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Original Article

Metal contamination of commercial fish feed and quality aspects of farmed tilapia (*Oreochromis niloticus*) in Bangladesh

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ABSTRACT: In this study, we evaluated the heavy metal load of the formulated commercial fish feeds and heavy metal burden in cultured tilapia (*Oreochromis niloticus*) in Muktagacha, Mymensingh, one of the concentrated aquaculture regions of Bangladesh. Three of the most commonly used commercial fish feeds namely Quality, Mega, Rupshi, manufactured by Quality feeds limited, Spectra hexa feeds limited., and Rupshi fish feed limited respectively and tilapia were collected from nine aquaculture farms selected randomly from the Muktagacha, Mymensingh region. Heavymetal (Cu, Fe, Pb, Cd, Na, Cr, Ni) concentrations were measured using Atomic Absorption Spectrometer (AAS) in edible muscle from fish and in fish feeds. We observed higher concentrations of Cd, Fe, Na and Pb in fish feeds and in fish muscles, all of which exceeded the World Health Organization's or other standard limits for food safety. The highest target hazard quotient (THQ) value was estimated for Na (1.51) followed by Cd (1.45), which were higher than the acceptable guideline of US Environmental Protection Agency (USEPA). In case of target cancer risk (TR), consumption of tilapia at current accumulation level is safe but continuous and excess consumption for a lifetime of more than 70 years are in risk of cancer.

KEYWORDS: Fish Feed, Tilapia, Metal contamination, Aquaculture

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INRODUCTION

In Bangladesh, fisheries and aquaculture sector is one of the major component of agricultural activities and plays a crucial role in economic development by ensuring food security and stimulating the growth of a number of subsidiary industries. About 11% of the local population directly and indirectly depends on fisheries for their livelihood[1]. Bangladesh has established milestone in aquaculture development and in 2014, the country was ranked 6th in global farmed fish production[2]. With the increasing demand for fish and the decline in capture fisheries production, aquaculture in Bangladesh is heading towards intensification. The Nile tilapia, (*Oreochoromis niloticus*) is a widely cultured fish because it can grow and reproduce in a wide range of environmental conditions and tolerate stress induced by handling[3]. Production of Nile tilapia from ponds of Bangladesh was 8,221 MT which was only 1.52% of total pond fish production in the year 2001-2002. This production has increased to 98,758 MT which is 8.10% of total pond fish production in the year 2010-11[4]. In Bangladesh, fish farmers shift gradually from no feed, through the use of farm-made feeds, to factory-made feeds[5]. The success of intensive and semi-intensive fish culture depends on a large extent to the application of suitable feeds. Scaling up of fish culture in Bangladesh has expanded the fish feed industry in recent years and some ten well-known industries are Rupshi Feed, Mega feed, Saudi-Bangla Feed, Paragon Feed, Quality Feed, ACI Feed, C.P Feed, Kayer Feed, AIT Feed, New Hope Feed. On the other hand, hundreds of small-scale non-commercial and on-farm feed industries produce fish feed throughout the country.

Besides environmental pollutants, chemical contamination in food fish has become a global concern. Fish as contributing more than 50 % of animal protein source of Bangladeshi diets[2] gets highest concern. Heavy metals contamination comprises significant portion of the problem as these metals known for their bioaccumulation and bio-magnification, which cause various health hazards to human[6]. Fish bioaccumulate chemicals directly from polluted water by diffusion through gill and skin or they ingest with food[7]. In wild fish a wide range of organo-chemical and metal contaminants are reported[8] and aquaculture fish also found to be contaminated. Polluted fish could be a dangerous dietary source of certain toxic heavy metals to human[9].

