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Bioaccumulation of heavy metals in different tissues of Nile tilapia (*Oreochromis niloticus*) in Bangladesh

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ABSTRACT

Introduction: The culture of Nile tilapia *(Oreochromis niloticus*) has become wide spread because of its high productivity over a short period of time. Its production partially fulfills the demand for food in rural people in Bangladesh. However, the accumulation of toxic heavy metals in the human body through consumption of fish contaminated by it causes various diseases. The aim of this study was to evaluate the bioaccumulation of five heavy metals, namely, cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) and copper (Cu) in cultured Nile tilapia in the Noakhali region of Bangladesh. **Methods:** Fish were collected from three different fish farms in the Noakhali region and samples of gill, muscles and liver of tilapia were assayed for Cd, Cr, Pb, Ni and Cu using atomic absorption spectroscopy. Proximate composition of the tilapia was also determined. **Results**: Metal accumulation in different tissues was as follows: liver > gill > muscle. The accumulation of metals in the muscle, gill and liver was Ni > Pb > Cr > Cu > Cd, Pb > Ni > Cu > Cr > Cd and Pb > Cu > Ni > Cr > Cd, respectively. The bioaccumulation of lead was significantly increased in liver and gill while muscle showed the lowest value. **Conclusion:** It can be concluded that bioaccumulation of Pb, Cr and Ni in Nile tilapia in this study exceeds the permissible limits set for heavy metals by Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA)-407. This is potentially risky for consumers.

Keywords: Bioaccumulation, heavy metal, gill, muscles, liver

INTRODUCTION

Malnutrition in Bangladesh remains a challenge. Fish is the largest source of animal food accounting for approximately 60% of animal protein intake, at 18.1kg consumed per person per year (Belton *et al.*, 2014). It contributes to a healthy diet that provides high value amino acids, nutrients and essential omega-3 fatty acids. The American Heart Association has recommended that fish should be eaten at least twice per week in order to reach the required daily intake of omega-3 fatty acids (Kris-Etherton,

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Harris & Appel, 2002). Fish meat is an important source of such nutrients as protein, unsaturated fatty acid and minerals (Zhao *et al.,* 2010).

Fishery is an important industry in Bangladesh. Fish is important from the aspect of national food security since it is estimated that 60% of animal protein is afforded through fish. Furthermore 11% of total population of the country obtained their livelihood through fisheries or related activities. Nearly 2% of national foreign currency earning is contributed by fisheries sector. Over the last five years, fish production has grown by 5.9% (DoF Bangladesh, 2014).

Nile tilapia (*Oreochromis niloticus*), is one of the most important fish species that is consumed and is very important in world fisheries (FAO, 2011). It is extensively cultured in Bangladesh to meet the protein demand from all walks of life because of its cost-effectiveness. Fish muscle contains 19.5g/100g protein, 2g/100g fat, 0.70g/100g vitamin B_{12}, 6.3g/100g vitamin D_3 and 0.40g/100g vitamin E, in the raw edible parts. Apart from proximate composition, Nile tilapia contains iron, zinc, calcium, iodine, selenium, phosphorus, magnesium, sodium, potassium, manganese, sulphur, copper (Cu) and at the levels of 1.1mg, 1.2mg, 95mg, 11g, 26g, 190mg, 26mg, 81mg, 280mg, 0.052mg, 240mg and 0.031mg per 100g raw edible part, respectively (Bogard *et al.*, 2015). Bangladesh is currently generating about 0.5 million metric tons (MT) of Nile tilapia and Pangus annually together from its fresh water and brackish water aquaculture system. According to the Yearbook of Fisheries Statistics of Bangladesh, there was a rapid development in Nile tilapia farming which showed an increase in annual production in this country from 2,140 MT to 370,017 MT during the period 1999 to 2016-2017 (FRSS, 2017). The production of Nile tilapia was 16.7% of total pond fish production in the year of 2016-2017.

