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RESEARCH ARTICLE

Assessment of toxic and essential metals in fish feed ingredients available in different areas of Bangladesh

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ABSTRACT

Contamination of heavy metals in fish feed ingredients is regarded as a major crisis globally especially in developing countries because it may be the source of toxicity in the food chain. Fish farming in Bangladesh is growing very rapidly and fish available in the markets are mostly coming from fish farms where fish farmers mostly use commercial fish feeds manufacturing with different ingredients. In this study, several types of ingredients based on different origins were collected from several areas and after measuring dry weight these were digested by a mixture of acids. This study is concerned to access the toxic and essential metals in different fish feed ingredients which are frequently used to produce commercial fish feed in Bangladesh. The concentration (mg kg^{-1} dry weight) range of toxic metals such as; Pb (0.56- 1.73), Cd (0.12-0.97), Cr (0.15-0.88), As (BDL), Hg (BDL), Ni (1.10-2.50) and essential metals such as; Fe (13.57-48.96), Cu (10.11-28.09), Zn (10.60-26.25), Na (12.07-35.00), K (13.06-28.97), Ca (10.00-47.96) in selected fish feed ingredients were recorded by Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Optima 7000DV) with significant variation ($P<0.05$). The analyzed toxic and essential metal concentrations in most of the ingredients were within the safe limit proposed by World Health Organization, Food and Agricultural Organization and European Union indicating no health risk.

Keywords: Essential metal, fish feed ingredients, health risk, toxic metal

1. INTRODUCTION

Bangladesh has one of the highest population densities and is also the 7th most populous country in the world. A large number of folks directly or indirectly rely on agriculture [1]. Aquaculture and fisheries are one of the major sectors of agricultural activities and play a significant role to boost the country's economy by ensuring fish demand and encouraging the establishment of a large number of by-product industries which have a great contribution to the national economy, giving 3.69% to the Gross Domestic Product (GDP) of this country's economy and 22.60% to the agricultural GDP [1, 2].

Civilization rapidity, high population rate and inclination to industrialization, developing countries are becoming the shelter of many of the world's most contaminated air, water and solid waste problems. Previous studies have reported that the level of particular heavy metals is increasing in water bodies around the world, especially in rivers. The industrial procurement and waste generated from these industries are mainly responsible for this worldwide pollution [3]. Bangladesh is widely recognized for producing leather from raw hides and skins and the tanning industries are highly reliable to chrome tanning method which is liable for chromium pollution both in liquid and solid waste. Moreover, groundwater is polluted severely due to arsenic contamination in many regions of this land [4, 5]. Industrial disposals and mining extraction can be a potential source of heavy metal exposure in the aquatic ambiance [6]. Besides, environmental contaminants in food processed with fishes and even in fish have become a global concern. Due to high biological accumulation and magnification rate, heavy metal contamination in fish can impose a detrimental

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effect on human health [7]. Exposure of metals in water bodies has attracted global concern because of its exuberance, consistency and environmental toxicity. Pollution because of toxic metals has a detrimental health effect on invertebrates, fish, and humans. Cultivated fishes and aquacultures largely depend on artificial feeds. Commercial feed manufacturers failed to fulfill the standards required for cultivated fish production, and the origin of ingredients for the manufacturing of the feeds tends to be contaminated with heavy metals and other pollutants [8, 9]. The common sources of fish feed ingredients are maize, rice, rice polish, wheat, soybean grits, mustard oil cake, coconut oil cake, lentil bran, molasses, etc. [10].

