

Bioaccumulation of Toxic Heavy Metals in Fish after Feeding with Synthetic Feed: A Potential Health Risk in Bangladesh

Md. Abdul Mannan^{1*}, Md. Saddam Hossain¹, Md. Al-Amin Sarker², Md. Motahar Hossain¹, Liton Chandra¹, ABM Hamidul Haque¹ and Md. Kudrat-E-Zahan¹

¹Department of Chemistry, Rajshahi University, Rajshahi, Bangladesh

²Department of Fisheries, Rajshahi University, Rajshahi, Bangladesh

Abstract

This present research was conducted to assess the bioaccumulation of toxic heavy metals in fish before and after six months feeding with synthetic feed. Here in concentrations of eight toxic heavy metals such as chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn), iron (Fe) and manganese (Mn) were determined by atomic absorption spectrophotometric (AAS) method. Before feeding, the concentrations in fish were found to be 19.5 ppb, 61.8, 30.5, 35.3, 123.3, 121.9, 197.7 and 543.2 ppb, respectively. And after feeding with synthetic feed, the concentrations were found to be 22.1 ppb, 101.5, 37.7, 102.0, 147.6, 214.1, 175.8 and 508.0 ppb, respectively. Contents of the trace metals in the feed, fresh water and the water after addition with synthetic feed were also evaluated. The *Labeo rohita* locally known as "Rui fish" has been selected for this study since it is a very popular fish in the rural as well as in urban peoples of Bangladesh. The accumulation of beneficial elements *i.e.*, Cu, Zn, Fe and Mn were found to be within the permissible limit in both the fishes of before and after feeding with the synthetic feed. Concentrations of potential toxic metals *i.e.*, Cd, Pb and Ni (37.7 ppb, 102.0, and 147.6 ppb, respectively) accumulated in the fish after six months feeding were found to be much higher than that of the recommended permissible limit. Concentrations of Cd, Pb and Ni (21.6 ppb, 102.0 and 114.0 ppb, respectively) in the synthetic feed used for feeding were also found to be much higher than the WHO/EU recommended value. Considering the potential health risk issues, it is recommended that the use of natural feed should be encouraged for feeding despite the synthetic feed in the fish farming project.

Keywords: Heavy metals; *Labeo rohita*; Atomic absorption spectroscopy; Wet digestion; Synthetic feed

Introduction

The fish and fisheries are integral part of the culture and heritage of Bangladesh. This sector is quite significant in the country as it offers an important source of earning livelihood, foreign exchange and food supply. This sector is the second largest part-time and fulltime employer in the rural areas. Bangladesh is being considered as one the most suitable region for aquaculture and fisheries in the world since the country has huge area of coastal belt. Fish is the second most valuable agricultural crop and its production contributes to the livelihoods and employment of millions of people of Bangladesh. About 10% of the population directly or indirectly depends on fisheries. The culture and consumption of fish, therefore, has important implication for national income and food security. Sustainable fisheries and aquaculture play a crucial role in food and nutrition security and in providing for the livelihoods of million people of Bangladesh. Nutrition, obviously, plays an important role in the maintenance of a healthy and marketable product.

In the recent year, the concentrations of heavy metals have been increased in the environment than that of the natural abundance. This situation has arisen as a result of the rapid growth of population, increased urbanization, and expansion of industrial activities, exploration and exploitation of natural resources, extension of irrigation and other modern agricultural practices as well as the lack of environmental regulations. This increase of heavy metal in environment is great alarming for the human and animals. This can be visualized especially in aquatic ecosystems, since the production of fish in fish farm has been increased rapidly [1]. In the fish farming, enough synthetic feed has been used as fish feed that contain toxic trace heavy metals such as Pb, Cd, Cr, Cu, Zn, Ni etc. [2]. Accumulation of heavy metals in fish is a clear indication for the contamination. Contamination of heavy metals