Heavy metals are high-density non-biodegradable metals and metalloids with prolonged toxic effects, which, upon accumulation in the aquatic environment, are transferred to the aquatic biota through various pathways (Khalifa *et al.*, 2010). This ecotoxicity can impact changes on species diversity and the ecosystem (Türkmen *et al.*, 2009; Storelli *et al.*, 2005). Heavy metals eventually enter the food chain and their bioaccumulation and magnification can cause physiological and morphological alterations not only in aquatic animals but in human beings as well (Vinodhini & Narayanan, 2008). Bioaccumulation results in an increase in the concentration of a toxic xenobiotic substance in an organism with time that parallels the xenobiotic concentration in the environment. These substances can, in turn, have carcinogenic, cytotoxic and mutagenic effects on humans who consume these organisms (Rauf, Javed & Ubaidullah, 2009). Although fish is highly nutritious, its high consumption rate can have significant deleterious effects on human health because of accumulated toxic metals beyond permissible safety limits.

The production of this fish was 93,132 MT in the Noakhali region of Bangladesh, during the period 2016-17. The aim of the present study was to evaluate the level of contaminant heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) and Cu of Nile tilapia from three different fish farms of Noakhali region, at the three organ sites namely muscles, gill and liver, and, in addition, in the feed and sediments.

MATERIALS AND METHODS

Sample collection

Nile tilapia fingerlings were cultured in three different commercial farms in the

Noakhali region for one year from August 2015 to August 2016. The average pH, temperature, turbidity and dissolved oxygen in different ponds were 7.1, 30^0C, 117 Nephelometric Turbidity Units (NTU) and 4.7mg/L respectively. The fingerlings in each farm were fed once a day at a fixed feeding rate. In the first 20 days of the experiment, feeds were given at 10% of body weight, 5% in next 20 days and 1% for the remaining experimental days. The fish were sampled by following an appropriate sampling procedure. Eighteen samples of fish were randomly collected from August 23, 2015 to August 28, 2016 through cast-net and stored at -20^0C to prevent deterioration. After recording the wet body weight (150-200g) and total body length (22-25 cm), the samples were washed, preserved in ice boxes, and transported to the Bangladesh Council of Scientific and Industrial Research (BCSIR) for heavy metal analysis where they were stored in a freezer. Feed, sediment and water samples were also simultaneously collected in plastic containers from each farm. The physicochemical parameters of the farm water, the heavy metal content of the feed, sediment and water samples have been discussed in our previous paper (Das *et al.*, 2017).

Sample preparation

The frozen fish samples were thawed at room temperature and dissected using stainless steel scalpels. Muscle, the entire liver and gill from each sample were dissected for analysis. For the proximate components (protein, fat, moisture, ash), standard analytical methods as per the Association of Official Analytical Chemists (AOAC) were used. The determination of protein (block digestion - AOAC 981.10), fat (acid hydrolysis - AOAC 948.15), moisture (air drying – AOAC 950.46), ash (direct method – AOAC 920.153) were performed in triplicate.

The dissected samples were taken separately and kept in an oven at 70^0C for about 24 hours. The samples were then ground into a fine powder.

Digestion processes

Fish samples were digested according to the AOAC (1995) technique. A 0.5g dried powder of muscle, liver and gill from each sample were separately weighed in different glass beakers, to which was added 10ml of concentrated nitric acid and then covered with a watch glass. The samples were digested on a hot plate at 80-90°C. After 2 hours when solutions became almost transparent, the watch glasses were removed from beaker. The beakers were then removed from hot plate. When solution became cooler, 5ml perchloric acid was added after 5-15 minutes. All beakers were then placed on the hot plate under fume hood at 100-150°C temperature till the solution achieved a jelly-type steadiness. After complete digestion, the samples were cooled to room temperature and diluted to 25ml with distilled water. A blank solution was prepared in the similar way. Solutions were then filtered and collected into sterilized plastic bottles, before the analysis for heavy metals.