Bangladesh is well known for the cultivation of fish to fulfill the protein demand of the countrymen and fishes are the most pivotal organisms in the aquatic food chain, which are very sensitive to metal contamination. Different metals are accumulated in fish tissues at different concentration levels. The metal concentration differs from fish to fish due to the affinity of metals to fish tissues, uptake rate, extraction and absorption rate [11, 12]. Metals such as Pb, Cd, As, Hg, Cr are toxic as well as accumulation of these metals for a long period in the bodies of aquatic organisms may be responsible for severe diseases [13]. Heavy metal toxicity can be responsible for mental distress and nervousness, energy declining, and blood composition irregularity, breakdown of the function of lungs, kidneys, liver and other vital organs [14]. The adverse effect of metals when intakes above the standard levels can be considered as chronic or acute toxic, and heavy metals can be regarded as neurological toxic, carcinogenic, mutagenic. The primary diseases of humans related to toxic metals such as; Cd, Pb, As, Hg consumption may cause vomiting, convulsions, immobility, ataxia, hemoglobinuria, gastrointestinal diseases, stomatitis, tremor, diarrhea, depression and respiratory diseases [15].

Essential metals are needed in trace amounts for the proper ramification of enzyme profile, hemoglobin development and vitamin production in humans [16]. Iron helps to bind, transport, and spread oxygen into the flow of blood in animals. Some of the trace elements control important biological processes [17]. Zinc and copper are essential to life. It plays a vital role in lipid, protein and carbohydrate metabolism. Copper is involved in iron metabolism, the formation of bones, connective tissues and maintaining the integrity of the myelin sheath of nerve fibers. The deficiencies of zinc and copper cause growth retardation of animals [18, 19]. Sodium and Potassium are the prime dietary metal requirements of humans to keep a good balance of physical fluids system and to active nerve and muscle properly [20], [21]. Calcium is a key nutrient in the human body that mainly helps the bones rebuild properly and stay strong [22]. Although essential metals play important physiological and biochemical roles in the body and either their deficiency or excess can lead to disturbance of metabolism and therefore causes to create various diseases [23]. Since raw materials of different origins are used to produce commercial fish feeds, proper observing for heavy metal accumulation in feeds will have to go a far way for manufacturing standard feeds that will help to produce a large number of cultivated fishes and keep humans safe from different diseases [24].

Though several studies are done previously on heavy metal determination in fishes, we focused to identify the preliminary sources of these metals in fish tissues. Besides, along with heavy metals, we assessed the concentration of essential metals in the different fish feed ingredients which are regularly used by feed producers around the country. Consequently, this study was designed to find the concentration of toxic metals (Pb, Cd, Cr, As, Hg, Ni) and essential metals (Fe, Cu, Zn, Na, K, Ca) in selected fish feed ingredients available in the local market of Bangladesh.

2. MATERIALS AND METHODS

2.1. Sample collection

Samples of feed ingredients were collected from different renowned fish feed manufacturing companies located in Bangladesh. Ten major feed ingredients including carbohydrate (Wheat, maize, rice), animal origin (Chicken Bone, Fat) and plant origin (Mustard oil cake, Linseed, Duck Weed, Water hyacinth) were collected for their assessment of heavy metal contents. All homogeneous samples were collected in an insulated small container that was dry and clean without any leakages. Each sample was labeled with proper name, weight, physical type (animal or vegetable origin), and collection date by using a waterproof marking pen on a strip of masking tape (WHO, 2002) [25].

2.2. Digestion and analysis

All collected fish feed ingredient samples were cut into small species and dried in a micro-oven at 105oC until a constant weight was achieved and finally powdered. Samples also were dried in a similar way 1 g of the homogenate of each sample (dry weight) was taken into a quick fit round bottom flask and a 15 mL mixture of concentrated HNO_3, H_2SO_4 and $HClO_4$ in (4:1:1; v/v) was added into the flask. A condenser was set up with the flask and the mixture was stirred at 85°C for 3 hours until the proper digestion was completed and the solution became clear [26]. Then the digested samples stayed to cool for 30 minutes. Filtration of all digested samples was done with Whitman no. 42 filter paper and the filtration was transferred into the volumetric flask, then 20 mL deionized water was added and rinsed well and finally diluted to 50 mL with deionized water. Samples were stored at ambient temperature until metal analysis. All chemicals used were Merck, Germany analytical grade, including standard stock solutions of known concentrations of different metals. The entire samples were analyzed by Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with the following detection limit [Table 1].