Aquaculture relies heavily on formulated feed and among animal protein sources fishmeal is a common ingredient in most fish feed formulation. Some of the commercial feed producers failed to meet up with standards for the requirement of fish and in many ways, the source of raw material for the production of the feeds tends to be contaminated with heavy metals[10]. There is lack of information on the heavy metals load of fish feeds used in aquaculture in Bangladesh. Although contamination of animal feeds by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health. In the present study, we aim to evaluate the presence of heavy metals in commercial fish feeds and their accumulation in tilapia fish tissues in order to assess the carcinogenic and non-carcinogenic risk associated with consumption of the farmed tilapia fish in Bangladesh.

MATERIALS AND METHODS

Collection of samples

Fish feed and fish (*Oreochromis niloticus*) samples were collected from Muktagacha Upazilla of Mymensingh, Bangladesh. Three most commonly used feeds brands, namely: Quality, Mega, and Rupshi were identified from key informant interviews in the region. Fish feed were collected in sealed sampling bag. Three categories of fish feeds (nursery, starter, and grower) of each brand were sampled from nine Tilapia farms. Adult freshwater Tilapias of 100 g to 150 g were collected from tilapia farms. After collection, the samples were cleaned with deionized-distilled water, stored in pre-cleaned plastic bags, and transported in icebox to the laboratory and stored in freezer (-20 ^{o}C) for further analysis.

Preparation of the samples

The fish muscles (0.4 to 0.5 g) were separated and dried in an oven at 80 ^{0}C until constant weight was obtained. Afterwards, samples were homogenized and placed in a teflon digestion vessel with 7 ml of ultra-pure HNO_3 and 1 ml of H_2O_2. Sealed containers were placed in a microwave oven and heated according to the digestion program (Power 1600W (100%), Ramp time 15 min, Temperature 200º C, Hold time 15 min and cooling time 10 min). After digestion, sample solutions were cooled to room temperature and transferred quantitatively into acid cleaned 100 ml standard volumetric flasks and made up to 100 ml with double distilled deionized water. Microwave digestion is used instead of classical methods because of its shorter time, less acid consumption, and ability to retain volatile compounds in the solutions[11].

Analysis of heavy metal concentrations

Fish muscle content of lead (Pb), nickel (Ni), copper (Cu), iron (Fe), sodium (Na), and chromium (Cr) were determined with a flame atomic absorption spectrophotometer (Model Shimadzu AA-7000) using acetylene gas as fuel and air as an oxidizer. All the equipment used in the experiment were acid washed for 24 h in diluted nitric acid (10% v/v); afterwards, were rinsed several times with deionized water. 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. Flame atomic absorption spectroscopy technique has been widely employed for elemental analysis in a number of matrices such as soils, water, nuts wine and wine products[12]. The technique is based on the principle of ground state metals absorbing light at specific wavelength and relies on Beer

Lambert's law[13]. The atoms of lead (Pb), nickel (Ni), copper (Cu), iron (Fe), and chromium (Cr) are known to absorb radiations at wavelengths of 217 nm, 232 nm, 324.8 nm, 248.3 nm 228.8 nm and 357.9 nm, respectively from a hollow cathode lamp[14]. 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 = (mg/kg) R× dilution factor

Where:
(mg/kg)R = AAS Reading of digest
Dilution Factor = Volume of digest used ⁄ Weight of digested sample

Health risk estimation

Target hazard quotient

The target hazard quotient (THQ) is an estimate of the risk level (non-carcinogenic) due to pollutant exposure. To estimate the human health risk from consuming metal-contaminated fish, the target hazard quotient (THQ) was calculated as per USEPA Region III Risk-Based Concentration Table[15]. The equation used for estimating THQ was as follows:

$$THQ = \frac{EF \times ED \times FIR \times Cf \times CM}{WAB \times ATn \times RfD} \times 10^{-3}$$

Where THQ is the target hazard quotient, EF is the exposure frequency (365 days/year), ED is the exposure duration (30 years for non-cancer risk as used by USEPA 2011), FIR is the fish ingestion rate (49.5 g/person/day)[16], Cf is the conversion factor (00.208) to convert fresh weight (Fw) to dry weight (Dw) considering 79 % of moisture content in fish, CM is the heavy metal concentration in fish (mg/kg dry weight.), WAB is the average body weight (bw) (70 Kg), ATn is the average exposure time for non-carcinogens (EF×ED) (365 days/year for 30 years (i.e., ATn=10,950 days) as used in characterizing non-cancer risk[15], and RfD is the reference dose of the metal (an estimate of the daily exposure to which the human population may be continuously exposed over a lifetime without an appreciable risk of deleterious effects).