Heavy metal analysis

The concentrations of Cd, Cr, Cu, Pb and Ni were determined using an atomic absorption spectrophotometer (Simadzu: AA-7000). Standard solutions for calibration were prepared from commercially available chemicals. The heavy metal concentration in the unknown was determined by using following formula:

Actual concentration of heavy metal (mg/kg) in sample = Reading X Dilution Factor

where,
Reading = AAS reading of digest
Dilution Factor = volume of digest used / weight of digested sample

Bioaccumulation factor

The bioaccumulation factor (BAF) is an important parameter of environmental contamination. It is the extent to which from water pollutants get deposited into aquatic organisms such as fish. The BAF is the ratio of the pollutant concentration in the fish to that in water (Chiou, 2002). It was calculated as follows:

$$\text{BAF} = \frac{\begin{array}{c}\text{Concentration of heavy metals} \\ \text{in dry \% of fish muscle} \\ (\text{mg kg}^{-1})\end{array}}{\begin{array}{c}\text{Concentration of heavy metals} \\ \text{in water (mg kg}^{-1})\end{array}}$$

Statistical analyses

All samples were collected and analysed in duplicate. Data were analyzed using Statistical Package for the Social Sciences (SPSS) software (version 15) (IBM Corp., Armonk, NY). The t-test for duplicate paired samples (at 95% significance) was performed to determine if the individual results in each pair were statistically similar. Differences in metal concentrations in the samples of fishes studied were determined using analysis of variance (ANOVA). Significant level was determined at $p<0.05$. Other calculations and graphs were performed by Microsoft Excel 2010 and origin 8.5.

RESULTS

The proximate composition of Nile tilapia fish was as follows: protein 19.46g, fat 2.10g, moisture 77.02g and ash 1.20g per 100 g raw edible parts. The results of our present study were consistent with those of Bogard *et al.* (2015).

Metal contamination in Nile tilapia fish is an important issue as this fish is an important source of nutrients for poor as well as rich people. A previous paper (Das *et al.*, 2017) had identified heavy metal contamination in this fish. Before remedial measures could be taken, the identification of the route by which heavy metals enter into fish tissues needed to be done. Since the level of heavy metals was below detection limit in three different farmed waters, they had to come from either feed or sediment. The content of heavy metals (Cd, Cr, Pb, Ni and Cu) in feed, sediment and Nile tilapia that were collected from three different fish farms and the permissible limits set by the International Atomic Energy Agency (IAEA)-407 (Wyse, Azemard & Mora, 2003) are presented in Table 1.

Table 1 shows the concentrations of heavy metals in feed, sediment and fish. The highest concentration of metal in feed sample was Cu which was present at a concentration of 12.14±0.60mg/kg and the lowest was Cd. In sediment sample, order of metal concentrations was Ni (25.00±1.10 mg/kg) was present in the highest concentration while Cd was the lowest. The heavy metal present in the highest concentration in the fish was Pb (3.56±0.71 mg/kg) followed by Ni, Cu, Cr and Cd. By comparing concentration of heavy metals in the Nile tilapia samples with the tolerable

Table 1. Concentration (mg/kg) of heavy metal in various tissues of farmed tilapia, feed and sediment, with IAEA-407 values for comparison

Heavy metals	*Feed* †	*Sediment* †	*Fish* †	*IAEA-407*
Cd	0.25±0.02	0.28±0.05	0.34±0.04	0.18
Cr	5.99±1.06	8.09±0.77	1.67±0.19	0.73
Pb	1.29±0.22	7.07±0.46	3.56±0.71	0.12
Ni	2.00±0.48	25.43±1.10	2.59±0.62	0.60
Cu	12.14±0.60	23.67±1.90	2.13±0.28	3.28

†Values are shown as mean±standard errors, n=3

Table 2. Comparison of heavy metal content in various tissues of fish sample (mg/kg) from our study and from other studies reported in the literature (Rajeshkumar & Li, 2018).