Table 1. Information about the ICP-OES spectrometer

Metals	Wavelength (nm)	Detection limit (mg kg^{-1})	Assigned Value (mg kg^{-1})	Obtained Value (mg kg^{-1})	Percent Recovery (%)
Pb	220.35	0.01	0.50	0.4815	96.3
Cd	228.80	0.001	0.50	0.4923	98.5
Cr	267.71	0.001	0.50	0.4693	93.4
As	193.69	0.01	0.50	0.5132	102.6
Hg	253.65	0.01	0.50	0.5242	104.8
Ni	245.78	0.01	0.50	0.4953	99.06
Fe	238.21	0.001	0.50	0.5232	104.6
Cu	327.39	0.001	0.50	0.5245	104.7
Zn	206.20	0.001	0.50	0.4876	97.5
Na	245.65	0.01	0.50	0.5123	102.5
K	213.45	0.01	0.50	0.4987	99.7
Ca	220.34	0.01	0.50	0.5143	102.8

2.3. Statistical analysis

All statistical analyses were performed using Microsoft Excel (version 2016). The Pearson Correlation Coefficient in the present study is also done to calculate the interrelationships among the analyzed elements at a level of > 0.5 or < -0.5. A positive value indicates, one metal increase in value, other value also rises. Besides, negative value implies the opposite relationship. If the value is greater than 0.8, there is a strong relationship between the two variables whereas if the value is lesser than 0.5 means a weak relationship. Analysis of variance (Two-way ANOVA) and correlation matrix was employed to examine the statistical significance of differences in the mean concentration of metals in the collected fish feed ingredients. A probability level of $P < 0.05$ was considered statistically significant [27].

3. RESULTS AND DISCUSSION

The summary of the concentrations of toxic metals such as Pb, Cd, Cr, As, Hg and Ni found in different commercial fish feed ingredients are presented in Table 2. Table 3 shows that the highest concentration of Pb was 1.73 mg kg^{-1} found in chicken bone and the lowest concentration (2.15 mg kg^{-1}) was in Animal fat. The mean concentration of Pb in these selected ingredients was 1.05 mg kg^{-1} which is lower than the permissible limit of 5.0 mg kg^{-1} proposed by (WHO/FAO, 2004) [28] [Fig 1] but the recorded result for Pb in the present study was higher than the previously recorded value which was 3.19 mg kg^{-1} and 4.18 mg kg^{-1} respectively [30, 31]. The mean concentration of Pb in the present study was lower than 0.30 mg kg^{-1} reported by Adeniji et al. [10].

Table 2. Concentration of toxic metals (mg kg^{-1} dry weight) in different fish feed ingredients

No	Ingredients	Pb	Cd	Cr	As	Hg	Ni
01	Wheat	0.80	0.12	0.63	BDL	BDL	1.50
02	Maize	1.11	0.97	0.50	BDL	BDL	1.10
03	Duck Weed	1.05	0.43	0.33	BDL	BDL	2.50
04	Water- hyacinth	1.13	0.70	0.40	BDL	BDL	2.50
05	Linseed	1.07	0.13	0.18	BDL	BDL	2.00
06	Rice & Rice bran	0.76	0.40	0.15	BDL	BDL	1.50
07	Mustard oil cake	1.13	0.18	0.68	BDL	BDL	1.70
08	Molasses	1.15	0.83	0.40	BDL	BDL	1.60
09	Chicken Bone	1.73	0.58	0.88	BDL	BDL	1.80
10	Animal Fat (cow)	0.56	0.15	0.63	BDL	BDL	1.40
Safe Limit (mg kg^{-1})	FAO/WHO, 2004 [28]	5.00	1.00	1.00	1.00	0.50	----
	EU, 2010 [29]	2.00	2.00	1.00	2.00	1.00	----

