# Heavy Metal Contamination in Feed based Catfish Culture in North-West Part of Bangladesh: A Consumer Health Concern

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#### Abstract:

Background: Heavy metal contamination in water is an increasing world-wide environmental concern. Heavy metals are capable of being bio-accumulated in high concentrations in fish tissues to cause potential health risks to the consumers. Culture fishes can easily be contaminated by different heavy metals through polluted culture environment and contaminated artificial feeds. Since studies on heavy metal contamination in feed based catfish culture are scare in Bangladesh, we have selected this study to evaluate the level of heavy metal contamination in feed based catfish (Pangasius sutchi, Clarias batrachus and Heteropneustes fossilis) culture for assessing the status of risks level for human consumption of these species.

Materials and Methods: For this study, nine catfish culture ponds (3 for P. sutchi, 3 C. batrachus and 3 for H. fossilis) were selected in different areas of Rajshahi region, Bangladesh. In this study, Pb, Ni, Cd, Cr and Zn contents in the fish feeds, sediment soil and water of the study ponds, and the accumulations of these metals in fish muscles were estimated through Atomic Absorption Spectrophotometer (AAS).

**Results:** The fish feeds contained relatively higher concentrations of Zn and Pb followed by Ni whereas Cr and Cd concentrations are lower. Pb and Ni contents in sediment soil and water of the study ponds are relatively higher than others metals (Cd, Cr and Zn). The raw and cooked muscles of the fish contained relatively higher concentrations of Pb than other metals (Ni, Cr, Cd and Zn). The traditional cooking method had little decreasing effects on heavy metal in catfish muscles.

**Conclusion:** This study stated that heavy metals were accumulated in fish muscles from the sediment soil and water, and also from the fish feed. This study indicated that Pb concentrations in the raw and cooked muscles of the catfish exceed the permissible limits (according to different standard guidelines) which may cause adverse health problems in consumers.

Key words: Feed based, catfish, heavy metal, accumulation, fish muscles, health risk

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# I. Introduction

The fish production in Bangladesh is increasing day by day but this industry faced pollution problems especially heavy metal pollution from various sources. The heavy metals are regarded as most toxic substances to humans, fishes and environment <sup>[1]</sup>. Heavy metal contamination in water is an increasing world-wide environmental concern <sup>[2]</sup>. The heavy metals are considered as critical contaminants of aquatic ecosystems due to their high potential to enter and accumulate in food chains <sup>[3]</sup>. Fish can accumulate toxic metals which they absorb from contaminated sediments and water and from organisms consumed by the fish <sup>[4]</sup>.

Heavy metals like lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), zinc (Zn) etc. may become toxic to fishes if their concentrations exceed certain levels. Heavy metals alter the physiological activities and biochemical parameters both in tissues and blood of fish <sup>[5]</sup>. Pb has significant toxic effects on fish <sup>[6]</sup> and it impairs both the respiratory and digestive systems and suppresses the immune system of fish. Ni contamination causes changes in fish behavior, respiratory manifestation and white discoloration of the skin <sup>[7]</sup>. Cr has adverse effects in fish at hematological level and mostly decline in the contents of glycogen, lipids and proteins <sup>[8]</sup>. In fish, cadmium can cause a number of structural and pathomorphological changes in various organs <sup>[9]</sup>. Zinc exhibits accumulation in the tissues and organs of freshwater fish <sup>[10]</sup> and may have negative effects in haematological and immune parameters of the fish <sup>[11]</sup>. Heavy metals are capable of being biomagnified in the aquatic food chain/web and bioaccumulated in fish tissues to the detriment of fish consumers. Fish production in contaminated water takes up heavy metals in large quantities enough to cause potential health risks to the consumers <sup>[12]</sup>.

However, the success of fish culture depends on a large extent to the use of suitable feeds. Scaling up of fish culture in Bangladesh has expanded the fish feed industry in recent years. Unfortunately, the sources of raw materials used for the manufacturing of feeds tend to be contaminated with heavy metals and finished feed contain large amount of heavy metals. In the fish farming, enough artificial feed has been used as daily ration that contain toxic trace heavy metals <sup>[13]</sup>. Thus, aquaculture environment and fish can be contaminated by heavy metals through the contaminated feeds used for fish production.