in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem. Fish is an important source of protein and it also provides essential omega-3 fatty acids (docosahexaenoic and eicosapentaenoic acids) that help to maintain cardiovascular health by playing a major role in the regulation of blood clotting and vessel constriction [3]. Trace metals are essential for human health and they prevent diseases, but when these elements are in high concentration then they would play carcinogenic action to human health [4]. For example, lead (Pb) has carcinogenic properties, and it impairs both the respiratory and digestive systems and suppresses the immune system. This metal is particularly harmful in children and damaging their intelligence and nervous systems [5,6]. Cadmium (Cd) accumulates easily in the circulatory system, kidney (especially the renal cortex), lung, and heart, and is toxic to bones and gonads. These risks are recognized by the International Agency for Research on Cancer and the National Toxicology Program, and Cd has been classified as a group-1 carcinogen. Chromium (Cr) can exist in several oxidation states [7-9]. Among them the hexavalent chromium (VI) is highly soluble and mobile and is harmful to the skin, liver, kidney, and respiratory organs, causing various diseases, such as dermatitis, renal tubular necrosis,

***Corresponding author:** Dr. Md. Abdul Mannan, Associate Professor, Department of Chemistry, Rajshahi University, Bangladesh, Tel: +8801753841119; E-mail: mannan.chem@ru.ac.bd

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perforation of the nasal septum, and lung cancer etc. and therefore, *Cr* is categorized as group-1 carcinogen. Excessive copper (*Cu*) intake can cause nausea, vomiting, kidney failure, blood cell damage, and central nervous system inhibition. Nickel (*Ni*) is primarily accumulated in the spinal cord, brain, and organs due to its mutability and carcinogenicity and is also categorized as group-1 carcinogen [10,11]. Therefore, contamination with heavy metals that might be accumulated in the various organs of fishes; and such accumulation may affect humans and other species that depend on such fish as food.

In this study, we have analyzed the concentration of heavy metals bioaccumulated in fish before and after feeding with synthetic feed. We also analyzed the metal concentrations in the supplied feed and in water sample before and after addition of the feed in the experimental pond. The data were then compared against the recommended maximum permissible levels allowed in food.

Material and Methods

Chemicals

Analytical grade nitric [Aldrich, 69% (w/w)] and hydrochloric [Aldrich, 36% (w/w)] acids were used for the digestion of fish samples. Standard solutions of *Cr*, *Cu*, *Cd*, *Pb*, *Fe*, *Mn*, *Ni*, and *Zn* were obtained from Merck Germany. The standard solution was diluted to 0.5, 1.0, 1.5 and 2.0 ppm with double distilled water. The absorbance of the solutions was measured immediately and finally, a calibration curve of concentration vs absorbance was constructed.

Selection of pond

This study was conducted through the cage culture system placed in pond. The pond was located at the west side of Rajshahi University residential area, Bangladesh. The study was carried out for the period of 180 days (six months) from December 2016 to May 2017. The average depth of the pond was 2 m, square in shape of 250 m², well exposed to sunlight, independent and completely free from aquatic vegetation. The pond has inlet and outlet facilities. The main source of water was rainfall but there are facilities to supply water from a deep tube-well whenever required.

Preparation of cage

Two cages each of 1 m³ size were made to run this experiment. A photograph of preparation of a typical cage is shown in Figure 1. The frame of the cages were made of metallic iron rod and covered by synthetic special nylon knotless net (Makorsha brand) with 5 mm mesh size. The mesh size of the net was selected as it required to prevent the fishes from escaping from the cage and to keep water passing easily through it. An opening was kept at the top of each of the cages for supplying feed and handling of the fishes. The sinking feed was supplied through this opening. The cages were submerged 15 cm above of the pond bottom. Two vertical and a horizontal bamboo poles were used to hang the cages. Nylon rope was used to tie the cages to make it float with the help of the bamboo poles.

Feeding, cage management and collection of fish samples

A very popular and well-known fish *Labeo rohita* local name "Ruhi" is selected for this study. According to fish size and weight a required amount of feed were given into the experimental cages. The synthetic feed purchased from the local market was given twice in a day (in the morning at 9:00 AM and in the evening at 5:00 PM). The net of the cage was cleaned regularly during feeding time and dead fish if observed was removed to keep the environment good for fish. The