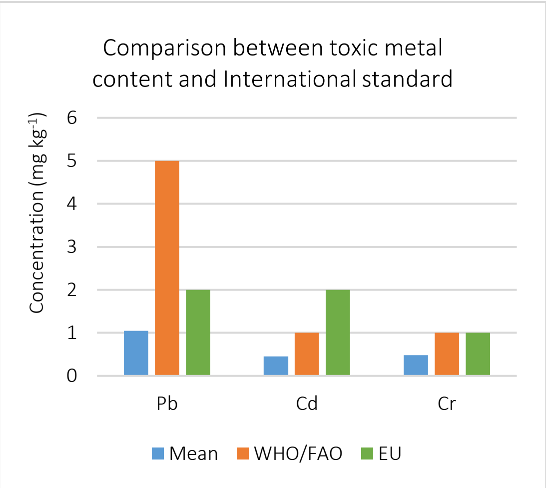
BDL= Below Detection Limit; FAO= Food and Agricultural Organization; WHO= World Health Organization; EU= European Union.

Table 3. Statistical data analysis of toxic metals

Statistical data analysis of Toxic Metals						
Metals	Pb	Cd	Cr	As	Hg	Ni
Maximum value	1.73	0.97	0.88	---	---	2.50
Minimum value	0.56	0.12	0.15	---	---	1.10
Mean value	1.05	0.45	0.48	---	---	1.76
Standard Deviation	0.31	0.31	0.23	---	---	0.46
P value	0.003*	0.001*	0.008*	---	---	0.60
F value	0.71	0.21	0.76			1.12
F-critical value	0.20	0.28	0.43			1.64
Adeniji et al. [10]	0.30	0.50	0.20	---	---	
Alexieva1 et al. [30]	3.19	0.54	1.53	0.108	.0002	3.07
Islam et al. [31]	4.18	0.32	3.5	1.00	----	2.70

*Significant at P< 0.05

The mean concentration of Cd in these selected ingredients was 0.45 mg kg^{-1} which is beyond the safe limit of 1 mg kg^{-1} [28] and 2 mg kg^{-1} [29] [Fig 1]. The maximum Cd concentration was 0.97 mg kg^{-1} recorded in Maize whereas the least content was 0.12 mg kg^{-1} found in Wheat. The average Cd content was 0.32 mg kg^{-1} in a previous study reported by Islam et al. [31], Adeniji et al., [10] and Alexieva1 et al. [30] found Cd content 0.50 mg kg^{-1} and 0.54 mg kg^{-1} respectively in their study which was higher than the present study. A noticeable amount of Cr was found in Chicken bone, Animal fat and Mustard oil cake. The highest concentration of Cr was 0.88 mg kg^{-1} recorded in Chicken bone and the lowest concentration was 0.15 mg kg^{-1} in Rice and Rice bran. The recorded mean value of Cr was 0.48 mg kg^{-1} within the maximum limit of 1.00 mg kg^{-1} [28, 29] [Fig 1]. Islam et al. [31] and Alexieval et al. [30] found the Cr concentration 3.50 mg kg^{-1} and 0.54 mg kg^{-1} in their study which is higher than the present study but the mean concentration of Cr in this study was higher than 0.20 mg kg^{-1} recorded by Adeniji et al. [10]. As and Hg concentration was below detection limit whereas the maximum limit 1.00 mg kg^{-1} for each metal proposed by WHO/FAO, 2004 [28] and EU, 2010 [29] though some studies traced the concentration of As and Hg in their study. The highest concentration of Ni (2.50 mg kg^{-1}) was found in Water hyacinth and Linseed and the lowest concentration (1.10 mg kg^{-1}) was recorded in Maize. The average concentration of Ni was 1.76 mg kg^{-1}, which is lower than 3.07 mg kg^{-1} and 2.70 mg kg^{-1} in the two separate studies carried out by Alexieva1 et al. [30] and Islam et al. [31].



