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The presence of toxic metals in popular farmed fish species and estimation of health risks through their consumption

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ABSTRACT

This study concerns an assessment of essential and toxic metals (Zn, Cu, As, Cr and Cd) in some popular farm fishes which are largely consumed by the populations of the Southern region in Bangladesh. Three different species of fish *(T. nilotica, P. pangasius and L. rohita)* were collected from four representative farms located in the Fatickchari, Hathazari, Patiya and Raozan Upazila of Chittagong district. Flame Atomic Absorption Spectrometer (FAAS) and Graphite Furnace Atomic Absorption Spectrometer (GFAAS) were used to measure the metal concentrations. The order of concentration of metals in flesh was Zn > Cu > Cr > As > Cd with values of 16.205 ± 0.303 > 0.874 ± 0.037 > 0.590 ± 0.05 >0.042 ± 0.003 > 0.004 ± 0.00 (mg/kg dw) in *T. nilotica*, 20.324 ± 0.697 > 1.035 ± 0.050> 0.577 ± 0.074> 0.045 ± 0.005 > 0.006 ± 0.000 (mg/kg dw) in *P. pangasius* and 22.270 ± 0.745 > 0.953 ± 0.525 > 0.623 ± 0.060 > 0.035 ± 0.002 > 0.004 ± 0.000 (mg/kg dw) in *L. rohita.* Measured data lie within the permissible limits recommended by WHO/FAO. Potential metal toxicity to human health following the consumption of the studied fishes was estimated via a number of hazard parameters: Daily intake of metal (DIM), Target hazard quotient (THQ), Hazard index (HI) and Target risk (TR), all of the data show values within the recommended level given by regulatory bodies. Estimated TR for potential carcinogenic metals As, Cr and Cd were found in the range (10^{-6} - 10^{-5}), which lies within the US-EPA risk range of 10^{-6} - 10^{-4}. Note that, fish consumption forms a minor part of the total diet while the US-EPA risk range is for the dietary intake from all foods. Therefore the estimated risk may not be totally neglected. Moreover, considering the non-biodegradability of toxic metals and their potential uptake in fish tissues, reduction in metal supplementation in fish feed should be introduced and periodic monitoring of fish may help to mitigate non-essential metal toxicity to consumers.

1. Introduction

An increasing concern on ecological and public health associated with environmental pollution via toxic materials has been seen in recent years [1]. Major contaminant includes persistent organic pollutants, radioactive materials, toxic heavy metals, residues from extractive industries, pathogens, litters and debris etc. [2]. Among different pollutants, heavy metal pollution has become a great concern due to their potential toxicity, non-biodegradable nature, long biological half-life and tendency to bio-accumulate [3]. Heavy metals are naturally occurring elements that have a high atomic weight and a density of at least 5 times greater than that of water. They are found throughout the earth's crust, but indiscriminate human activities have drastically altered their geological cycles and biochemical balance [4], and allow them to enter the human body via ingestion, inhalation and dermal contact. Among the various heavy metals, some are biologically essential for living organisms and play an important role in metabolism [5]. There are some heavy metals that have no known beneficial effect on a living organism [6]. Therefore, heavy metals are classified as essential (Zn, Cu, Fe, Mn etc.) and non-essential or toxic heavy metals (As, Cd, Pb, Hg etc). Toxic heavy metals are very harmful even at low concentrations when ingested over a long time. The essential metals can also produce toxic effects when their intake is excessive [7,8]. During recent years the concentration of toxic metals in many eco-systems are reaching unprecedented levels [1]. Especially, aquatic ecosystems are more sensitive to heavy metal pollutants and gradual increases in the levels of such metals in aquatic

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environment, mainly due to anthropogenic sources, becomes a problem of primary concern [9].