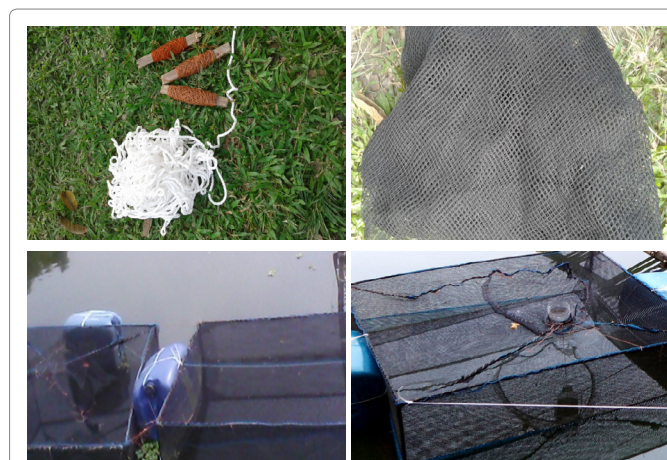


Figure 1: Photograph of preparation of cage which was placed in the experimental pond for farming of *Labeo rohita*. Synthetic feed was used for feeding of the fish during the experimental period of six months.

fishes were sampled initially and after six months interval with feeding. The initial (200 g) and final body weights (900 g) of the fishes were recorded and the growth rate was determined in terms of increase of its weight. After six months, the fish was sampled and dissected its edible parts using stainless steel knife and quickly wrap with plastic bags. The bags were frozen in icebox until digestion. The samples were then dried overnight on oven at 105°C. The dried specimen of the fish tissue was then powdered in a blending machine.

Digestion of fish and feed samples

Digestion of the fish and the synthetic feed materials were performed by wet ashing digestion method [12]. The dried specimen was transferred to the furnace oven and completely dried at 550°C. The dried sample was then powdered using mortar and pestle and made ready for digestion process. 2.0 g of the powdered sample was placed in a 100 mL round bottom flask with ground glass joint and mineralized under reflux using a mixture of 12.0 mL nitric acid; 4.0 mL perchloric acid. The digestion procedure took 5 h to obtain clear solution. The solution was then filtered with whatmann filter paper and transferred to a 100 mL volumetric flask and the volume was made to mark. Digestion of the feed materials was same as discussed for the fish sample.

Digestion of water samples

The collected water samples were filtered with whatmann filter paper and acidified with 2.0 mL conc. HNO₃ acid. The acidified water samples were then transferred to a 100 mL volumetric flask and the volume was made to mark.

Instrumentation and calibration

Eight toxic heavy metal concentrations in the fish sample such as: *Cr*, *Cd*, *Cu*, *Pb*, *Fe*, *Mn*, *Ni*, and *Zn*, were determined by atomic absorption spectrophotometer (SHIMADZU, AA-6800) equipped with flame and graphite furnace at the Central Science Laboratory of Rajshahi University. Detailed instrumental measurement conditions are outlined in Table 1. All the analyses were performed using an air-acetylene flame for the atomization of metal content. Calibration was accomplished using a linear fit for all the elements. The data were rounded off suitably according to the value of standard deviation from measurements in triplicate.

Physico-chemical water quality parameters

The water quality parameters such as temperature (°C), transparency (cm), pH, dissolved oxygen (mg L⁻¹) and total alkalinity (mg L⁻¹) were measured on the spot at the fortnightly interval at 10:00 AM. The results are shown in Table 2. Toxic heavy metal concentrations were analysed in the Central Science Laboratory of Rajshahi University and are shown in Table 3.

Results

During the experiment, water quality of the experimental pond has been evaluated. The results of the physico-chemical properties are shown in Table 2. It has been observed that the water quality physico-chemical parameters were not significantly changed after six months feeding of the synthetic fish feed. Concentrations of the typical eight toxic harmful trace metals such as *Cr*, *Cu*, *Cd*, *Pb*, *Ni*, *Zn*, *Fe* and *Mn* of water of the experimental pond as well as in the synthetic feed were analyzed. The results are shown in Table 3. It has been found that the concentrations of three metals viz. *Cd*, *Pb* and *Ni* in the feed samples are exceeded the recommended value. The concentrations of *Cd*, *Pb* and *Ni* are 21.6, 102.0 and 114.0 ppb, respectively. These three trace elements are found to be very toxic for the human health. The other five elemental concentrations of *Cr*, *Cu*, *Zn*, *Fe* and *Mn* were not exceeded the WHO and EU standard. The concentrations of other elements viz. *Cr*, *Cu*, *Zn*, *Fe* and *Mn* in the feed sample were found to be 19.5, 38.2, 85.5, 197.0 and 520.7