Sample	*Description*		*Heavy metals*					*Reference*
			Pb	*Cr*	*Cd*	*Cu*	*Ni*	
1	Noakhali fish farm, Bangladesh	Muscle	2.26	1.99	0.25	1.58	2.39	Our study
		Gill	2.59	1.26	0.10	1.50	2.32	
		Liver	4.63	0.77	0.72	2.96	2.68	
2	Dhanmondi Lake, Bangladesh	Muscle	2.08	-	-	-	-	Begum *et al.*, 2005
3	Taihu lake fish sample, China	Muscle	0.61	0.34	0.12	0.21	-	Rajeshkumar & Li, 2018
		Gill	0.49	0.16	0.12	0.24	-	
		Liver	0.60	0.35	0.12	1.45	-	
4	Kolleru lake, Kerala, India	Muscle	1.84	11.00	0.11	-	-	Sekhar *et al.*, 2004
		Gill	2.98	19.00	0.22	-	-	
		Liver	3.77	30.00	0.37	-	-	

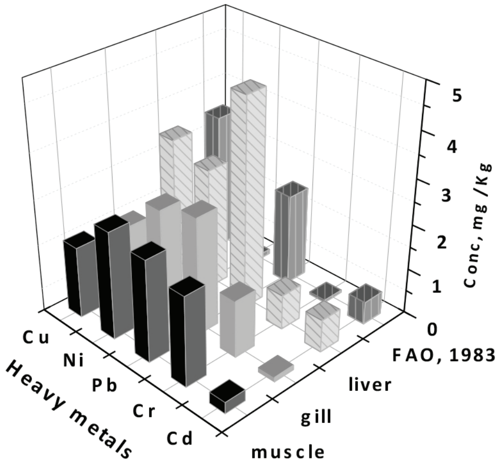

Figure 1. Concentrations of Cd, Cr, Pb, Ni and Cu in the different tissues of Nile tilapia compared with permissible values by FAO

values stated by IAEA-407, it was found that accumulated metals had reached beyond permissible levels for human consumption. The concentration of Pb showed a value that was very harmful to human health as well as other aquatic organisms also and thus is a matter of concern.

Table 2 depicts the comparison of Cd, Cu, Cr, Ni and Pb content in mg/kg in the muscle, gill and liver tissue of fish that were observed in this and other published studies by different authors. The concentrations of Cd, Cr, Pb, Ni and Cu with acceptable values by Food and Agriculture Organization (FAO) are presented in Figure 1. Lead is a toxic metal and its adverse health effects are well known. Of the different parts of Nile tilapia, the liver accumulated the highest Pb concentration at 4.63±0.54mg/kg, while muscle accumulated the lowest at 2.26±0.89mg/kg Pb. According to FAO (1983), the permissible level of Pb was 2mg/kg. The present study showed that the Pb levels in all the analyzed fish parts were well above the acceptable limit for human consumption. Concentrations of Cd in this study were low with the highest recorded value being 0.72±0.18mg/kg in liver followed by 0.25±0.03mg/kg in muscle and 0.10±0.04mg/kg in the gills. The Cd concentration in liver exceeded the permissible values by FAO. The presence of Cr in the diet is essential, due to its active involvement in metabolism. According to FAO, the recommended maximum permitted value of Cr is 0.05mg/kg. In this study, the muscle accumulated the highest amount (1.99±0.09mg/kg) of Cr and liver accumulated the lowest amount (0.77±0.17mg/kg), which are several fold higher than the acceptable value. Copper is an essential trace element. However, very high levels of Cu can cause acute toxicity. Concentration of Cu in muscle, gill and livers were 1.58±0.24mg/kg, 1.50±0.49mg/kg and 2.96±0.15mg/kg, respectively. The safe level for Cu that is recommended by the FAO is 3mg/kg, which is slightly higher than our determined values. The concentration of Ni in the environment is normally very low, but can cause a variety of adverse health effects. The FAO prescribed the permissible limit of Ni to be 0.1mg/kg. The maximum (2.68±0.52mg/kg) concentration of Ni was measured in the liver and the minimum concentration (2.32±1.53mg/kg) was found in the gill. Our values for Ni were higher than the safety levels established by the FAO. Overall, the heavy metal accumulation in the muscle, gill and liver was Ni > Pb > Cr > Cu > Cd, Pb > Ni > Cu > Cr > Cd and Pb > Cu > Ni > Cr > Cd, respectively.

The bioaccumulation factor, which is an indicator of aquatic pollution, was not from water to fish as heavy metal content in water was below detection limit.