Catfishes (*Pangasius sutchi*, *Clarias batrachus* and *Heteropneustes fossilis*) are of commercially important culture species in Bangladesh as their higher growth rate and nutritional values. These fishes can easily be contaminated by different heavy metals through polluted culture environment and directly through taking contaminated feeds. For this reason, estimation of heavy metals in different sources and its accumulation in muscle of these fish is crucial to assess the risks level for consumer health. Although, a numbers of studies have been conducted on heavy metal contamination of fish in different parts of the world [14][15][16][17][18] but in Bangladesh, studies on heavy metal pollution in feed based catfish culture system are so scare. Therefore, the present study was conducted to estimate the level of heavy metals in feeds and culture environment, and the accumulation of heavy metals in the muscle of catfish cultured in Rajshahi regions of Bangladesh for assessing the risks status for human consumption of these species.

#### II. Materials and Methods

# 2.1 Sample collection site

The triplicate samples (Feeds, sediment soil, water and fish) were collected from 9 catfish culture ponds (3 for *Pangasius sutchi*, 3 for *Clarias batrachus* and 3 for *Heteropneustes fossilis*) located at the Parila, Kutibari and Majhigram in Rajshahi district of Bangladesh.

# 2.2 Sample collection, preparation and preservation

The collected feed samples were dried in oven, after drying, the samples were grinded and homogenized, and then preserved in room temperature. The sediment soil samples of the study ponds were also collected randomly. The collected sediment soils were dried in oven and homogenized and then preserved in room temperature. The water samples were collected randomly in plastic bottles and acidified immediately with 2 ml of HNO<sub>3</sub> per liter of water and preserved in room temperature. The adult catfish fish were collected from the study ponds. After washing, the muscles of the fish were taken with a knife and preserved at -4°C. After cooking (using traditional cooking method, gravy fried), the cooked muscles of the fish were also taken and preserved at -4°C. The preserved muscles (raw and cooked) were dried in an oven at 85°C to constant weight. After cooling, the dried muscle samples were grinded and pastel to make powder and homogenized. The homogenized samples were finally stored in pre-cleaned dry plastic pots and preserved in refrigerator for further analysis. For the quantitative analysis of heavy metal, all samples were digested.

# 2.3 Digestion of samples

The homogenized samples were digested in the Quality Control Lab in the Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. Sub-sample from each stock sample (sediment soil, feeds and muscle) were weighted (1 mg, dry. wt) and were burned using muffle furnace at 550°C within separate crucible. After 6 hours, samples were taken out from muffle furnace and were cooled to room temperature and then digested in acid cleaned jars with hot concentrated 1N HCl to obtain release of heavy metals. All organic materials in each sample were completely digested. The digests were allowed to cool, filtered through Millipore membrane filter, transferred to 25 ml volumetric flasks and made up to mark with 1% nitric acid and diluted with double distilled water to 25 ml. The digests were kept in plastic bottles. For digestion of water sample, added distilled water to the volume of 25 ml filtered water sample and final volume of 100 ml. 95 ml of each water sample was taken to 250 ml beaker and added 3 ml nitric acid and 2 ml hydrochloric acid (3:2).

#### 2.4 Determination of heavy metal content

The heavy metal contents were determined in the Central Lab, University of Rajshahi 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 analytical technique used to determine heavy metal levels in all samples was thermo element Solar S4 Atomic Absorption Spectroscopy. The technique is based on the principle of ground state metals absorbing light at specific wavelength and relies on Beer Lambert's law (Skoog, and West, 1982).

Beer's law: A = abc

[Where, "A" is the absorbance, "a" is the molar absorptivy 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 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 =  $(\mu g/g) R \times dilution$  factor [Where,  $(\mu g/g)R = AAS$  Reading of digest and Dilution Factor = Volume of digest used/Wt of digested sample]

#### 2.5 Statistical analysis

The means and standard deviations of the metal concentrations in the samples were calculated. Significance of the differences in mean concentrations of heavy metals among different samples for each metal were tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS 16.0. For all the tests, the significance was determined at the level of P<0.05.

### III. Results

#### 3.1 Heavy metal contents in fish feeds

The estimated heavy metal concentrations in feeds used for catfish culture are given in Table-1. The feeds contained relatively higher concentrations of Zn and Pb followed by Ni whereas Cr and Cd concentrations were lower. On the basis of mean value, the fish feeds were enriched with metals in following order: Zn>Pb>Ni>Cr>Cd.