ppb, respectively. Similarly, to feed sample, concentrations of *Cd*, *Pb* and *Ni* in both the water samples were also found to be exceeded the recommended value. It was found that the concentration exceeded the recommended value before addition of feed in the pond. The concentration of *Cu*, *Cd*, *Pb*, *Ni*, *Zn*, *Fe* and *Mn* are increased from 28.3 to 38.7; 23.3 to 29.2; 15.7 to 47.10; 114.10 to 141.0; 10.6 to 18.4; 51.2 to 57.5 and 503.9 to 551.7 ppb, respectively after addition of the synthetic fish feed in the pond. Comparable concentration of *Cr* was found in both the samples which was not exceeded the standard value [13].

The metal concentrations were determined in fish before and after six months feeding of the synthetic feed. The results are shown in Table 4. It is seen from the table that all the metal concentrations are increased in both the fish-1 and fish-2 samples. Before feeding, the *Cd*, *Pb* and *Ni* concentrations 30.5, 35.3 and 123.3 ppb, respectively, in fish-1 was found to be enough higher than that of the recommended value. In fish-2, the concentration increased from 30.5 to 37.7; 35.3 to 102.0 and 123.3 to 147.6 ppb, respectively. The other metal concentrations of *Cr*, *Cu* and *Zn* were increased significantly from 19.5 to 22.1; 61.8 to 101.5; and 121.9 to 214.1 ppb, respectively after six months feeding of the fish. The concentration of *Fe* and *Mn* were not increased but decreased from 196.7 to 175.8 and 543.2 to 508.0 ppb, respectively. The release of *Fe* and *Mn* might be due to the incorporation of the digestive functions of the fish body.

Elements flow	Wavelength (nm)	Current (mA)	Burner height (mm)	Burner angle (degree)	Slit width (nm)	Fuel gas flow (L/min)	Lighting mode	Type of oxidant
Fe	248.3	10	7	0	0.2	2.0	BGC-D2	Air-acetylene
Mn	279.5	10	7	0	0.2	2.0	BGC-D2	Air-acetylene
Ni	232.0	12	7	0	0.2	2.2	BGC-D2	Air-acetylene
Zn	213.9	8	7	0	0.5	2.0	BGC-D2	Air-acetylene
Cr	357.9	10	7	0	0.5	2.0	BGC-D2	Air-acetylene
Cu	324.8	6	7	0	0.5	1.8	BGC-D2	Air-acetylene
Cd	228.8	8	7	0	0.5	1.8	BGC-D2	Air-acetylene
Pb	217.0	12	7	0	0.5	2.0	BGC-D2	Air-acetylene

Table 1: Operating conditions for the determination of the heavy metals in the fish samples.

Properties	Water-1	Water-2
Temperature (°C)	26.03 ± 0.06	27.83 ± 0.29
Transparence (cm)	31.67 ± 0.58	32.33 ± 1.53
pH	7.17 ± 0.29	7.03 ± 0.06
Dissolve oxygen (mg L ⁻¹)	5.03 ± 0.81	5.90 ± 0.20
Alkalinity (mg L ⁻¹)	125.67 ± 2.52	111.00 ± 2.65

Table 2: Variations in values of physico-chemical properties of water. The samples water-1 and water-2 are designed as the initial and after six months addition of synthetic feed of the experimental pond.

Metals	Feed (ppb)	Water-1 (ppb)	Water-2 (ppb)	WHO Standard (ppb) [13]	EU Standard (ppb) [13]
Cr	19.50	35.20	35.20	50	50
Cu	38.20	28.30	38.70	2000	2000
Cd	21.60	23.30	29.20	3	5
Pb	102.00	15.70	47.10	10	10
Ni	114.00	114.10	141.00	20	20
Zn	85.50	10.60	18.40	3000	N/M
Fe	197.00	51.20	57.50	N/M	200
Mn	520.70	503.90	551.70	500	50

N/M= not mentioned

Table 3: Metal concentrations of the synthetic feed, water samples of before addition of the feed (Water-1) and after six months addition of the feed (Water-2). Feed was given twice (morning and in the evening) in a day with the amount of around 300 g in each time.