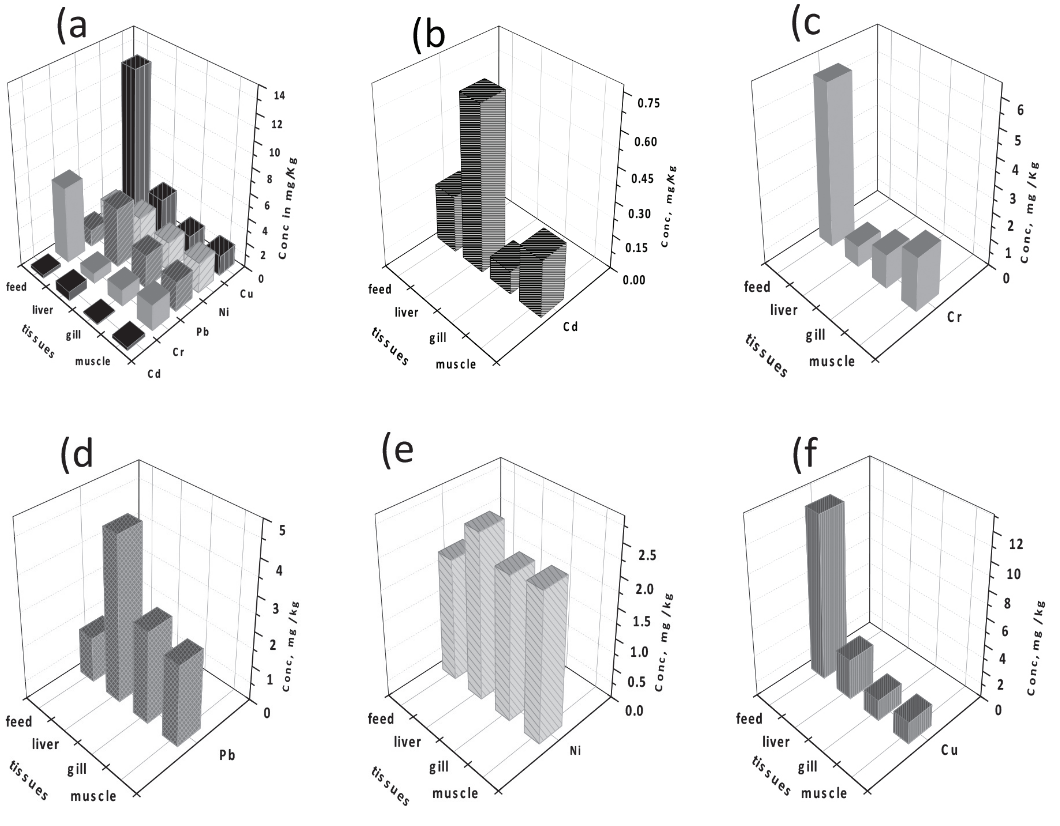

Figure 2. Bioaccumulation from feed to different tissues in Nile tilapia fish of (a) five selected heavy metals; (b) Cd; (c) Cr; (d) Pb; (e) Ni; and (f) Cu

