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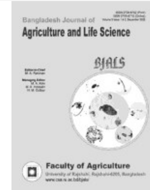

Heavy Metal Contamination in Feed-based Tilapia (*Oreochromis niloticus*) Culture in the Rajshahi Region

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ABSTRACT

As culture fish can easily be contaminated by different heavy metals through the polluted environment and contaminated feed, a study was conducted to investigate the heavy metal contamination in feed-based tilapia (*Oreochromis niloticus*) culture in the Rajshahi region. For this study, samples of sediment-soil and pond water, fish feed, and fish muscle were collected from nine farmers. The collected samples were prepared for metal analysis following the standard methods. The concentrations of Pb, Cd, Ni, and Cr in the samples were estimated through acidic digestion and an atomic absorption spectrophotometer. During this study, the concentrations of Pb, Cd, Ni, and Cr were found to be significantly higher in the feed and fish's muscle, followed by the sediment-soil and pond water. On the basis of the mean value, the samples were enriched with heavy metals in the order of Pb>Ni>Cr>Cd. The results of this study indicated that the heavy metals were hardly being accumulated in the fish's body from the sediment-soil and pond water but rather accumulated in large amounts by the fish feed. The study concluded that the muscle of the fish was found to contain heavy metals above the FAO/WHO/EC's standard limits, which may cause problems for consumer's health.

Introduction

Feed-based pond aquaculture, a common fish farming system in Bangladesh, has tremendous potential. However, this promising industry faces various problems, such as low-quality feed, contaminated feed ingredients, and pollution, especially heavy metal pollution. In aquatic ecosystems, heavy metals have received considerable attention due to their toxicity and accumulation in biota and fish, which leads to biomagnifications in the food chain.

The accumulation of heavy metals in the food chain may lead to an increased stock in biota, thereby magnifying the human dose (EC, 2003). Consumption of fish is one of the important routes through which heavy metals enter the food chain and finally into the human system (APHA, 1981). Fish cultured in contaminated waters take up heavy metals in large quantities enough to cause potential health risks to consumers (Copat et al. 2012).

Contamination of food sources by heavy metals, which are potentially toxic elements (PTEs), is a serious environmental and health

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concern because of the negative health effects of ingestion of PTE-contaminated foodstuffs. Due to their persistence in the environment, PTEs such as cadmium, chromium, nickel, and lead accumulate in the environment, get transferred into the food chains, and consequently lead to adverse health effects in consumers, especially in humans (Ali *et al.* 2019). Heavy metals like lead, cadmium, chromium, nickel, copper, arsenic, mercury, etc. exhibit toxic effects on aquatic organisms. Raised levels of lead in the water can cause generative damage in aquatic life and cause blood and nervous changes in fish and other animals (Kalay *et al.* 1999). Cadmium can damage the liver and affect the skeleton development of fish, whereas nickel affects the gill and kidney of fish (Hadeed *et al.* 2008). A decrease in the total erythrocyte, hemoglobin, glycogen, lipids, and protein content of fish is observed due to chromium contamination (Aslam and Yousafzai, 2017).

Fish can accumulate both essential and toxic metals that they absorb from contaminated sediments and water through their gills and skin, as well as from organisms that are consumed by the fish (Saha and Zaman, 2013). If there is heavy metal in sediment-soil and water in large amounts, fish get contaminated by that water. Again, one of the major sources of heavy metals in aquaculture is commercial feed, which provides various heavy metals. In fish farming, enough artificial feed has been used as a daily ration that contains toxic trace heavy metals (Anhwange *et al.* 2012). Thus, the aquaculture environment and fish can be contaminated by heavy metals through the contaminated feeds used for fish production.

Tilapia (*Oreochromis niloticus*) is an important culture species as it is an omnivore species with a higher growth rate and is easy to culture. It gets contaminated by heavy metals from various sources like sediment-soil, water, feeds, etc. However, a number of studies have been conducted on the heavy metal contamination of fish in different parts of the world (Abdel-Baki *et al.* 2011; Ahmed *et al.* 2019; Baharom and Ishak, 2015; Javed and Usmani, 2011; Kundu *et al.* 2017). But the studies on heavy metal contamination in feed-based tilapia culture in Bangladesh are scarce. Therefore, the study was conducted to assess the concentration of some toxic heavy metals in the environment, feed, and flesh of the tilapia culture in the Rajshahi region of Bangladesh.

Materials and methods

Sampling site

The triplicate samples of feed used by the farmers, sediment-soil and pond water and fish were collected from nine tilapia culture ponds of three farmers at the Chokdhadas under Puthia Upazilla in the Rajshahi district.