Bangladesh is a small country in South Asia that is overpopulated. While the presence of tropical climate and prevalence of diverse inland water bodies such as rivers, haors, baors, beels, canals, ponds, ditches, etc. provide very suitable natural habitats for fish, the socio-economic condition has made Bangladesh one of the most fish consuming nation in the world. As a result, fish plays a crucial role in the daily diet for Bangladeshi by providing necessary animal protein and micronutrients to fulfill their nutrients requirement. However, due to the very high population density, the natural aquatic resources of fish are facing great difficulties to meet the increasing demand. As a result, fish farming has been experiencing dramatic growth over the past few decades following the increasing domestic consumption and foreign export [10]. According to an FAO report, Bangladesh is the fourth largest fish producer in the world for inland fisheries. In Bangladesh, fish supply about 60% of animal protein as well as being a key source of the essential minerals, vitamins, and fatty acids [11]. As fish constitute an important part of the human diet, it is not surprising that the quality and safety aspects of fish are of particular interest, especially for the farm/cultured fishes. This is because, fish farming has shifted gradually from no feed to the use of farm-made feeds, and factory-made feeds. Consequently, manufactured feeds become an important part of modern commercial aquaculture. Although factory-made feeds are manufactured in order to provide the balanced nutrition needed by farmed fish, however, the presence of toxic heavy metals in such feeds cannot be neglected. In this regard, over the last few decades, concentrations of heavy metals in fish have been studied in various parts of the world. Like other countries, the aquatic organism in Bangladesh is also suffering from heavy metal pollution. Since the diet is the main route of human exposure to heavy metal, thus the edible species form the greater part of metal toxicity in the human body [12]. Once heavy metals enter the human body, they tend to accumulate in human tissues and pose chronic toxicity. Chronic assimilation of heavy metals is a known cause of cancer [13]. Since heavy metals are non-biodegradable, cannot be metabolized, and not break down in harmless form, the measurement of toxic metal concentrations in soft tissues of fish shows a great demand [14]. It is worth mentioning that, Chittagong is the second largest fish producing division for inland fisheries in Bangladesh [15]. But unfortunately, there is not enough information on the heavy metal contents in cultured fishes of the Chittagong area. Therefore, the aim of this study was to determine the heavy metal concentrations in some popular farm fishes of Chittagong, Bangladesh. The measured data are compared with the available literature data and also with the maximum allowed levels in fish recommended by different international organizations. In addition, potential health risks due to the consumption of these fishes were also assessed.

2. Materials and methods

2.1. Study area

Chittagong lies in the southeastern region of Bangladesh. It is located from 22°20′15″ north to 91°50′20″ east. The annual consumption of fish and fish products in Bangladesh is about 12 kg per capita. However, Chittagong having the highest consumption rate of 17 kg. Meanwhile, the global consumption rate is 19.2 kg per capita [15]. Four sampling sites of Chittagong district named Fatickchari, Hathazari, Patiya and Raozan were chosen to conduct the present study (see Fig. 1). This is because in recent years fish farming has been increased tremendously in this area which plays an important role in the economy. A large number of people earn their livelihood from fish farming and other sectors which are directly associated with this.

2.2. Sample Collection and preparation

Following their relatively low prices, about 60% of Bangladeshi people consume *P. pangasius, L. rohita and T. nilotica* on a daily basis, and this constitutes approximately 5% of their daily diet. That is why these three species were selected in the present study. A total of 32 samples of three species of marketable size were collected from different fish farms of the selected study sites during May–June 2016. No gender difference was considered in this study and similar sized fishes were taken to avoid any difference in result due to metal residence timing inside the fishes. All the fishes were thawed and dissected very carefully with a special ceramic knife, scissors, and plastic forceps to avoid metal contamination from laboratory equipment. Muscular tissues on the dorsal surface of each fish were taken out and homogenized. Then the samples were dried in a microwave oven at 60 °C for two days until they reach a constant weight and then grind into a fine powder.

2.3. Reagents and digestion procedure

All the plastic and glassware used were rinsed and soaked in 2% HNO_3 overnight. They were rinsed 5 times with de-ionized water and oven-dried prior to use. All acids: Nitric acid (HNO_3), Hydrochloric acid (HCl), Potassium iodide (KI) and oxidant: Hydrogen peroxide (H_2O_2) were obtained in the highest purity form from Merck Germany. About 5 g of the homogenized muscle of each specimen were taken. At first 6.0 ml of 65% HNO_3 and then 2 ml of HCl was added. Then the samples were placed in a hot plate and heated at 60°C for half an hour. After that, 4 ml H_2O_2 (35%) was added and digested at 90°C for two hours until a clear solution was obtained [12]. Then all the samples were filtered with Whatman filter paper (40) and stored into 50 ml vial until analysis.

