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Assessment of heavy metal contamination in fish feed available in three districts of South Western region of Bangladesh

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Abstract
This study focused on the heavy metal content of fish feed locally available in the south western region (Satkhira, Khulna and Bagherhat districts) of Bangladesh from June to August 2016. Assessment of heavy metals was done using atomic absorption spectrophotometer. Mainly five heavy metals were assessed namely Lead (Pb), Cadmium (Cd), Cromium (Cr), Aersenic (As) and Mercury (Hg). Graphite Furnace was used for Pb, Cd and Cr analysis. On the other hand, hydride generator system was used for As and cold vapor hydride generator system was used for Hg. The analytical procedure was checked using certified reference material DORM- 4 Fish protein for heavy metals. As and Hg were found below the detection limit. Cd concentration was found within the acceptable limit (0.29±0.08 mg/kg). On the other hand, the average Pb and Cr concentration in feed sample were 8.49±3.66 and 8.57±3.47 mg/kg respectively which are much higher than maximum consumption limit. EU maximum level for both Pb and Cr in fish feed is set at 5.0 mg/kg. The range of elemental concentrations (mg/kg) of fish feed were Cd (0.13-0.46), Pb (2.49-14.87), Cr (2.83-15.45) respectively.

Keywords: fish feed, acceptable limit, arsenic, lead, mercury, chromium, cadmium

1. Introduction
In the last decades, contamination of aquatic systems by heavy metals has become a global problem (Yilmaz, 2009) [1]. Heavy metals such as mercury, plutonium and lead are toxic as well as their accumulation; over a period of time in the bodies of animals can cause serious illness (Duruibe *et al.*, 2007) [2]. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs (Tchounwou *et al.*, 2002) [3]. Long-term exposure may affect in the physical, muscular and neurological degenerative processes (Thomas and Mohaideen, 2014) [4]. Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the levels of such metals in aquatic environments has become a problem of primary concern. Fishes are the aquatic inhabitants which can be highly affected by heavy metals (Ayas, 2007) [5]. The existence of metal ion in the environment mediation increased the mucus like secretion from gill, excessive excretion, anorexia and also the fin movement (Islam *et al.*, 2007) [6]. However, fish feed in aquaculture system might be a source of heavy metal contamination in fish body. Therefore, we must ensure that fish feeds are not contaminated with heavy metals or should not cross their acceptance limit.

Khulna, Satkhira and Bagherhat districts are very famous for brackish water aquaculture in the south western region of Bangladesh. Satkhira and Bagherhat are very famous for shrimp (*Penaeous monodon*) and brackish water fish culture surrounded by the world's largest mangrove forest, Sundarban. In contrast, Khulna is famous for both shrimp (*Penaeous monodon*) and prawn (*Macrobrachium rosenbergii*) culture due to its geographical location. As aquaculture practice has evolved in this region, the push towards higher yields and faster growth has involved the replacement of natural live foods with prepared diets or supplemental feeding. The main cost factor in aquaculture is the cost of feed. Moreover, high amounts of feed ingredients from marine sources have been of concern both environmentally and economically. Therefore, the objective of this study was to assess the abundance of some heavy metals namely Lead (Pb), Cadmium (Cd), Chromium (Cr), Mercury (Hg) and Arsenic

(As) in fish feeds available in these three districts of the south western region of Bangladesh.

2. Materials and Methods
2.1 Study area and study period
The present study was conducted in the south western part of Bangladesh. Fish feed samples were collected from Khulna, Satkhira and Bagherhat district from June to August 2016.

2.2 Sampling
Forty feed samples were collected from different places of Khulna (15 samples), Satkhira (12 samples) and Bagherhat (13 samples) districts. Immediately after collection, the feed samples were placed in acid cleaned polyethylene bottles and brought to Quality Control Laboratory of Department of Fisheries (DoF), Boyra, Khulna for analysis. Consistently, experiment was done with three replications.