Table-1: Heavy metal contents in feeds used for the catfish culture

Heavy metals content (ug/g, +SD, N=9)

Feed Sample	Heavy metals content (μg/g, ±SD, N=9)						
reed Sample	Pb	Ni	Cr	Cd	Zn		
FP	0.585±0.095 <sup>a</sup>	0.398±0.066 <sup>a</sup>	0.044±0.025 <sup>a</sup>	0.016±0.007 <sup>a</sup>	0.608±0.013 <sup>a</sup>		
FC	0.589±0.091 <sup>a</sup>	0.426±0.064 <sup>a</sup>	0.044±0.028 <sup>a</sup>	0.019±0.004 <sup>a</sup>	0.590±0.039 <sup>a</sup>		
FH	0.590±0.087 <sup>a</sup>	0.432±0.058 <sup>a</sup>	0.042±0.025 <sup>a</sup>	0.016±0.002 <sup>a</sup>	0.598±0.047 <sup>a</sup>		

<sup>\*</sup>FP= Feed used for P. sutchi, FC=Feed used for C. batrachus and FH= Feed used for H. fossilis. Values in the same column with same superscripts are not significantly different (P<0.05).

## 3.2 Heavy metal contents in sediment soil of the catfish ponds

Heavy metal contents in sediment soil of the catfish ponds are given in Table-2. From the estimated values, it was observed that sediment soil contained relatively higher concentrations of Pb and Ni followed by Zn whereas Cr and Cd concentrations were lower. On the basis of mean value, sediment soils of the catfish ponds were enriched with heavy metals in following order: Pb>Ni>Zn>Cr>Cd.

**Table-2:** Heavy metal contents in sediment soil of the catfish ponds

Sediment soil	Heavy metals content (μg/g, ±SD, N=9)					
sample	Pb	Ni	Cr	Cd	Zn	
SSPP	0.309±0.053 <sup>b</sup>	0.345±0.063 <sup>a</sup>	0.050±0.025 <sup>a</sup>	0.019±0.005 <sup>a</sup>	0.173±0.040 <sup>a</sup>	
SSPC	0.383±0.059 <sup>a</sup>	0.360±0.096 <sup>a</sup>	0.029±0.005 <sup>a</sup>	0.021±0.004 <sup>a</sup>	0.160±0.035 <sup>a</sup>	
SSPH	0.398±0.038 <sup>a</sup>	0.364±0.099 <sup>a</sup>	0.037±0.016 <sup>a</sup>	0.020±0.005 <sup>a</sup>	0.150±0.028 <sup>a</sup>	

<sup>\*</sup>SSPP= Sediment soil in the ponds of P. sutchi, SSPC= Sediment soil in the ponds of C. batrachus and SSPH= Sediment soil in the ponds of H. fossilis. Values in the same column with different superscripts are significantly different (P<0.05).

#### 3.3 Heavy metal contents in water of the catfish ponds

The estimated heavy metal concentrations in water of the catfish ponds are given in Table-3. It was observed that Pb and Ni concentrations in water were relatively higher whereas Cd, Cr and Zn concentrations were lower. On the basis of mean value, water samples of the catfish ponds were enriched with heavy metals in following order: Pb>Ni>Cr>Zn>Cd.

**Table-3:** Heavy metal contents in water of the catfish ponds

Water sample	Heavy metals content (μg/g, ±SD, N=9)						
	Pb	Ni	Cr	Cd	Zn		
WPP	0.410±0.027 <sup>b</sup>	0.333±0.032 <sup>a</sup>	0.058±0.011 a	0.017±0.005 <sup>a</sup>	0.046±0.011 <sup>a</sup>		
WPC	0.402±0.083 <sup>b</sup>	0.360±0.029 <sup>a</sup>	0.055±0.010 a	0.016±0.003 <sup>a</sup>	0.049±0.012 <sup>a</sup>		
WPH	0.506±0.067 <sup>a</sup>	0.305±0.037 <sup>b</sup>	0.053±0.007 a	0.019±0.007 <sup>a</sup>	0.047±0.016 <sup>a</sup>		

\*WPP= Water in the ponds of P. sutchi, WPC= Water in the ponds of C. batrachus and WPH= Water in the ponds of H. fossilis. Values in the same column with different superscripts are significantly different (P<0.05).

