna contained very high concentrations of Pb (maximum 37.98 mg/kg) and Cr (maximum 52.5 mg/kg) compared with poultry stool samples (Figures 2 and 3) from Gopaganj and Madaripur.

In conclusion, although our study revealed a moderate level of heavy metal contamination in commercially farmed fishes, it also suggested that indiscriminate production and usage of heavy metal-contaminated fish feeds can impose significant noncarcinogenic and carcinogenic health risks on the population in the long run. Our study also suggested that, compared with commercial feed-nurtured fish farms, integrated farming techniques do not impose any additional risk from the perspective of heavy metals.

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