2.3 Equipments
All the matrixes were analyzed for Pb, Cd, Cr, Hg and As by atomic absorption spectrophotometer (Model ZEEnit 700P# 150Z7P0110, Analytikjena, Germany) using Graphite Furnace for Pb, Cd and Cr, Hydride Generator system for As and Cold Vapor Hydride Generator system for Hg.

2.4 Feed digestion for metal analysis
From each feed sample 2.0 g sample was taken in 100 ml beaker and place on the hotplate. Thereafter, 15 ml of concentrated HNO_3 was added and heated at 120^0 C (at nearly dry). Followed samples were allowed to cool at room temperature. After that 5 ml of concentrated HCL added and heated until 1-2 ml remains. For mercury and arsenic analysis 1 ml of H_2O_2 added and heated another 30 minutes and made the final volume with DI (De-ionized water) in 100 ml volumetric flask. Further dilution were made as per required. Finally the samples were examined with AAS (Atomic Absorption Spectrophotometer) for metal estimation followed by respective Equipment Standard Operating Procedure (Shamshad *et al*., 2009) [7].

2.5 Analysis of As (Dural *et al*., 2007) [8].
Step 1: 12.5-ml digested sample aliquots, 1.5 ml HCl were dispensed into 50 ml volumetric flask. Then 1 ml of ascorbic acid and KI mixture was added and final volume was made to 50 ml. Consequently, it took 2 hour to complete the reduction reaction of As.

Step 2: 5-ml prepared sample aliquots were dispensed into reaction vessel and reading were recorded.

2.6 Analysis for Hg (Dural *et al*., 2007) [8].
Step 1: 25-ml digested sample aliquots, 1.5 ml HNO_3 were dispensed into 50 ml volumetric flask. Then 0.5 ml of 1% $K_2Cr_2O_7$ was added and final volume was made to 50 ml.

Step 2: 5-ml prepared sample aliquots were dispensed into reaction vessel and reading were recorded.

2.7 Analytical technique and accuracy check
All the matrixes were analyzed for Pb, Cd, Cr, Hg and As by atomic absorption spectrophotometer (Model ZEEnit 700P# 150Z7P0110, Analytikjena, Germany) using GFAAS and Hydride Generator system (Biller and Bruland, 2012) [9]. All the methods are in-house validated following EC657/2002. Analytical conditions for the measurement of the heavy metals in sample using AAS were tabulated in Table 1. The instrument calibration standards were made by diluting standard (1000 ppm) supplied by Sigma Aldrich, Germany. The analytical procedure was checked using certified reference material DORM- 4 Fish protein for heavy metals. The standard deviations of the means observed for the certified materials were between 0.015–0.64% and the percentage recovery was between 83.17–102.64% as shown in Table 2. The results indicated a good agreement between the certified and observed values.

Table 1: Analytical conditions for measurement of heavy metals in aqueous solution using AAS

Elements	Wavelength (nm)	Slit (nm)	Lamp Current (mA)	Mode	Calibration Range (µg / kg)	Detection limit (mg/ kg)
As	193.7	0.8	6.0	HGAAS	2.5-20	0.05
Cr	357.9	0.8	4.0	GFAAS	4-32	0.019
Cd	228.8	1.2	3.0	GFAAS	0.4-1.2	0.03
Pb	283.3	0.8	4.0	GFAAS	5-40	0.025
Hg	253.7	1.2	3.0	CVHGAAS	5-20	0.02

Table 2: Concentrations of metals found in Certified Reference Materials by AAS

Parameters	Assigned Value(mg/kg)	Obtained Value(mg/kg)	Recovery %
Arsenic	6.80±0.64	5.76	84.70
Cadmium	0.306±0.015	0.266	86.93
Chromium	1.87±0.16	1.81	96.79
Lead	0.416±0.053	0.427	102.64
Mercury	0.410±0.055	0.341	83.17

2.8 Statistical analysis
The data were statistically analyzed by the statistical package, SPSS 17.0 (SPSS, USA). The means and standard deviations of the heavy metal concentrations in feed were calculated by using Microsoft office 2007.