Hazard index

The hazard index (HI) from THQs is expressed as the sum of the hazard quotients[15].

HI=THQ(Fe) + THQ(Cr) +THQ(Cd) +THQ(Na)+ THQ(Cu) +THQ(Ni)+ THQ(Pb)

Where HI is the hazard index, THQ (Fe) is the target hazard quotient for Fe intake, and so on.

Target cancer risk

Target cancer risk (TR) was used to indicate carcinogenic risks. The method to estimate TR is also provided in USEPA Region III Risk-Based Concentration Table[15]. The model for estimating TR was shown as follows:

$$TR = \frac{EF \times ED \times FIR \times CF \times CM \times CPSo}{WAB \times TAc} \times 10^{-3}$$

Where TR is the target cancer risk, CM is the metal concentration in fish (μg/g), FIR is the fish ingestion rate (g/day), CPSo is the carcinogenic potency slope, oral (mg/kgbw/day), and ATc is the averaging time, carcinogens (365 days/year for 70 year)[15]. Since CPSo values were known for Ni, Cd, and Pb, so, TR values were calculated for intake of these metals.

Statistical analysis

The data were statistically analyzed using the statistical package, SPSS 16.0 (SPSS, USA). The means and standard deviations of the metal concentrations in fish species were calculated. Multivariate post hoc Tukey's tests were performed to examine the statistical significance of the differences in mean concentrations of trace metals among different fish for each metal.

RESULTS

Concentration of heavy metals in fish feed and tissue

Mean concentrations and standard deviation of heavy metals in different fish feed and edible tissue (dorsal portion) of tilapia fed with those feed are presented in Table 1. Cu, Fe, Pb, Cd and Na were found in all feed and fish samples, whereas, no Cr and Ni were found within the detection limit (0.0001 mg/kg). There was no significant ($p < 0.05$) difference in metal concentrations among feeds. Concentration of all metals in fish tissue fed with different feeds were also not significantly ($p< 0.05$) different.

Copper (Cu)
Copper concentration in fish feed were in a range of 22.618 to 38.480 mg/kg (Table 1).The highest mean concentration of Cu (38.48 mg/kg) was found in Mega (nursery), while the Quality (starter) had the lowest (22.61 mg/kg). Cu content in tilapia flesh ranging from 19.073 to 25.343 mg/kg in all fish farms (Figure 1 A), which is below the permissible limit (30 mg/kg) of Cu proposed by WHO and FAO[17].

Iron (Fe)
The mean concentrations of iron (475.878 to 10004.855 mg/kg) were found above the WHO standard for feed (Table 1). The Rupshi starter feed had the highest mean concentration of 10004.86 mg/kg. Iron content in fish samples also exceed the permissible limit (100 mg/kg) proposed by WHO and FAO[17]. Iron content in fish varied from 136.241 to 200.26 mg/kg, with the highest content found in Quality feed fed tilapia (Figure 1 B).

Lead (Pb)
The mean concentrations of lead in the different brands of feeds were in the range of 7.671 to 12.232 mg/kg (Table 1). The highest mean concentration (12.232 mg/kg) was observed in the Mega-nursery brand. The Quality brand had lowest concentrations for the grower. All of the feed samples exceeded the maximum acceptable limit (5mg/kg) for lead in feed as stipulated by European Union[18]. Lead was detected in all examined fish samples and its concentration ranged from 6.787 to 16.386 mg/kg, with the highest content found in Mega feed fed tilapia (Figure 1 C). The permissible limit of Pb proposed by WHO and FAO is 2 mg/kg fresh weight[17].