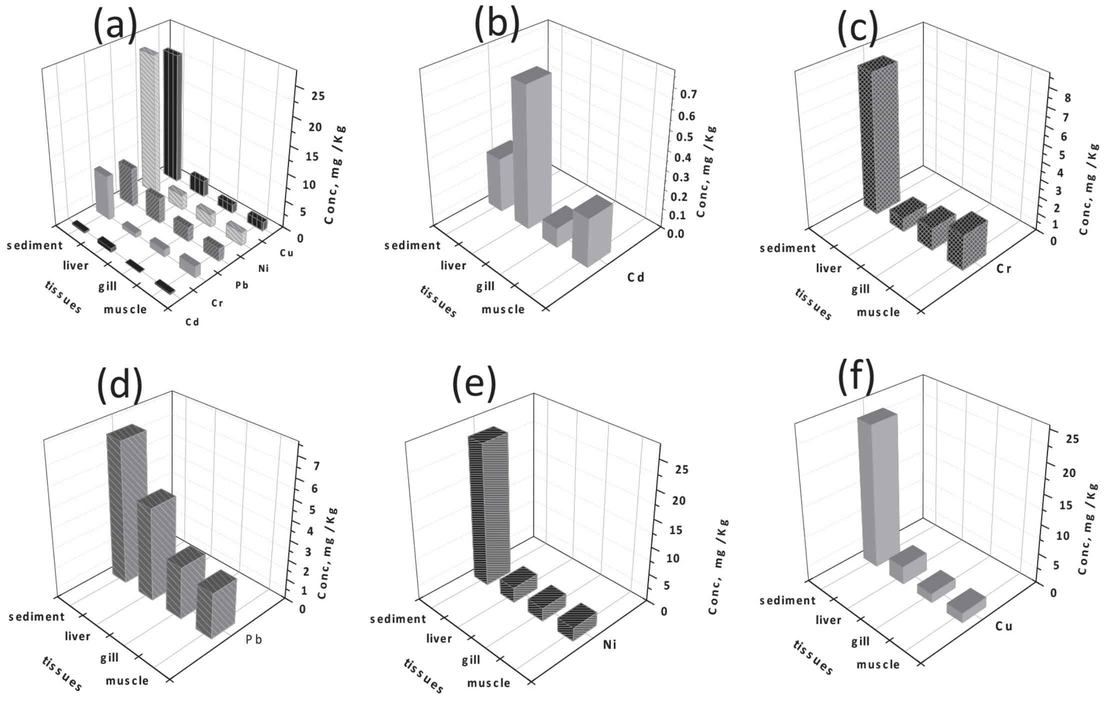

Figure 3. Bioaccumulation from sediment to different tissues in Nile tilapia fish of (a) five selected heavy metals, (b) Cd, (c) Cr, (d) Pb, (e) Ni and (f) Cu

There were significant differences in the muscle, gill and liver of the Nile tilapia as a result of the selective bioaccumulation by the tissues. Figure 2a shows the bioaccumulation of five selected trace metal from feed to different tissues of fish. As shown in Figure 2b, the liver accumulated the highest concentration of Cd followed by muscle and gill. Based on the distribution of heavy metals in various tissues of Nile tilapia, the sequence seems to be as follows: muscle > gill > liver (Figure 2c). Figure 2d shows the distribution of Pb in various tissues and it is in the following order liver > gill > muscle. Figure 2e shows the order of metal concentrations in the various tissues, which is as follows: liver > muscle > gill. In case of Cu distribution in different tissues, the sequence is shown in Figure 2f, which is liver > muscle > gill. In general, concentration of heavy metals is higher in feed than various tissues of fish. However, the scenario is reversed in case of Cd (Figure 2c), Pb (Figure 2d) and Ni (Figure 2e) where the concentration of the metal is higher in tissues than in feed. Thus, feed is not only source of heavy metals accumulation in fish. Another source of metal distribution in fish is sediment. Figure 3a shows the bioaccumulation of five selected trace metals from sediment to different tissues of fish. With the exception of Cd, the distribution of remaining four metals is several fold higher in sediment than in fish tissues. As shown in Figure 3b, the order of Cd distribution in various tissues is liver > muscle > gill. The concentration Cr in different tissues is liver > gill > muscle (Figure 3c). Based on the distribution of Pb in various tissues of fish, the sequence is shown in Figure

3d and the concentrations are liver > gill > muscle. Ni accumulation in fish tissue from sediment is shown in Figure 3e and it is liver > muscle > gill. As shown in figure 3f, liver accumulated the highest concentration of Cu followed by muscle and gill accumulated the lowest.

In general, the concentration of heavy metals was lower in the muscle tissues compared to other organs (liver, gill) of Nile tilapia.

DISCUSSION

The accumulation of heavy metals in fish occurs in many of its organs. Among the different organs, the liver accumulates higher concentrations of metals and has been used widely to investigate the process of bioaccumulation. Liver, kidney and gills were found to have the highest concentrations of heavy metals (Golovanova, 2008). The different organs in the body accumulate a specific metal to a high level though others do not accumulate the metal though present in the medium (Al-Kahtani, 2009). In present study, Pb accumulated in the order gill > liver > muscle. The gill is the one of sites which creates a path for the heavy metal to enter into the fish body (Bols *et al.,* 2001). Our results show that the liver accumulates highest level of metals followed by the gill and muscle. Bioaccumulation of heavy metal in muscles is lower. Fish liver and gills, both of which are metabolic active tissues, showed significantly higher abilities for the accumulation of all metals than the muscle. Similar results were also described by Jabeen, Javed & Azmat (2012). Yacoub (2007) reported this may be as the result of elevated metal–binding protein synthesized in gills and liver.