Sample collection, preparation and preservation

The collected feed samples were dried in an oven; after drying, the samples were grinded and homogenized, and then preserved at room temperature. The sediment samples from the study ponds were also collected randomly. The collected sediment-soils samples were dried in an oven, homogenized, and then preserved at room temperature. The water samples were collected randomly in plastic bottles, acidified immediately with 2 ml of HNO_3 per liter of water, and preserved at room temperature. The adult fish were collected from the selected study ponds. After washing, the muscles of the fish were taken with a knife and preserved at -4ºC. The preserved muscles were dried in an oven at 85ºC to a constant weight. After cooling, the dried muscle samples were grinded and pastelized to make powder and homogenized. The homogenized samples were finally stored in pre-cleaned dry plastic pots and preserved in the refrigerator for further analysis. For the quantitative analysis of heavy metals, the soil, feed, and fish muscle samples were digested.

Digestion of samples

The prepared samples were digested at the BCSIR Lab in Rajshahi. Digestion of soil, feed, and muscle samples was performed using an oxi-acidic mixture of $HNO_3/HClO_4$ (4:1, 10 ml for a 1.0 g sample) in a 100-mL beaker inside a hood. This mixture was heated up to 120˚C for 3 hours and brought to a volume of 25 mL with deionized water. Blank digestion was also carried out in the same way. For water, the volume of 25 ml also included distilled water and a final volume of 100 ml. 95 ml of each water sample was taken to 250 mL beaker, and added 3 ml of nitric acid and 2 ml hydrochloric acid (3:2). Then the sample was evaporated to near-dryness on a hot plate. Then the sample was cooled and added another 5 ml concentrated nitric acid. Samples were returned to hot plate and continued heating, adding additional acid as necessary until digestion was completed. This digestion decomposed organics. Then washed down the beaker walls with deionized water and filtered the sample into a 100 ml volumetric flask to remove silicate and other insoluble materials. Then each sample was made up to the mark with deionized water.

Determination of heavy metal content

The heavy metal contents were determined with a flame atomic absorption spectrophotometer (Model Shimadzu AA-7000) using acetylene gas as fuel and air as an oxidizer. Digested samples were aspirated into the fuel-rich air acetylene flame and the metal concentrations were determined from the calibration curves obtained from standard solutions. The technique is based on the principle of ground state metals absorbing light at specific wavelength and relies on Beer Lambert's law.

Beer's law: $A = abc$

[Where, "A" is the absorbance, "a" is the molar absorptive constant, "b" is the path-length of absorption and "c" is the concentration of the absorbing species]

Standard stock solutions of the targeted heavy metals were prepared by diluting each single element stock with deionized distilled water containing 1% (v/v) nitric acid. At each step of the measurement process, acid blanks were performed to ensure that the chemicals used were not contaminated with metals, and the measurements were corrected for the blanks. The actual concentration of each metal was calculated using the following formula:

Actual concentration of metal in sample = (μg/g) R× dilution factor

[Where, (μg/g)R = AAS Reading of digest and Dilution Factor = Volume of digest used ⁄ Wt of digested sample]

Data analysis

Statistical analysis of the collected data was carried out using Microsoft Excel 2020. The mean and standard deviation were carried out and presented in the table. After confirming the homogeneity of the data using Levene's test, a one-way ANOVA was performed to assess if there was any significant difference (P=0.05) for each particular heavy metal.

Results

Heavy metals in sediment-soil

In the sediment-soil of the study ponds, the mean concentrations of estimated heavy metals, viz., Pb, Cd, Ni, and Cr, were found to be 7.41±2.66, 0.45±0.19, 3.88±2.17, and 0.56±0.33μg/g, respectively. On the basis of the mean value, the sediment-soil samples were enriched with these metals in the order of Pb>Ni>Cr>Cd.

Table 1. Heavy metal concentrations in different samples

Samples	Heavy metal concentration (±SD, μg/g)			
	Lead	Cadmium	Nickel	Chromium
Sediment-soil	7.41 ± 2.66^{b} (5.33-10.41)	0.45 ± 0.19^{c} (0.28-0.66)	3.88 ± 2.17^{b} (2.39-6.37)	0.56 ± 0.33^{b} (0.21-0.87)
Pond water	1.34 ± 0.39^{c} (0.97-1.74)	0.30 ± 0.17^{c} (0.19-0.49)	1.14 ± 0.64^{c} (0.58-1.84)	0.25 ± 0.14^{c} (0.13-0.40)
Fish feed	11.53 ± 3.08^{a} (8.45-14.61)	1.13 ± 0.27^{a} (0.82-1.32)	7.76 ± 2.52^{a} (4.47-9.11)	5.05 ± 1.14^{a} (3.95-6.23)
Fish muscle	8.84 ± 2.74^{b} (6.02-11.49)	0.99 ± 0.35^{b} (0.59-1.24)	6.82 ± 2.36^{a} (4.37-9.08)	5.27 ± 2.14^{a} (3.43-7.61)

Values in the same column with different superscripts are significantly different (P=0.05).