Cadmium (Cd)
The concentrations of cadmium observed were in a range of 8.082 to 9.771 mg/kg. The Mega-Nursery had comparatively higher mean concentration of Cd compared to the other feed types while the Quality-Nursery had the lowest. However, all feed had higher mean concentration of cadmium comparing with the maximum acceptable limit of 2 mg/kg stipulated by European Union[18]. Cadmium was detected in all examined fish samples at a high level; ranged from 9.083 to 10.453 mg/kg (Figure 1 D) which is far exceeded the permissible level of 1 mg/kg fresh weight[17]. The highest Cd content found in Quality feed fed tilapia.

Sodium (Na)
We observed a very high level of sodium (891.046 to 1079.131 mg/kg) in all feed tested. The Rupshi starter had the highest mean concentration of 1079.131 mg/kg. Sodium concentration ranged from 821.33 to 885.13 mg/kg in tilapia (Figure 1 E), with the highest content found in Mega feed fed tilapia.

Health risk estimation
The risk associated with the carcinogenic effects of target metal is expressed as the excess probability of contracting cancer over a lifetime of 70 years. The target hazard quotient (THQ) estimated for individual metal through consumption of different fish species is presented in Table 2. The acceptable guideline value for THQ is 1[15]. THQ values were less than 1 for all individual heavy metal in all three brands feed fed tilapia indicating no non-carcinogenic health risk from ingestion of a single heavy metal through consumption of these fishes. The highest THQ value was estimated for Na (1.51) followed by Cd (1.45) contaminated in all farms. Cd, Cu, Na and Pb contributed the most in the HI in all farms. Target cancer risk (TR) values were estimated for the metals reported with known carcinogenic effects. The TR values for Cd and Pb ranged from 9.12E-03 and 1.24E-05 in Quality 8.74E-03 and 1.85E-05 in Mega, and 9.07E-03 and 1.47E-05 in Rupshi, respectively (Table 3).

DISCUSSION

We observed that all three selected commercial feeds have metal contamination and there is no significant difference of metal contamination among the feeds tested. In general, it is very difficult to compare the metal concentrations even between the same tissues in different fish. This is because of the difference in the aquatic environments, feed, intensity and type of water pollution. The ability of fish to accumulate heavy metals depends on ecological needs, metabolism, and degree of pollution in sediment, water and food, as well as salinity and temperature of water[19]. Metal concentration and several intrinsic factors of fish such as organism size, genetic composition and age of fish also play role in their metal accumulation[20]. Different fish species also accumulate metals in their tissue in significantly different values[21]. Differences of concentrations of metals between fishes also reported to vary depending on their feeding habits, the bio-concentration capacity of each species and to the biochemical characteristics of the metal[22].

Copper is an essential element that is carefully regulated by physiological mechanisms in most organisms[23]. Copper is an essential part of several enzymes and is necessary for the synthesis of hemoglobin[24]. However, studies have shown that Cu is highly toxic in aquatic environments and has effects on fish, invertebrates, and amphibians, with all three groups equally sensitive to chronic toxicity. Copper accumulate in many different organs in fish and mollusks[20]. The copper concentration measured in fish feeds in this study was far below the FAO guideline of 100 mg/kg[17]. The values found from this study were also lower than that obtained for poultry feed in Pakistan[25]. The concentrations of Cu in the fish samples analyzed ranged from 21.13±1.44 to 23.76±1.41mg/kg. The permissible limit of Cu proposed by WHO and FAO is 30 mg/kg fresh weight of fish[17]. However, there are some reports on high concentration of Cu (575.34±61.86 mg/kg) in prawn from the Buriganga River in Bangladesh[26].