3. Results
The experiment was carried out on different types of fish feeds that are available in the south western region of Bangladesh. In the present study, 5 types of heavy metal was assessed namely Arsenic (As), Mercury (Hg), Lead (Pb), Cadmium (Cd) and Chromium (Cr).

3.1 Lead
The mean lead in feed sample was 8.49±3.66 mg/kg (Table 3) which is higher than maximum consumption limit and the range was 2.49 to 14.87 mg/kg. In most cases, the lead concentration in the studied feed samples were found higher

than the EU maximum level for lead in fish feed which is set at 5.0 mg/kg.

Table 3: Average concentration level and range between highest and lowest amount of lead among feed samples.

Concentration of Lead (Pb) in fish feed	
Average	8.49±3.66 mg/kg
Lowest value	2.49 mg/kg
Highest value	14.87 mg/kg

3.2 Cadmium
The cadmium concentration in studied feed samples was less than 0.5 mg/kg (Table 4) and the EU maximum level for cadmium in fish/shrimp feed is 0.5 mg/kg.

Table 4: Average concentration level and range between highest and lowest amount of Cadmium among feed samples.

Concentration of Cadmium (Cd) in fish feed	
Average	0.29±0.08 mg/kg
Lowest value	0.13 mg/kg
Highest value	0.46 mg/kg

3.3 Mercury
The mercury content in studied feed samples was lower than the detection limit of the method that means all feed samples contain mercury level below 0.02 mg/kg.

3.4 Arsenic
The detection limit of the method for arsenic is 0.05 mg/kg. The arsenic content in studied feed samples that are mostly used in the south western region of Bangladesh was below the detection limit.

3.5. Chromium
All the feed samples were found contaminated with chromium (Table 5). The allowed chromium content in feed is 5 mg/kg. But among our 40 feed samples, 31 feed samples contained chromium that exceeds the maximum consumption level.

Table 5: Average concentration level and range between highest and lowest amount of chromium among feed samples.

Concentration of Chromium (Cr) in fish feed	
Average	8.57±3.47 mg/kg
Lowest value	2.83 mg/kg
Highest value	15.45 mg/kg

4. Discussion
4.1 Lead
Lead compounds may have a variety of hazards within the nervous system both for fishes and for humans causing neurotoxicity (Bondy, 1988) [10]. The average lead content in our 40 feed samples was 8.49±3.66 mg/kg. Assessment showed that most of the samples had lead content greater than the maximum consumption limit (5 mg/kg). This is very alarming to us. Lead poisoning in humans causes severe dysfunction in the kidney, reproductive system, liver, brain and central nervous system (Martínez-Quintana and Penagos-Corzo, 2012) [11]. Lead causes loss of neurons' myelin sheaths, reduces numbers of neurons, interferes with neurotransmission and decreases neuronal growth (Rudolph *et al*., 2004) [12]. Alexieva *et al.*, (2007) [13] reported the average concentration of lead content in different animal feed samples (59 samples) was 4.77 mg/kg, ranging between 1 to 9.5 mg/kg, which is similar to our study. On the other hand, Shamshad *et al.*, (2009) [7] reported that the average lead content in shrimp feed that is mostly used in Bangladesh was 3.58 mg/kg. Adamse *et al*., (2017) [14] reported that the highest percentage of feed samples with lead concentrations exceeding the maximum limit (of 30 mg/ kg) were from feed additives belonging to the functional groups of binders and anti-caking agents. This concerned complementary mineral feeds, pre-mixtures, fish meal, manganous oxide and 'other feed additives'. Most of the branded fish feed in Bangladesh are costly and mostlfree from metal pollution (Shamshad *et al.*, 2009) [7]. However, feeds those are made locally with available ingredients are cheap but not tested properly to assess the contamination level with different heavy metals. As most of the fish farmers are poor, they typically use local feeds which are often contaminated with different harmful heavy metal like lead.