# 3.4 Heavy metal contents in raw muscles of the catfish

Heavy metal contents in raw muscle of the catfish species are given in Table-4. Pb concentrations in raw muscle of all catfishes were relatively higher followed by Ni and Zn whereas Cr and Cd concentration were lower. On the basis of mean value, raw muscles of the catfish were enriched with heavy metals in following order: Pb>Ni>Zn>Cr>Cd.

**Table-4:** Heavy metal contents in raw muscles of the catfish

Raw muscle	Heavy metals content (μg/g, ±SD, N=9)					
sample	Pb	Ni	Cr	Cd	Zn	
RMP	0.682±0.125 <sup>a</sup>	0.383±0.024 <sup>a</sup>	0.054±0.027 <sup>a</sup>	0.016±0.003 <sup>a</sup>	0.122±0.014 <sup>a</sup>	
RMC	0.625±0.114 <sup>a</sup>	0.355±0.019 <sup>b</sup>	0.043±0.007 <sup>b</sup>	0.016±0.004 <sup>a</sup>	0.134±0.018 <sup>a</sup>	
RMH	0.604±0.109 <sup>a</sup>	$0.386\pm0.026^{a}$	0.037±0.011 <sup>b</sup>	0.014±0.003 <sup>a</sup>	0.130±0.020 <sup>a</sup>	

<sup>\*</sup>RMP= Raw muscles of P. sutchi, RMC= Raw muscles of C. batrachus and RMH= Raw muscles of H. fossilis. Values in the same column with different superscripts are significantly different (P<0.05).

# 3.5 Heavy metals content in cooked muscles of the catfish

Heavy metal contents in cooked muscles of the catfish are given in Table-5. The cooked muscles of the fish contained relatively higher concentrations of Pb followed by Ni whereas Cr, Cd and Zn concentrations were relatively lower. On the basis of mean value, cooked muscles contained heavy metals in following order: Pb>Ni> Zn>Cr>Cd.

Table-5: Heavy metal contents in cooked muscles of the catfish

Raw muscle sample	Heavy metals content (μg/g, ±SD, N=9)						
	Pb	Ni	Cr	Cd	Zn		
CMP	0.666±0.118 <sup>a</sup>	0.369±0.102 <sup>a</sup>	0.048±0.024 <sup>a</sup>	0.015±0.004 <sup>a</sup>	0.078±0.011 <sup>a</sup>		
CMC	0.608±0.096 <sup>a</sup>	0.339±0.030 <sup>a</sup>	0.039±0.012 <sup>a</sup>	0.015±0.003 <sup>a</sup>	0.085±0.012 <sup>a</sup>		
CMH	0.587±0.125 <sup>a</sup>	0.367±0.093 <sup>a</sup>	0.034±0.019 <sup>a</sup>	0.013±0.005 <sup>a</sup>	0.066±0.019 <sup>a</sup>		

<sup>\*</sup>CMP = Cooked muscles of P. sutchi, CMC = Cooked muscles of C. batrachus and CMH = Cooked muscles of H. fossilis. Values in the same column with same superscripts are not significantly different (<math>P < 0.05).

# 3.6 Reduction of heavy metal due to cooking (Gravy fried)

Reduction of heavy metal concentrations from the raw muscles of catfish due to cooking is shown in Table-6. On the basis of % reduction due to cooking, the heavy metals decreased follow the order: Zn>Cr>Cd>Ni>Pb.

**Table-6:** Reduction of heavy metals from raw muscles due to cooking

Fish Species	Sample	Mean value of heavy metals (μg/g)						
	Sample	Pb	Ni	Cr	Cd	Zn		
P. sutchi	Raw muscles	0.682±0.125	0.383±0.024	0.054±0.027	0.016±0.003	0.122±0.014		
	Cooked muscles	0.666±0.118	0.369±0.102	0.048±0.024	0.015±0.004	0.078±0.011		
	% Reduction	2.35	3.66	11.11	6.25	36.07		
C. batrachus	Raw muscles	0.625±0.114	0.355±0.019	0.043±0.007	0.016±0.004	0.134±0.018		
	Cooked muscles	0.608±0.096	0.339±0.030	0.039±0.012	0.015±0.003	0.085±0.012		
	% Reduction	2.72	4.51	9.30	6.25	35.82		
H. fossilis	Raw muscles	0.604±0.109	0.386±0.026	0.037±0.011	0.014±0.003	0.130±0.020		
	Cooked muscles	0.587±0.125	0.367±0.093	0.034±0.019	0.013±0.005	0.066±0.019		
	% Reduction	2.81	4.92	8.11	7.14	49.23		