Lead is a non-essential element and it is well documented that lead can cause neurotoxicity, nephrotoxicity, and many others adverse health effects[27]. All of the feed samples exceeded the European Union limit for lead of 5 mg/kg[18]. However, the values obtained in this study were lower than 23.2–32.6 mg/kg reported in analysis of poultry feed used in Pakistan[25]. The concentrations of Pb in the fish samples analyzed ranged from 9.92±3.51 to 14.83±1.36 mg/kg, which is higher than the permissible limit of 2 mg/kg fresh weight[17]. A high Pb content (10.27 mg/kg) also reported in open water fish (*Coricasoborna*) from Bangshi River, Bangladesh[28]. Pb in chapila (*Gudusiachapra*) fish reported to be 13.52 mg/kg from the Buriganga River, Bangladesh [29]. Wild fish polluted with high Pb does not directly link the observed high level of Pb in culture fish or in fish feed. However, trash fish used as fishmeal in feed manufacture could be a potential source of such high Pb level in fish feed and cultured tilapia.

Iron is a critical nutrient for the proper functioning of the body organs. It is a component of the respiratory pigments and enzymes concerned in tissue oxidation and it is essential for oxygen and electron transport within the body. When the body absorbs more iron than it can take, the excess iron cannot be discarded naturally and is stored in the body tissues of the liver, pancreas and heart. The concentrations of iron (Fe) in the feed samples analyzed ranged from 607.67±146.20 to 831.16±351.06 mg/kg, which was higher than the European Union guideline of 500 mg/kg[18]. The concentrations of Fe in the fish samples analyzed ranged from 151.49±24.27 to 193.98±7.17 mg/kg. The permissible limit of Fe proposed by WHO and FAO, was 80 mg/kg fresh weight [17]. Some studies reported Fe level of 36.211mg/kg[30] and 6.570mg/kg[31] in fishes from Turkey, 27.22 mg/kg in some common fishes from Cambodia[32] and 8.819 mg/kg in fishes from Italy[33].

The level of contamination of fish with Cadmium is largely affected by environmental pollution. Cadmium occurs naturally in low levels in the environment and is also used in batteries, pigments, and metal coatings. Industrial processes such as smelting or electroplating and the addition of fertilizers can increase the concentration of Cd in the pond (environment). The concentrations of Cd in all feed samples exceeded the WHO/FAO limit[17]. The concentrations of Cd in the fish samples analyzed ranged from 9.43±0.37 to 9.84±0.55 mg/kg. The permissible limit of Cd proposed by FAO, was 1 mg/kg fresh weight[17]. However, the values obtained in this study were lower than 3.8–33.6 mg/kg obtained by Mahesar *et al*. (2010) in poultry feed[25]. Cadmium content of canned tuna fish in Saudi Arabia ranged between 0.08 and 0.66 mg/kg, which is much lower than the present findings[34]. The concentrations of Sodium in the feed samples analyzed were surprisingly high ranging from 930.21±34.18to 1029.00±62.37 mg/kg and in the fish samples varied from 821.33±42.13 to 885.13±22.45 mg/kg. Sodium relatively non-toxic to animals and toxic to plants at higher levels[35].

All the samples contained sodium much exceeding the nutritional requirement. But as no standards were fixed for maximum permissible limit as contaminant, it cannot be shown whether the contents in the samples were harmful. In present study, concentrations of non-essential elements (Cd, Cr and Pb) in fish muscles were found lower than those of essential metals (Fe, Na, Ni and Cu).This result is in consistent with the observation that the accumulation levels of the essential metals in fish are generally higher and more homeostatic than the non-essential metals[36]. Huang (2003), found the order of concentrations of four heavy metals in common benthic fishes as: Fe> Cu > Cd >Pb, however in fish tissue from Lake Manzala, Egypt the average concentrations of the metals exhibited the order: Fe>Cu>Pb>Cd[37]. These results were in agreement with results from present study in which concentrations of the metals followed the order Na>Fe> Cu >Pb> Cd in fish tissue.