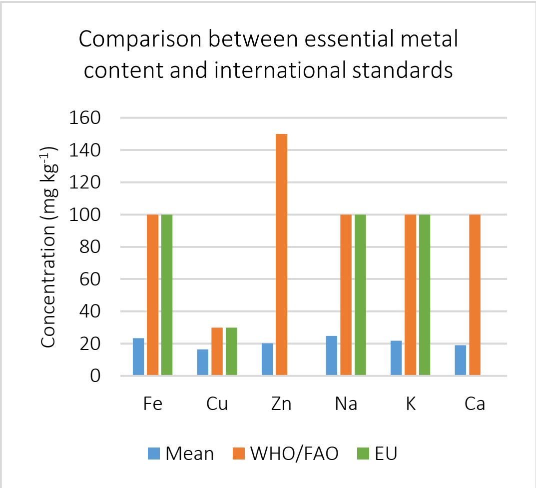


Fig 1. Comparison between recorded content of traced metals and international Safe limit

The concentration of essential metals such as; Fe, Cu, Zn, Na, K and Ca in selected fish feed ingredients are represented in Table 4. Table 5 summarizes that the highest concentration of Fe was 48.96 mg kg^{-1} recorded in the chicken bone which is considered as animal origin whereas the lowest concentration of Fe was 13.57 mg kg^{-1} found in Linseed as plant origin. The average concentration of Fe was 23.30 mg kg^{-1} which is within the safe limit of 100 mg kg^{-1} proposed by FAO, 2016 [32]and EU, 2010 [29] [Fig 1] as well as the found average value of Fe in the present study is lower than 34.78 mg kg^{-1} in a past study by Godfred et al., 2020. The average concentration of Cu was 16.41 mg kg^{-1} in this study whereas 29.00 mg kg^{-1} Cu was found in a study by Roy et al.[33]. The recorded value in the present study was in the acceptable limit of 30 mg kg^{-1} stated by FAO, 2016 [32]and EU, 2010 [29] [Fig 1].

Table 4. Concentration of essential metals (mg kg^{-1} - dry weight) in different fish feed ingredients

No	Ingredients		Fe	Cu	Zn	Na	K	Ca
01	Wheat		27.57	15.72	15.15	35.00	27.00	18.10
02	Maize		28.70	10.11	14.23	31.10	13.06	16.50
03	Duck Weed		18.73	18.37	24.27	29.00	24.30	10.00
04	Water- hyacinth		18.30	19.40	24.27	14.00	15.34	10.07
05	Linseed		13.57	11.80	25.00	24.00	27.80	12.62
06	Rice & Rice bran		19.40	17.98	26.25	23.30	17.65	19.06
07	Mustard oil cake		33.70	16.90	14.57	26.83	21.34	12.69
08	Molasses		33.08	15.08	23.08	30.60	27.12	24.16
09	Chicken Bone		48.96.	28.09	23.78	21.67	28.97	47.96
10	Animal Fat (cow)		16.67	10.67	10.60	12.07	15.78	18.41
Safe Limit (mg kg^{-1})		WHO, 2004 & FAO, 2016 [28, 32]	100	30	150	100	100	100
		EU, 2010 [29]	100	30	....	100	100	---

Table 5. Statistical data analysis of essential metals

Statistical data analysis of Essential Metals						
Metals	Fe	Cu	Zn	Na	K	Ca
Maximum value	48.96	28.09	26.25	35.00	28.97	47.96
Minimum value	13.57	10.11	10.60	12.07	13.06	10.00
Mean value	23.30	16.41	20.12	24.76	21.84	18.96
Standard Deviation	10.22	5.25	5.76	7.38	5.97	11.12
P-value	0.005*	0.07	0.001*	0.004*	0.003*	0.50
F-value	0.31	0.85	0.61	0.47	0.53	1.16
F-critical value	0.15	1.38	0.21	0.12		1.49
Roy et al., 2019 [33]	----	29.00	24.50	---	---	----
Godfred et al., 2020 [34]	34.78	----	14.95	---	---	----