There are two reasons for lower metals accumulation in muscle: 1) The presence of a mucous layer coating the fish skin surface creates barrier that protects fish muscle tissue by forming complexes with the heavy metals that are present in surrounding environment (Uysal, 2008); 2) Jagakumar & Paul (2006) have stated that detoxification does not directly occur in the muscle. Hence, other tissues do not transport heavy metals to muscle.

Our results showed that the highest concentration of heavy metals are accumulated in the liver. This may be due to interaction of metals with the constituents of the target organ (Sorensen, 1991). As fish liver was seldom consumed, it was thus less of a threat to human health.

The presence of heavy metals in sediment and feed was found to be higher than in the different part of fish body. The Cu and Ni were in higher concentrations in both compared to the other three heavy metals. Besides their presence in fish feed, chemicals that are used near the fish culture pond for agricultural crops and vegetables also contain heavy metals that leach and contaminate them. The consequent accumulation in fish in turn then harms humans who consume them. In this study, Cd was abundant in fish feed and high levels of Cd were also detected in fish farm sediments. This finding is in agreement with that of a previous study by Kalantzi *et al.* (2013). The concentration of Cd in liver is higher than in feed and sediment. When fishes survive in high level of metal ions in aquatic environment, their tissues tend to take up these metal ions through various routes from their surroundings. In general, metals occur in very low concentrations (in nanogram to microgram per liter concentrations) amount in the natural aquatic ecosystems. Diet and water are two main pathways of metal accumulation in fish (Bury, Walker & Glover, 2003). The present study has shown that Nile tilapia accumulates metals to concentrations many times higher than that which is present in their surrounding water.

CONCLUSION

Fish is widely consumed as a main source of nutrition in many coastal communities like Noakhali, in Bangladesh. The unconscious consumption of metal contaminated fish may result in severe chronic and acute diseases. This consumption is the main route for human exposure to heavy metals compared to other routes such as inhalation and dermal contact, if the fish contain excessive amount of toxic metals. The pollution of the aquatic environment by heavy metals has become a worldwide problem in recent times. In this study undertaken in the Noakhali region, the order of heavy metal accumulation in the muscle, gill and liver was Ni > Pb > Cr > Cu > Cd, Pb > Ni > Cu > Cr > Cd and Pb > Cu > Ni > Cr > Cd, respectively. In case of feed and sediment it was Cu > Cr > Ni > Pb > Cd and Ni > Cu > Cr > Pb > Cd, respectively. Of all the heavy metals, the bioaccumulation of lead was significantly increased in gill and liver of Nile tilapia. This study determined the concentration of heavy metals (Cd, Cr, Pb, Ni and Cu) of Nile tilapia sediment and fish feed. The highest levels of all the metals in the present study were detected in sediment and followed by feed. As such, there was a high possibility of bioaccumulation through commercial fish feed and sediment. Among the fish tissues, higher levels were found in liver and gill while muscle showed the lowest value of metal accumulation.

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Authors' contributions

HMK, one of the principal investigator, conceptual and designed the study, prepared the draft of the manuscript and reviewed the manuscript; conducted the study, data analysis and interpretation, assisted in drafting of the manuscript and reviewed the manuscript; MMG, another principal investigator, conceptual and designed the study, prepared the draft of the manuscript and reviewed the manuscript; IS, led the data collection in the Noakhali region, and sample digestions and analysis, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript and reviewed the manuscript; PA, led the data collection in the Noakhali region and sample digestions and analysis; DSS, led the data collection in the Noakhali region and sample digestions and analysis and reviewed the manuscript; MJL, advised on the data analysis and interpretation and reviewed the manuscript; SPD, MM, and SB performed the heavy metals analyses using the atomic absorption spectrometer.

Conflict of interest

There is no conflict of interest.

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