2.4. Analytical technique

Total 5 heavy metals, two essential (Zn and Cu) and three non-essential (As, Cr and Cd) were determined using Atomic Absorption Spectrometer (Model-Z (2000), Hitachi, Japan). Flame Atomic Absorption Spectrometer (FAAS) was used for determining Zn and Cu concentrations, and Graphite Furnace Atomic Absorption Spectrometer (GFAA) was used to determine Cr and Cd. An extra section which is called Hydride Generation System was used along with FAAS to determine the concentration of As. In this study, working standards were freshly prepared from the stock solution (1000 ppm, Fluka, Switzerland) by serial dilution. Each time acid blank was run along with the samples and its value was subtracted from sample concentration to get the actual concentration of metals in the fish sample. Concentrations of the studied metals (mg/kg) in samples were calculated following Eq. (1) [16].

$$\text{Concentration, C}\left(\frac{\text{mg}}{\text{kg}}\right)=\frac{\text{Concentration,}\ \ \text{C}\left(\frac{\text{mg}}{\text{l}}\right)\times\text{Volume,}\quad\text{V(l)}}{\text{Mass of the Sample,}\ \ \text{M}\ \ \text{(g)}}\times(1000) \tag{1}$$

In equation (1), V is the final volume after digestion; M is the mass (in grams) of the sample to be tested and C is the concentration (mg/l) of metal in the digested solution, and 1000 is the conversion factor for gram-to-kilogram.

2.5. Health risk assessment

The risk for human health as a result of consumption of the studied fish species was evaluated by using a number of relevant parameters such as Daily Intake of Metals, Hazard Index and Hazard Quotient etc. Detailed information of their calculations are as follows:

2.5.1. Daily intake of metals

The daily intake of metals (DIM) expresses the average daily loading of metals into the human body for a specified bodyweight of a consumer. Estimated DIM indicates the probable ingestion rate of metal into the human body by ingestion of fish per day. DIM was calculated by using Eq. (2) [17,18].

$$DIM = C_{fish} \times [D_{fish}/BW] \tag{2}$$

Where, C_{fish} = average heavy metals concentration in fish muscle (mg/kg wet weight), D_{fish} = the average daily fish consumption (g/day) per person which is 53 g/day for Bangladeshi people [19], BW = body weight (60 kg) of target population [20].

2.5.2. Non-carcinogenic and carcinogenic risk

The target hazard quotient (THQ) assessed the non-carcinogenic health hazards due to each individual metal through fish consumption. The THQ assumes a level of exposure (i.e. R_fD) below which is unlikely for even a sensitive population to experience adverse health effects [18]. On the other hand, HI (Target hazard index) represents the sum of all THQ's [21]. HI indicates the combined hazard of all metals [22]. If THQ is less than 1 the exposed population is unlikely to experience obvious adverse effects. If it is equal to or greater than 1, there is a potential health risk through the consumption of the individual metal.

For carcinogens, target risks were estimated as the incremental probability of an individual to develop cancer over a lifetime as a result of exposure to that potential carcinogen. The THQs, HI, and TR were estimated using equations (3), (5) and (6) respectively.

$$THQ = \frac{EF \times ED \times FIR \times CF \times CM}{(WAB \times ATn \times RfD)} \times 10^{-3} \tag{3}$$

$$HI = \sum THQ \tag{4}$$

$$HI = THQ\,(Zn) + THQ\,(Cu) + THQ\,(As) + THQ\,(Cr) + THQ\,(Cd) \tag{5}$$

$$TR = \frac{EF \times ED \times FIR \times CF \times CM \times CPSo}{WAB \times TAc} \times 10^{-3} \tag{6}$$

Where, EF is the exposure frequency (365 days/year), ED is the exposure duration (30 years) for cancer risk used by Ref. [21], FIR is the ingestion rate (53 g/day) for Bangladeshi people [19], CF is the conversion factor (0.208) to convert wet weight (W_W) to dry weight (D_W) considering 79 % of moisture content in fish, CM is the concentration of metal in fish (mg/kg dw), WAB is the average body weight (bw = 60 kg), AT_n is the average exposure time for non-carcinogens (EF × ED) (365 days/year for 30 years) i.e. AT_n = 10950 days as used in characterizing non-cancer risk [21], R_fD is the oral reference dose with values (Zn = 0.03, Cu = 0.04, As = 0.0003, Cr = 0.003, Cd = 0.001) according to Regional Screening level (RSL) summary table [23], AT_C is the average time for carcinogens (365 days/year for 70 years) as used by Ref. [21] and CPS_0 is the oral