The highest concentration of Cu (28.09 mg kg^{-1}) was found in Chicken bone and the lowest concentration (10.11 mg kg^{-}) was recorded in Maize. The average concentration of Zn was 20.12 mg kg^{-1} in this study which is within the permissible limit of 150 mg kg^{-1} proposed by WHO, 2004 [28] [Fig 1] and it is higher than 14.95 mg kg^{-1} recorded Godfred et al. [34] and lower than 24.50 mg kg^{-1} reported by Roy et al. [33]. The highest concentration of Na was 35.00 mg g^{-1} found in Wheat and the lowest concentration was 12.07 mg kg^{-1} in Animal fat recorded in Wheat. The average concentration of Na was 24.76 mg kg^{-1} in the present study which is within the maximum limit of 100 mg kg^{-1} proposed by WHO and EU [Fig 1]. The highest concentration of K and Ca was 28.97 mg kg^{-1} and 47.96 mg kg^{-1} both in Chicken bone. The average value of K and Ca was recorded 21.84 mg kg^{-1} and 18.96 mg kg^{-1} respectively in the present study which is acceptable according to the safe limit of 100 mg kg^{-1} WHO and EU [Fig 1].

Table 3 and Table 5 indicates, there is a significant variation ($P<0.05$) in metal concentration in all fish feed ingredients as P-value is lower than α value (0.05) except for the recorded concentration of Ni, Cu and Ca. Besides, the present study also found that F-critical values were lower than F-value which also indicated that there was significant variation in the concentration of Pb, Cd, Cr, Fe, Zn, Na and K. In contrast, P-value is higher than α value (0.05) and F-critical value is higher than F value in the concentration of Ni, Cu and Ca.

Table 6. Pearson correlation coefficient of selected metals in fish feed ingredients

	Pb	Cd	Cr	Ni	Fe	Cu	Zn	Na	K	Ca
Pb	1									
Cd	0.27	1								
Cr	0.50*	0.32	1							
Ni	0.14	-0.31	-0.27	1						
Fe	0.18	0.24	0.65*	0.17	1					
Cu	0.50*	-0.14	0.37	0.43	0.65*	1				
Zn	0.01	-0.08	-0.01	0.60*	0.36	0.51*	1			
Na	-0.15	-0.15	-0.06	0.33	-0.52	-0.58	0.62*	1		
K	-0.30	0.11	-0.73	0.06	-0.44	-0.26	0.16	0.37	1	
Ca	0.59*	0.57*	0.52*	-0.13	0.36	0.52*	0.02	0.03	-0.16	1

The Pearson Correlation Coefficient in the present study is used to describe the interrelationships between the elements analyzed at a level of > 0.5 or < -0.5 which are significantly correlated. Table 6 summarizes a high positive correlation between Pb-Cr (0.50), Pb- Ca (0.59) and Fe-Cu (0.65) which indicates a similar source of these metals in fish feed ingredients. The correlation between Pb-Cu, Cd-Ca, Cr-Fe, Ni-Zn, Zn-Na were above 0.5 and positively correlated. In Contrast, Cr-K, Fe-Na and Cu-Na are weakly correlated as their values were less than -0.5.

4. CONCLUSIONS

Overall, the total mean toxic and essential metal concentration of all fish feed ingredients in this study revealed an order of Ni>Pb>Cr>Cd>As, Hg and Na>Fe>K>Zn>Ca>Cu. A significant amount of all selected metals was assessed in different fish feed ingredients in this study. However, the recorded result revealed that the concentration of Pb, Cd, Cr, As, Hg, Ni, Fe, Cu, Zn, Na, K, Ca in the selected fish feed ingredients was within the permissible limit of WHO/FAO and EU and indicating no risk for fish cultivation as well as for human health in future. This study recorded that there was significant variation ($P<0.05$) in metal concentration in each fish feed ingredients. Since it is impossible to completely eliminate the presence of undesirable contaminant due to the environment so, regular training and enlightenment programs should be given to farmers, feed producers on the need for proper storage and handling of fish feed ingredients as these will reduce or preventing where possible the level of toxic metals in feed ingredients, fish and human the ultimate consumer. Policymakers, food regulator authority, farm owners can take initiative to check the quality and origin of ingredients which will use to manufacture the commercial feeds for fish cultivation in Bangladesh.

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