Discussion

In the recent year, production of fish has been increased tremendously because fish consumption in the rural as well as in the urban area has also been increased. This study was taken to investigate heavy metal concentrations in fish since a lot of synthetic feed has been used for feeding purposes. To our knowledge, so far, no study has been reported on the heavy metal concentrations in fish sample before and after feeding of the synthetic feed. The physico-chemical properties such as temperature, transparency, pH, dissolve oxygen and alkalinity of water quality of the experimental pond have been also studied. Our study revealed that the physico-chemical properties of the water quality are not changed appreciably after addition of the synthetic feed during the six months experiment Table 2. Therefore, it is presumably suggested, the water quality of the experimental pond would be quite good for the study.

The comparative study of harmful toxic heavy metal concentrations such as *Cr*, *Cu*, *Cd* and *Pb* and essential metal concentrations such as *Ni*, *Zn*, *Fe* and *Mn* in feed, water-1 and water-2 is shown in Figure 2. The level of metal concentrations recorded in feed and water-1 are generally low in comparison to that of the water-2. The concentration of *Cd*, *Pb* and *Ni* in all the samples has exceeded both the WHO and EU recommended value. The other metal concentrations has been exceeded the recommended value. All the metal concentrations have been increased in water-2 after six months addition of the synthetic feed.

The comparison study of the harmful metal concentrations in fish before feeding (fish-1) and after six months feeding (fish-2) is presented in Figure 3. It is revealed that all the metal concentrations have been increased in fish-2 except the *Fe* and *Mn*. The level of *Cd*, *Pb* and *Ni* were increased tremendously and exceeded the WHO and EU recommended value. These metals causes severe health hazard for human health.

Chromium (*Cr*) is known to be one of the most environmental toxic pollutants. In principle, *Cr* present in food in the trivalent form [*Cr* (III)] is usually an essential nutrient but the hexavalent form [*Cr* (VI)] is more toxic and normally not found in the food chain. The trivalent *Cr* is necessary for the normal metabolism of cholesterol, fat and glucose [14]. The concentration of *Cr* in both fish-1 and fish-2 is lower than that of the water samples. This means that *Cr* does not accumulate in the fish from feed and water.

Copper (*Cu*) is an essential trace element which is necessary for normal biological activities of amino acids and is required for some essential enzymes such as super oxide dismutase, cytochrome oxides,

Metals	Fish-1 (ppb)	Fish-2 (ppb)	WHO Standard (ppb) [15]	EU Standard (ppb) [15]
Cr	19.50	22.10	50	50
Cu	61.80	101.50	2000	2000
Cd	30.50	37.70	3	5
Pb	35.30	102.00	10	10
Ni	123.30	147.60	20	20
Zn	121.90	214.10	3000	N/M
Fe	196.70	175.80	N/M	200
Mn	543.20	508.00	500	5

N/M= not mentioned

Table 4: Metal concentrations of the fish samples of before feeding (Fish-1) and after six months feeding (Fish-2). Feed was given twice (morning and in the evening) in a day with the amount of around 300 g in each time.

lysyl oxides, etc. Excess of *Cu* might result in dermatitis, metallic taste in mouth, hair and skin decoloration etc. It is also essential for normal plant growth but it could be toxic at excessive level. The permissible limit set by FAO/WHO/EU was 2000 ~ 3000 ppb [13,15]. It plays an important role in some neurological conditions like Alzheimer's disease, Wilson's disease etc. Therefore, *Cu* has been considered as a toxic heavy metal. The estimated *Cu* concentration in the studied synthetic feed, water-1 and fish-1 samples (38.2 ppb, 28.3 and 61.8 ppb, respectively) were much lower than that of the recommended value. After six months feeding, the concentrations of *Cu* in water-2 and fish-2 samples were found to be 38.7 ppb and 101.5 ppb, respectively. These levels of concentrations are also lower than that of the permissible value. However, the concentration of *Cu* is increased in both water and fish samples after feeding. This means that *Cu* is accumulated in fish after feeding.