THQ values were less than 1 for all individual heavy metal in all three brands feed fed tilapia indicating no non-carcinogenic health risk from ingestion of a single heavy metal through consumption of these fishes. The highest THQ value was estimated for Na (1.51) followed by Cd (1.45) contaminated in all farms. This indicates that excessive consumption over a long time period might cause non-carcinogenic effect as the THQ values were higher than the acceptable guideline value of 1[15]. The TR values for Cd and Pb indicates that excessive consumption over a long time period might cause carcinogenic effect. Although the fish species under the present study were found safe for human consumption, but the probability of cancer is also present for continuous consumption for 70 years.

This study was performed to understand trace metal accumulation from feed in farmed fish. The results of the study revealed that the fish feeds analyzed contained some of the heavy metals in varying proportions. As a whole, the average copper, chromium and nickel concentration in the feeds used in Mymensingh, was considerably below the maximum allowed limit, permitting a less frequent control of this element. The average cadmium and lead content in feeds were higher than the maximum allowed concentrations. This signifies that practically, control for cadmium and lead amounts in commercial feed is necessary. The present study concludes that tilapia collected from different farms accumulates various metals at different concentrations. However, at current concentration level in fish no metal was found to pose potential carcinogenic health risk individually, but collectively, the metals were found enough to be considered as potential human health hazard. Further studies should be done to cover more locations/divisions of the country, and include other fish feeds available in the region for metal contamination screening.

Table 1: Mean (±SD) metal concentrations (mg/kg) in commercial fish feed and in edible tissue of cultured tilapia (*O. niloticus*)

Metal	Concentration of heavy metals (mg/kg dry weight)			Standard safety level (mg/kg)
	Quality Feed	Mega Feed	Rupshi Feed	
Cu	26.75±6.23	35.08±3.34	32.2±92.23	100 (WHO*)
Fe	607.67±146.20	709.55±108.53	831.16±351.06	500 (WHO)
Pb	9.34±1.78	10.12±1.83	9.25±0.49	5 (EU*)
Cd	8.20±0.12	9.21±0.52	8.64±0.01	2 (EU)
Na	930.21±34.18	1005.38±76.01	1029.00±62.37	700 (WHO)

*WHO (World health organization), *EU (European Union)

Table 2: Target hazard quotient (THQ) for different heavy metals and their hazard index (HI) from consumption of three feed brands fed tilapia collected from the Mymensingh, Bangladesh.

Metals	Reference dose of the metal (*RfD) (mg/kg)	Target hazard quotient (THQ)		
		Quality	Mega	Rupshi
Fe	0.7	4.08E-02	3.18E-02	3.35E-02
Na	0.08	1.51E+00	1.57E+00	1.63E+00
Cu	0.005	6.41E-01	6.99E-01	6.22E-01
Cd	0.001	1.45E+00	1.39E+00	1.44E+00
Pb	0.2	7.30E-03	1.09E-02	8.66E-03
HI	0.986	3.65E+00	3.70E+00	3.73E+00

*USEPA 2011

Table 3: Target cancer risk (TR) of heavy metals from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh.

Metals	Target cancer risk (TR)		
	Quality Feed	Mega Feed	Rupshi
Cd	9.12E-03	8.74E-03	9.07E-03
Pb	1.24E-05	1.85E-05	1.47E-05