4.2 Arsenic
Among different chemical forms, both inorganic and organic, inorganic arsenic compounds are the most toxic, particularly As_2O_3 (Ding *et al*., 2005) [15]. Arsenic is causally related to increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes (Abernathy *et. al*, 2001) [16]. Arsenic in feed and feed material originates from natural geological source, from pollution by industrial activities or specific feed additives (Adamse *et al*., 2017) [14]. Sloth *et al*., (2005) [17] reported that concentration in the range of 3.4-8.3 and 0.010-0.061 mg/kg for complete feedingstuffs were found for total arsenic and inorganic arsenic, respectively. But several of the complete feedingstuff samples had total arsenic concentration above the EU maximum content of 1 mg/kg in complete feedingstuff for fish. Choi *et al*., (2015) [18] stated that the levels of As in the different fish species varied substantially ranging from 0.02 µg/g (Isla eli carp) to 9.65 µg/g (Skate ray) with a median of 0.40 µg/g. However, Salawu *et al*., (2016) [19] examined six feed samples and found As in a range of 0.011 to 0.106 mg/kg for all fish feeds sampled. These concentrations were lowered than the maximum acceptable limit of 1 mg/kg for arsenic in fish feeds as stipulated by EU. Shamshad *et al*., (2009) [7] reported that arsenic content in shrimp feed that is mostly used in Bangladesh was 0.45 mg/kg wet weight and the range was from 0.138 to 0.909 mg/kg. As a whole, the average arsenic concentration in our studied feed samples, used in south western part of Bangladesh (Satkhira, Khulna and Bagherhat districts) was below the detection limit.

4.3 Cadmium
Cadmium is a nonessential nutrient may accumulate in the body, particularly in the kidney, liver, and to a lesser extent in the muscle (Li *et al*., 2005) [20]. The average cadmium content in our feed samples was 0.29±0.08 mg/kg. Shamshad *et al.*, (2009) [7] reported that the average cadmium content in shrimp feed that is mostly used in Bangladesh was less than 0.1 mg/kg. Ikem and Egilla (2008) [21] have reported that the average elemental concentration of Cd is 2.37 mg/kg in diet (dry wt.) of fish feed which is double than the acceptable limit. Adamse *et al*., (2017) [14] reported that the presence of elements like cadmium in the aquatic environment originates from natural (volcanic activity, weathering of bedrocks) or from anthropogenic sources such as mining activities, incineration of waste and agricultural use.

4.4 Mercury

The mercury content among the studied feed samples, used in south western part of Bangladesh was below detection limit. Shamshad *et al.*, (2009) [7] reported that the average mercury content in shrimp feed that is mostly used in Bangladesh was lower that the quantification limit for the analytical method (0.03mg/kg). The result is very similar to our study. Alexieva *et al.*, (2007) [13] reported the average mercury concentration in compound feeds for pigs and poultry, used in Bulgaria, was 0.0002 mg/kg that was very low compared to maximum allowed concentrations (0.1 mg/kg).

4.5 Chromium

Chromium is an essential nutrient. It facilities the action of insulin as well as helps to the metabolism and storage of carbohydrate, fat and protein (Anderson, 1997) [22]. But excessive level of chromium in fish feed damages the kidneys, the liver and blood cells through oxidation reactions (Dayan and Paine, 2001) [23]. The results obtained from our study were alarming. The average chromium content was found in our study was 8.57±3.47 mg/kg. The assessment showed that some of the feed samples contain chromium two or three times higher than the maximum chromium consumption level (5 mg/kg). Use of different types of feed additives and food color could be the reason of high chromium content in studied feed samples. This excessive level of Cr can hamper the function of DNA and can cause genotoxicity (Cohen *et al.*, 1993) [24]. The major concern about chromium is that it has carcinogenic behavior in humans (Baruthio, 1992) [25]. However, Ikem and Egilla (2008) [21] have reported that the average elemental concentration of Cr is 1.42 mg/kg in diet (dry wt.) of fish feed which is within the acceptable limit and lower than our findings.

5. Conclusion

According to the present study, it is clear that heavy metals contaminated with of lead and chromium in the feed samples in the investigated areas were very serious. It indicates that more attention should be paid on the formulation of fish feeds and ingredients which contain high concentration of heavy metals should be avoided. Additionally basic and application research on fish feed need to be performed and much perfect standard should be established.

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