Cadmium (*Cd*) is regarded as an awfully toxic trace element which biochemically replaces Zn that cause high blood pressure. Chronic exposure of *Cd* causes respiratory distress, lung and breast cancers, haemorrhagic injuries, anemia and cardiovascular disorders. It damages kidney and liver [16,17]. The concentration of *Cd* in feed, water-1 and fish-1 samples were found to be 21.6 ppb, 23.3 and 30.5 ppb, respectively. It is observed that the concentrations are much higher than that of the FAO/WHO/EU recommended value (2 ~ 5 ppb) [13,15]. After six months feeding, the concentrations in water-2 and fish-2 samples increased to 29.2 and 37.7 ppb, respectively. This

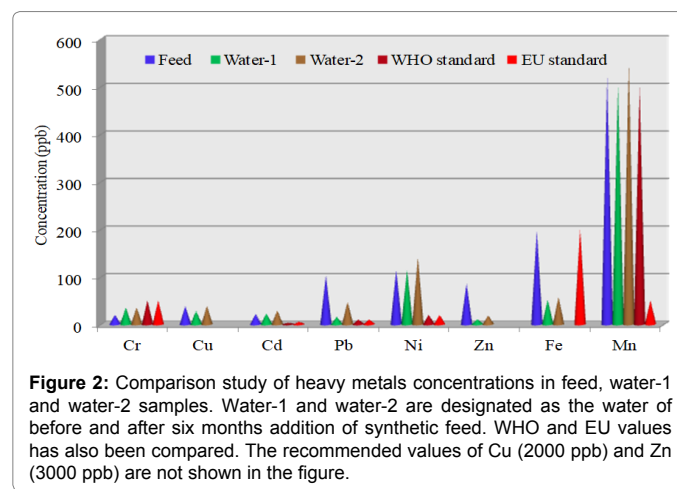


Figure 2: Comparison study of heavy metals concentrations in feed, water-1 and water-2 samples. Water-1 and water-2 are designated as the water of before and after six months addition of synthetic feed. WHO and EU values has also been compared. The recommended values of Cu (2000 ppb) and Zn (3000 ppb) are not shown in the figure.

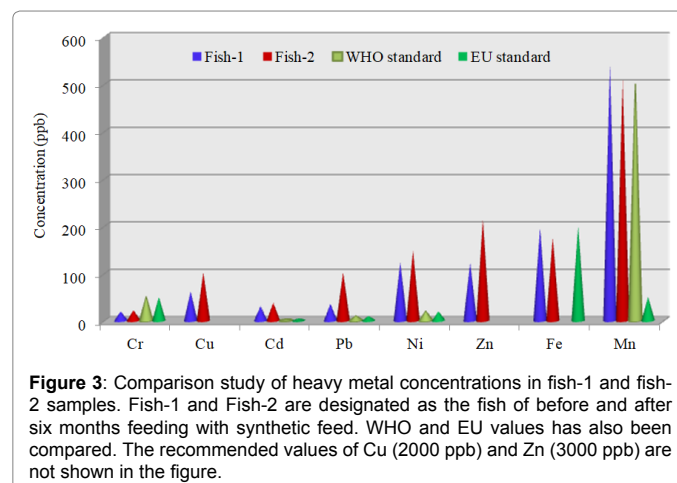


Figure 3: Comparison study of heavy metal concentrations in fish-1 and fish-2 samples. Fish-1 and Fish-2 are designated as the fish of before and after six months feeding with synthetic feed. WHO and EU values has also been compared. The recommended values of Cu (2000 ppb) and Zn (3000 ppb) are not shown in the figure.

indicated that concentration of *Cd* in water and fish has been increased after feeding. Since, the *Cd* level is increased rapidly in fish after feeding; therefore, it is suggested to avoid the *Cd* containing synthetic feed in fish culture. The *Cd* profiles evaluated in this study is much higher than the permissible limits, thus, consumption of fish obtained from fish farm could be *Cd*-induced health hazard.

Exposure of lead (*Pb*) is of great concern mainly because of its acute toxicity even at trace level. Number of studies has been revealed that *Pb* can adversely affect the central and peripheral nervous system, growth and development, cognitive development, renal system, blood circulation, mental retardation, reproductive health and even can cause death [18,19]. The recorded concentrations of *Pb* in feed, water-1 and in fish-1 samples were found to be 102.0 ppb, 15.7 and 35.3 ppb, respectively. After six months feeding, the concentrations of *Pb* in collected water-2 and in fish-2 samples were found to be recorded as 47.10 and 102.0 ppb, respectively. The permissible limit of *Pb* is only 10 ppb. This is indicated that the *Pb* is bioaccumulated in fish with greater extend after feeding of the synthetic feed. The increased level of *Pb* in fish might be due to the higher level of *Pb* in the feed sample.