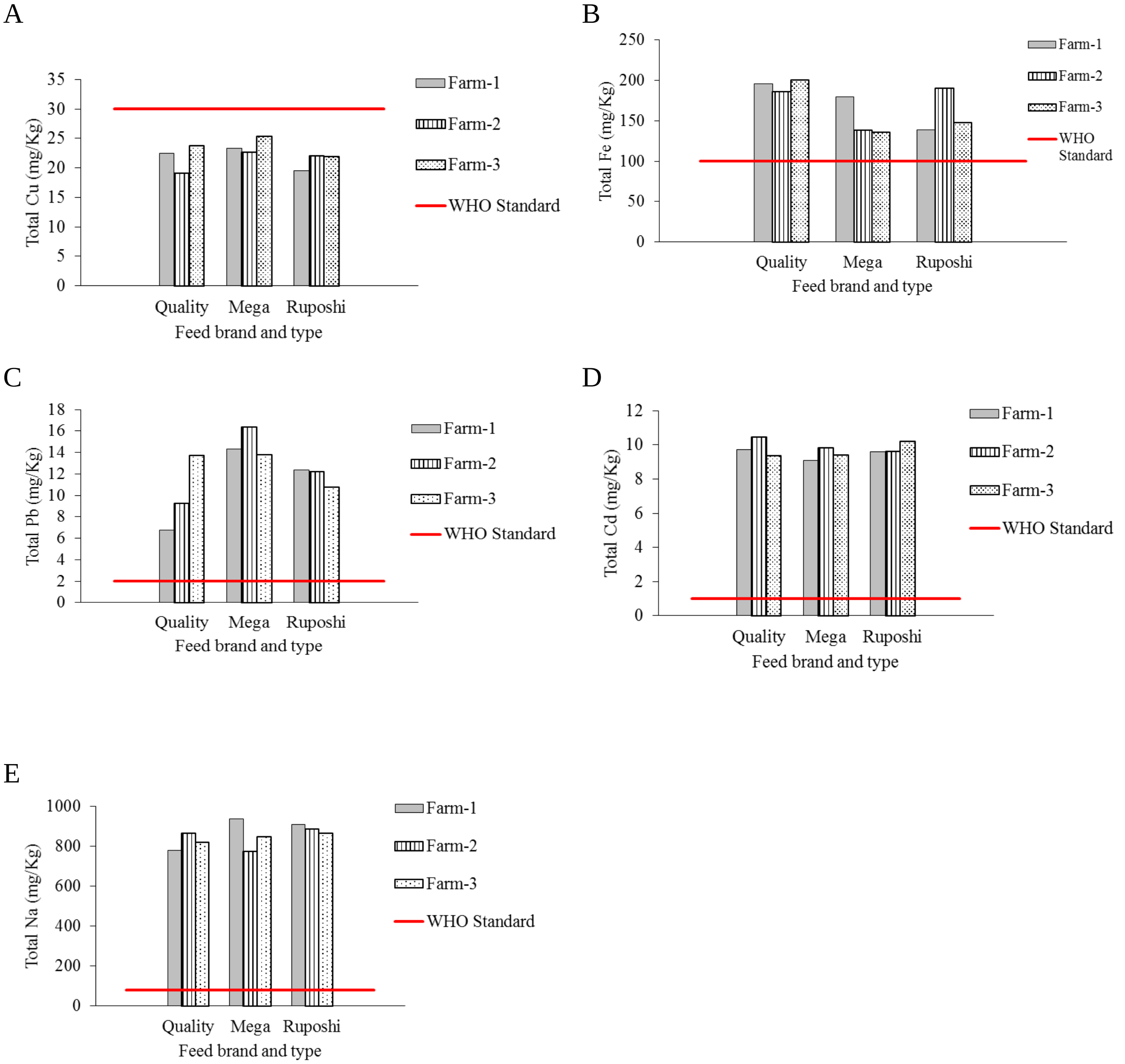

Figure 1. Concentrations (mg/kg) of Cu (A), Fe (B), Pb (C), Cd (D) and Na (E) in tilapia flesh, fed with three commercial fish feeds (Quality, Mega and Rupshi) in three fish farms of Mymensingh. Concentration of all metals in fish tissue fed with different feeds were not significantly ($p < 0.05$) different. WHO recommended values for each metal in fish tissue is shown as red horizontal line.

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