#### **IV. Discussion**

# 4.1 Heavy metals contents in the feeds

A number of studies indicate that fish feed contains different heavy metals in different levels  $^{[17]}$   $^{[20]}$   $^{[21]}$ . In the present study, the feeds used for catfish culture contained relatively higher concentrations of Pb (Range of mean value: 0.585-0.590µg/g) which are lower than the standard levels of European Union  $^{[22]}$ . Pb contents in the feed samples of our study were also lower than the guideline of FAO  $^{[23]}$  that proposed permissible limit of Pb in animal feed staff was 2µg/g. The permitted lead content in feed ingredients is 10 mg/kg  $^{[24]}$ . Alexieva *et al.*  $^{[25]}$  reported the average concentration of lead in different animal feed samples was 4.77 mg/kg which is higher than our study. The mean concentrations of Ni in the feeds used for *P. sutchi, C. batrachus* and *H. fossilis* were

 $0.398\pm0.066$ ,  $0.426\pm0.064$  and  $0.432\pm0.058$  µg/g, respectively whereas the Cr concentrations were  $0.044\pm0.025$ ,  $0.044\pm0.028$  and  $0.042\pm0.0025$  µg/g, respectively (Table-1). The concentrations of Ni and Cr in fish feed were lower than the permissible limit proposed by European Union <sup>[22]</sup>. The limits for the allowed nickel content in different animal foods range between 0.1 and 8 mg/kg <sup>[25]</sup> which is higher than our study. Ikem and Egilla <sup>[26]</sup> reported that the average concentration of Cr is 1.42 mg/kg in diet (dry wt.) of fish feed which is within the acceptable limit and higher than our result.

The maximum acceptable limit of Cd in fish feed is 2  $\mu g/g$  stipulated by European Union <sup>[22]</sup> whereas the permissible limit of Cd in fish feed proposed by FAO <sup>[23]</sup> is 1.0  $\mu g/g$ . The mean values of Cd contents in the feeds  $(0.016\pm0.007,\,0.019\pm0.004$  and  $0.016\pm0.002\,\mu g/g)$  were lower than the recommended guideline of EU and FAO. Ikem and Egilla <sup>[26]</sup> reported that the average concentration of Cd is 2.37 mg/kg in diet (dry wt.) of fish feed which is double than the acceptable limit. The mean concentrations of Zn in the feeds were less than 1.0  $\mu g/g$  but ranked first in the present study which is lower than the international acceptable limit. According to law and regulation relating to food in Sri Lanka, Bangladesh and Nepal, the permissible limit of Zn content in foodstuff is 100  $\mu g/g$ . EU <sup>[27]</sup> proposed total maximum contents are 150 mg Zn/kg in complete feed for salmonids and 100 mg Zn/kg in complete feed for all other species which is much higher than our study.

According to the present study, it is clear that heavy metals contamination in the feeds used for catfish culture in the study areas were relatively low. Shamshad *et al.* [28] reported most of the branded fish feed in Bangladesh are costly and mostly free from metal pollution whereas Kundo *et al.* [17] reported contrary report who observed higher concentrations of heavy metals in fish feeds that exceeded the WHO's or other standard limits for food safety. However, attention should be paid on the formulation of fish feeds with ingredients which contain high concentration of heavy metals should be avoid.

# 4.2 Heavy metal contents in sediment soil of catfish ponds

The Pb contents in the sediment soil samples  $(0.309\pm0.053, 0.383\pm0.059 \text{ and } 0.398\pm0.038 \text{ µg/g} \text{ in } P.$  sutchi, C. batrachus and H. fossilis ponds) were lower than the standard levels of USEPA <sup>[29]</sup>. The ranges of mean concentration of Ni and Cr in the sediment soil of the study ponds were  $0.345\pm0.063$  to  $0.364\pm0.099 \text{ µg/g}$  and  $0.029\pm0.005$  to  $0.050\pm0.025\text{µg/g}$ , respectively (Table-2). Both metals contents in the sediment soils recorded in the present study were lower than the standard level according EU <sup>[22]</sup>.