The major source of nickel (*Ni*) for human is food and uptake from natural sources, as well as food processing [20]. High intake of *Ni* can cause the cancer of the lung and nasal cavity. The *Ni* toxicity include skin rash, nausea, dizziness, diarrhea, headache, vomiting, chest pain, weakness and coughing. Contact with *Ni* vapour can cause to swelling of brain and liver, degeneration of the liver, irritation to the eyes and various types cancer [21]. The content of *Ni* concentrations (114.0 ~ 147 ppb) in feed, water and fish samples were also found to be exceeded the permissible limit. The bioaccumulation of *Ni* has been observed after the feeding of the synthetic feed.

Zinc (*Zn*) is an essential trace element for human health which is required for the normal development and metabolic functions. But if its concentration exceeded the physiological requirements, it can act as a toxicant and leading to decreased fertility [22]. *Zn* toxicity is rare, but at concentrations in water up to 4000 ppb might induce toxicity which characterized by the symptoms of irritability, muscular stiffness and pain, loss of appetite and nausea [23]. The concentrations of *Zn* in feed, water-1 and water-2 samples were found to be 85.5 ppb, 10.6 and 18.8 ppb, respectively. The estimated concentrations in fish-1 and fish-2 were 121.9 and 214.1 ppb, respectively. In fact, the *Zn* concentration was higher in the fish samples before feeding. Although the values are not exceeded the recommended permissible limit.

Iron (*Fe*) is one of the essential nutrients for the proper physiological functioning of the body organs. It is involved in the synthesis of haemoglobin in the red blood corpuscles of the blood and also helps with red blood cell production. Severe *Fe* deficiency may cause anaemia in humans. When the body absorbs more *Fe*, then the excess *Fe* will be stored in the liver, pancreas and in the heart. *Fe* was found to have the highest concentration (197.0 ppb) in the feed sample which is lower than the WHO/EU recommended value. After feeding, the concentration is little increased in the water-2 sample from 51.2 to 57.5 ppb, however, the concentration was found to have lowest in fish-2 (175.8 ppb) than from the fish-1 (196.7 ppb) sample. This decreased of the *Fe* concentration might be due to the biological functioning of the fish body, since *Fe* is needed for the production of red blood cells.

Manganese (*Mn*) is an essential micronutrient for animals as well as for plants. Its deficiency results severe skeletal and reproductive abnormalities in animals. It does not occur naturally as a metal but frequently associated with iron deposits. In aquatic environment, it has

been attributed to ferromanganese minerals, clay minerals etc. [24]. The levels of *Mn* in feed, water and fish samples are not significantly exceeded the permissible limit before and after feeding with the synthetic feed. Therefore, *Mn* is not concerned in the present study.

Conclusion

We have studied the bioaccumulation of toxic heavy metals in fish before and after six months feeding with synthetic feed. The results obtained in this research revealed that the highly concerned toxic *Cd* and *Pb* accumulated in fish with concentration much greater than the recommended value. The *Ni* concentration was also found to be higher than the permissible limit. The other metal concentration increased in fish after feeding at a certain level but not exceeded the WHO/EU recommended value. The metal concentrations in feed, fresh water, and water collected after six months feeding were almost lower than that of the WHO/EU value. The highest value of toxic metals accumulated in fish might be due to feeding with the synthetic feed. Since, the feed contains the high level of *Cd*, *Pb* and *Ni* concentrations. Finally, considering the safety aspects of human health concern, the use of synthetic feeding that containing high level of *Cd*, *Pb* and *Ni* must be avoided in the fish farming project rather than the natural feeding.

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Author Contribution

MAM conceived and designed the research proposal and wrote the final draft of the paper. MSH and LC performed the experimental works. MAS provided the fish samples of the study. MMH, ABMH and MKZ revised the draft of the paper. All authors read and approved the final manuscript.

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