Heavy metals in pond water

The water samples from the study ponds contained a significantly lower concentration of Pb, Cd, Ni, and Cr (Table 1). On the basis of the mean value, the pond water contained heavy metals in the order of Pb>Ni>Cr>Cd, and the mean values were 1.34 ± 0.39, 0.30 ± 0.17, 1.14 ± 0.64, and 0.25 ± 0.14, respectively (Table 1).

Heavy metals in fish feed

In the case of feed samples, the mean concentrations of Pb, Cd, Ni, and Cr were estimated as 11.53±3.08, 1.13±0.27, 7.76±2.52, and 5.05±1.14 µg/g, respectively (Table 1). On the basis of the mean value, the estimated metals in the feed samples were enriched in the order of Pb>Ni>Cr>Cd. From the analyzed data, it was observed that the mean concentration of the heavy metals was significantly higher in the feed than the sediment-soil and water samples of the study ponds.

Heavy metal in fish muscle

The estimated four heavy metals were also found to be significantly higher in the fish's muscle samples than the sediment-soil and water samples of the study ponds. The mean concentrations of these metals in fish's muscles were 8.84±2.74, 0.99±0.35, 6.82±2.36, and 5.27±2.14 µg/g, respectively (Table 1), and they were enriched in the order of Pb>Ni>Cr>Cd.

Discussion

Heavy metals in sediment-soil

Sediments are sources and sinks of contaminants and play an important role in mediating pollutants across environmental compartments of ecosystems. The mean concentration of Pb in the sediment-soil of the study ponds was 7.41±2.66 µg/g, which exceeded the standard levels of USEPA (2000), whereas the concentration of Cd (0.45±0.19) was lower than the standard value (0.487 mg/kg sediment) set by the EU (2003). The mean concentrations of Ni and Cr in the sediment-soil were 3.88±2.17 µg/g and 0.56±0.0.33µg/g, respectively (Table 1). Both metal contents in the sediment-soils of the study ponds were higher than the standard limits, according to the EU (2003) and WHO/FAO (2011). Moreover, the heavy metal contents in the sediment-soil were evaluated by the guidelines for sediment quality proposed by the CBSOG (2003). According to these criteria, it was observed that the concentrations of heavy metals were significantly present in the sediment of the study ponds.

Heavy metals in water

During the study period, the average Pb concentration in the pond water was 1.34±0.39 µg/ml. When the dissolved metal concentrations in the pond water were compared to international standards, the findings indicated that the Pb concentration in the pond water was higher than the USEPA (2000) and WHO (1993) guidelines. Furthermore, Pb levels in the urban pond water of Rajshahi varied from 0.14±0.12 to 4.92±1.66 mg/l, according to Flowra *et al.* (2014). Sultana *et al.* (2017) reported that Pb contents in the water of a fish culture pond in Mymensingh, Bangladesh, ranged from 0.039 to 0.066 mg/l, which is lower than the results of our investigation. The concentration of Cd in the pond water (0.30±0.17 µg/ml) was higher than the recommended level as stated by the WPCL (2004) and EPA (2002). According to TSE-266 (2005), WHO (1993), and WPCL (2004), the concentration of Ni (1.14±0.64 µg/g) in the water of the study ponds exceeded the allowable level (0.02 mg/l). The mean concentration of Cr in the water was found to be approximately 0.25±0.14 µg/ml, surpassing the polluted category (Cr>0.05 mg/L), as reported by WPCL (2004). Sultana *et al.* (2017) found 0.13–0.23 mg/L of Cr in the fish culture pond's water in Mymensingh, Bangladesh; this is more or less the same level of Cr found in our study. Moreover, our study's Cr level was higher than the EPA's (2002) standard permissible limit of 0.1 µg/ml.