The mean concentration of Cd in the sediment soil samples recorded in the present study  $(0.019\pm0.005, 0.021\pm0.004 \text{ and} 0.020\pm0.005)$  were lower than the standard value (0.487mg/kg sediment) set by EU <sup>[22]</sup>. The range of Zn concentration in the sediment soil was  $0.150\pm0.028$  to  $0.173\pm0.040$  µg/g which is lower than the report of Sarkar *et al.* <sup>[30]</sup>. Moreover, the metal contents in the sediments soil of the catfish ponds were evaluated by comparison with the sediment quality guideline proposed by USEPA <sup>[31]</sup> and CBSOG <sup>[32]</sup>. According to these criteria, it is a matter of great pleasure that the concentrations of heavy metals are not significantly presence in sediment of the catfish ponds.

# 4.3 Heavy metal concentration in water of the catfish ponds

The mean Pb concentrations in water sample of the catfish ponds were  $0.440\pm0.027$ ,  $0.402\pm0.083$  and  $0.506\pm0.067\mu g/g$  (in *P. sutchi, C. batrachus* and *H. fossilis* ponds). These values were under the limit of fishing water <sup>[33]</sup>. Moreover, Flowra *et al.* <sup>[34]</sup> reported the value of Pb varied from  $0.14\pm0.12$  to  $4.92\pm1.66$  mg/L in the water of urban ponds in Rajshahi which is higher than our study. Sultana *et al.* <sup>[35]</sup> reported that water of fish culture ponds in Mymensingh, Bangladesh contained 0.039-0.066 mg/L of Pb which is also lower than our study. The Pb contents in the water sample of the study ponds exceed the permissible level according to the WHO (0.01 mg/L) and USFDA (0.005 mg/L). The estimated mean values of Ni concentration (Range:  $0.305\pm0.037-0.360\pm0.029~\mu g/g$ ) in the water samples of the study ponds were exceed the permissible level (0.02 mg/L) according to the WHO  $^{[36]}$ , WPCL  $^{[37]}$  and TSE-266  $^{[38]}$ .

The mean concentration of Cr in the water samples of the study ponds was around 0.055  $\mu$ g/g (Range: 0.053-0.058  $\mu$ g/g) which was slightly higher than the polluted category (Cr>0.05 mg/L) described by EPA and WPCL <sup>[37]</sup>. Sultana *et al.* <sup>[35]</sup> reported that water of fish culture ponds in Mymensingh, Bangladesh contained 0.13–0.23 mg/L of Cr which is higher than our study. The mean Cd concentration in water samples were not more than 0.02  $\mu$ g/g which is not exceed the standard value as described by EPA <sup>[39]</sup> and WPCL <sup>[37]</sup>. In addition, the range of Zn concentration in water of catfish ponds was 0.046±0.011 to 0.049±0.012 which is within the range of fish culture (<sup>[33]</sup>. More or less similar observation for Zn in water was reported by Sultana *et al.* <sup>[35]</sup> who found Zn range 0.055–0.072 mg/L in freshwater aquaculture ponds of Mymensingh, Bangladesh.

#### 4.4 Heavy metal contents in the raw muscles of catfish

From the data, it was observed that Pb contents in raw muscle of catfish were found to higher than 0.50  $\mu$ g/g which is exceed the acceptable limit (0.5 mg/kg) according to FAO <sup>[23]</sup> and FAO/WHO <sup>[40]</sup> standards. Pb content in raw muscle of catfish was also higher than the Turkish Food Codex <sup>[41]</sup> and EC <sup>[42]</sup> detection limit (0.30mg/kg). The mean Ni contents in raw muscles were estimated as  $0.383\pm0.024$ ,  $0.355\pm0.019$  and

 $0.386\pm0.026\mu g/g$ , respectively. Ni contents in the raw muscles are lower than the permissible limit set by WHO/FAO <sup>[43]</sup> and USFDA <sup>[44]</sup> who recommended upper limits of Ni for food  $(10\mu g/g)$ .