Heavy metals in feed

Several investigations have demonstrated that varying concentrations of heavy metals are present in fish meal (Das et al. 2017; Kundu et al. 2017; Sabbir et al. 2018). The average Pb concentration (11.53 ± 3.08 µg/g) in the feed of our investigation exceeded the EU's recommended limits (2003). The levels of Pb in the fish feed examined in our study also exceeded the FAO's (1983) recommended permissible limit (2µg/g) for Pb in animal feed stuff. According to WHO (1995), animal feed ingredients may contain up to 10 mg/kg of Pb. According to Alexieva et al. (2007), the average Pb concentration in various animal feed samples was 4.77 mg/kg, which is less than what we found in our investigation.

The EU (2003) set a maximum allowable limit of 2 μg/g for Cd in fish feed, while the FAO (1983) suggested a permissible limit of 1.0 μg/g. The average levels of Cd in the feeds in the present study (1.13±0.27) were more or less in line with the EU and FAO's suggested values. According to Ikem and Egilla (2008), the fish feed has an average Cd concentration of 2.37 mg/kg, which is twice the allowable limit. The mean concentration of Ni in the fish feed was 7.76 ± 2.52 μg/g, while the mean concentration of Cr was 5.05 ± 1.14μg/g. Both metals in the fish feed were higher than the permissible limit proposed by the EU (2003). The limits for the allowed nickel content in different animal foods range between 0.1 and 8 mg/kg (Alexieva et al. 2007), which is higher than the levels found in our investigation. Compared to Ikem and Egilla's (2008) report, which stated the average concentration of Cr in fish feed is 1.42 mg/kg, the concentration of Cr in the feed samples from our study was higher.

The current investigation clearly showed that there was a comparatively higher level of heavy metal contamination in the feeds used for tilapia culture in the study areas. Higher levels of heavy metals in fish feeds that were above the WHO's or other accepted standards for food safety were also reported by Kundo et al. (2017). However, care should be taken when formulating fish diets with ingredients that are highly concentrated in heavy metals.

Heavy metals in fish muscle

Fish can absorb heavy metals orally through food and water, through their skin, or through their gills (Kahtani, 2009). Fish accumulate heavy metals depending on species and aquatic habitat (Canli and Atli, 2003). The heavy metals are transported through the bloodstream of the fish and are then either excreted, transformed, or stored (Ozturk *et al.* 2009). The fish bodies may contain more heavy metals due to the biomagnification process in different organs or tissues. From the results, it was observed that fish muscle had an average Pb concentration of 8.84±2.74μg/g, ranging from 6.02–11.19μg/g, which exceeded the WHO (2000) guideline value of 2.0μg/g for fish and fishery products. The Pb level found in fish muscle was also exceeding the permissible limit (0.5 mg/kg) set by FAO (1983) standards, the Turkish Food Codex (2008), and the European Commission (2006) detection limit (0.30 mg/kg). The current study's findings regarding the concentration of Cd (0.99 ± 0.35) in the fish muscle exceeded the limit set by the EU (2003), which suggested a threshold value of 0.05 μg/g for this metal in the fish muscle. Furthermore, the WHO (1992) established a threshold value of 1.0 μg/g for this metal in the fish muscle, which was not higher than the amount found in our study. The average Ni content in the fish muscle was 6.82±2.36μg/g, which was below the WHO/FAO (2011) and USFDA (1993) allowable limit of 10μg/g for food. Fish muscle had an average Cr content of 5.27±2.14 μg/g, which was above the FEPA (2003) allowed limit of 0.15 mg/kg.

However, from the results of the present study, it was assumed that the fish muscle did not absorb much heavy metal from the water, as less heavy metal was found in the water. Moreover, the concentration of heavy metals in fish feed was significantly higher, which indicated that fish might be more contaminated by the feed used for fish culture than the sediment and water of the study pond.

Conclusion

From the findings of the current study and FAO/WHO/EU-approved standard levels, and other related studies, it can be concluded that heavy metals were hardly being accumulated in the fish's body from the sediment-soil and water of the culture ponds but rather accumulated in large amounts by the fish feed. Additionally, the fish muscle contained heavy metals above the FAO/WHO/EU and other standard levels, which may cause problems in consumer's health. However, this study recommended that the monitoring and control of heavy metals in the commercial fish feed should be necessary. Moreover, further research should be done for assessing the heavy metals in fish feeds available in the markets and their accumulation in the fish's body. Such monitoring or assessment will be vital in preventing human health risks associated with heavy metal exposure through the consumption of metal-contaminated fish.

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Authors' contributions

Conceptualization, MTA and MMR; Methodology, MTA and MMR; Investigation, MTA; Write-up, MSA and MRS; Supervision, MMR. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interests.

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