The mean Cr contents in the raw muscles of catfish were less than  $0.055~\mu g/g$  whereas Cd contents were less than  $0.020~\mu g/g$ . The permissible limit set for Cr by FEPA <sup>[45]</sup> is 0.15~m g/k g which is higher than our study. Cr contents in raw muscles found in the present study were also much lower than the maximum limit (12-13 $\mu g/g$ ) stipulated by USFDA <sup>[44]</sup>. Cd contents in raw muscle were lesser than the limits of EC <sup>[42]</sup> that set the threshold value for this metal in fish muscle only  $0.05~\mu g/g$ . Cd content in the raw muscle was also less than the maximum permissible limit  $(1.0\mu g/g)$  set by WHO <sup>[46]</sup>. The range of Zn content  $(0.122~to~0.134~\mu g/g)$  in raw muscles found in the present study is much lower than the range  $(40-100\mu g/g)$  reported by Yamazaki *et al.* <sup>[47]</sup> and the permissible limit  $(30\mu g/g)$  set by FAO <sup>[23]</sup>. The permissible limit for Zn set by FAO/WHO <sup>[40]</sup> is 40 ppm which is also less than our study.

# 4.5 Heavy metal contents in the cooked muscles of catfish

From this study, it was observed that Pb contents in the cooked muscles were more than  $0.05\mu g/g$  whereas Ni contents were less than  $0.40~\mu g/g$ . Pb contents in cooked muscles found in the present study exceed the permissible limit  $(0.5\mu g/g)$  set by FAO <sup>[23]</sup> and FAO/WHO <sup>[40]</sup> but lower than the limit  $(1.0\mu g/g)$  of Turkish Food Codex <sup>[41]</sup>. The values of Ni content in cooked muscle were less than the USFDA <sup>[44]</sup> recommended upper limit  $(10\mu g/g)$  and report of Ersoy *et al.* <sup>[48]</sup>.

The Cr contents in the cooked muscles were below 0.05  $\mu g/g$  which were much lower than the maximum limit (12-13 $\mu g/g$ ) stipulated by USFDA <sup>[44]</sup>. Cr content in the cooked muscles of catfish not exceeds the permissible limit according to FEPA <sup>[45]</sup> who set the threshold value for this metal is 0.15 mg/kg in food. Cd contents (Range: 0.013-0.015 $\mu g/g$ ) in the cooked muscles found in the present study were below the permissible limit of 0.2 mg kg<sup>-1</sup> <sup>[49]</sup> and also lower than the limit (0.1 $\mu g/g$ ) of Turkish Food Codex <sup>[41]</sup>. In the present study, Zn content in the cooked muscles was not exceed 0.1  $\mu g/g$  which is much lower than the permissible limit (30 $\mu g/g$ ) set by FAO <sup>[23]</sup>. The permissible limits for Zn set by FAO/WHO <sup>[40]</sup> is 40 ppm which is less than the values found in our study. The permissible limit of Zn in fish was set at 50 mg kg <sup>-1</sup> <sup>[49]</sup> which is also higher than our study.

# 4.6 Reduction of heavy metal due to cooking

The different methods of cooking have been reported to significantly affect the heavy metal contents present in the final product <sup>[50]</sup>. In the present study, it was observed that cooking method (traditionally used for catfish cooking in Bangladesh) had relatively little effects on the reduction of Pb, Ni, Cd and Cr contents in catfish muscle but the reduction rate of Zn was relatively higher. A previous study on the effect of cooking methods on heavy metal contents of African catfish was done by Ersoy <sup>[51]</sup> correlates with our results. Bassey *et al.* <sup>[52]</sup> reported decreasing impact of cooking method on Zn content of fish muscles. However, heavy metal contents in the cooked muscles may be increased if the cooking materials, spices, water and oil are contaminated with heavy metals.

#### V. Conclusion

This study concluded that Pb contents in the raw and cooked muscles of catfish exceed the permissible limits of different standard guidelines. This study also indicated that traditional cooking method had little effects on the reduction of heavy metals from catfish muscles. The higher Pb content in the catfish muscles might be due to the relatively higher amount of this metal in the feeds and culture environment of the study ponds. Lead is a non-essential element and it can cause neurotoxicity, nephrotoxicity and many others adverse effects in human health. So, monitoring and control of heavy metals in fish feed and culture environment of the catfish farm should be necessary. Moreover, long-term further research should be done on the assessment of heavy metal content in fish feed and culture environment, and their accumulation in fish muscle. Such assessment will be vital in preventing human health risks associated with consumption of heavy metal contaminated fish.

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