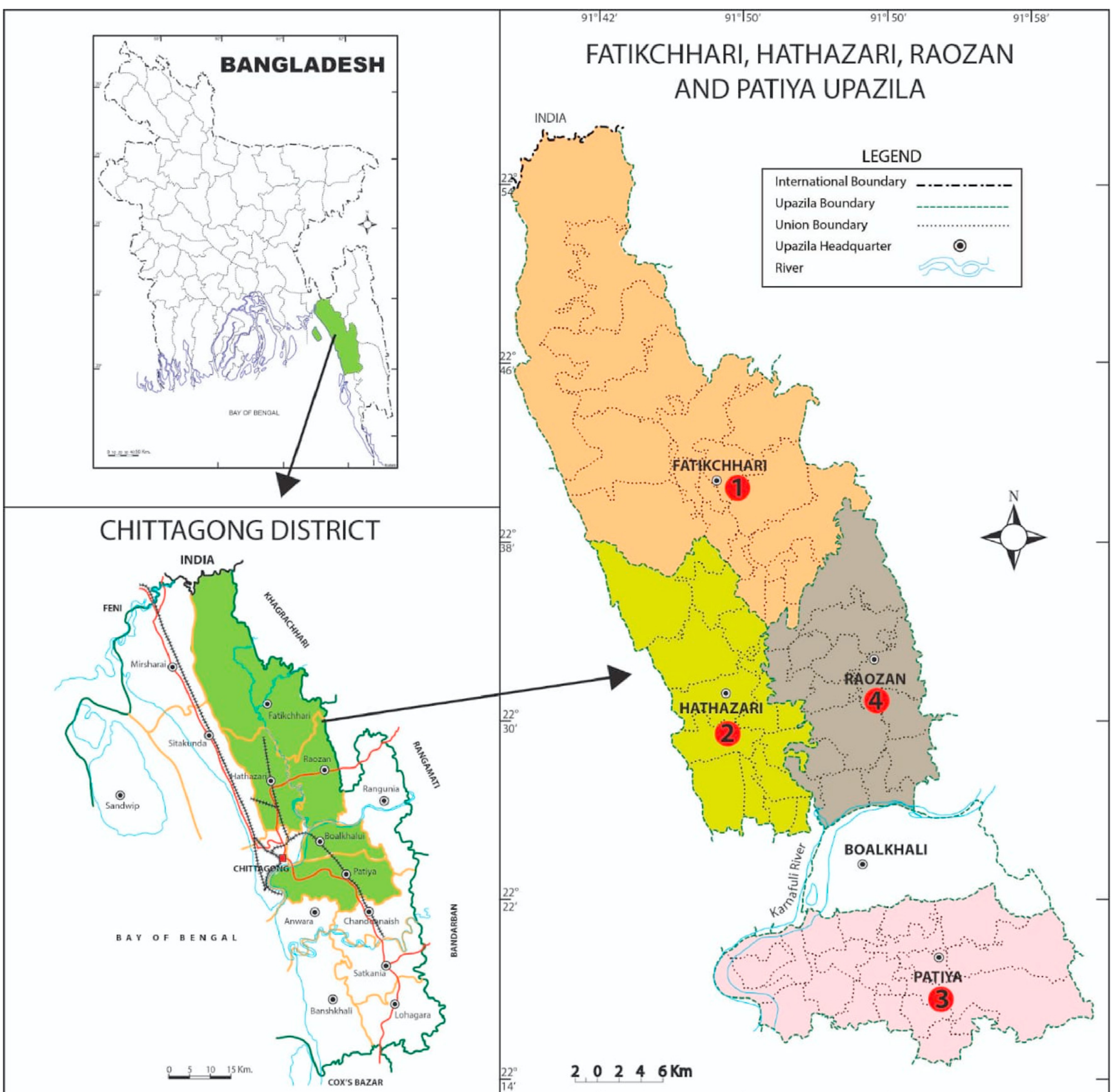

Fig. 1. Map of sampling location within in the Chittagong division in Bangladesh.

carcinogenic slope factor.

The acceptable risk level for carcinogens ranges from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1000000) [24].

3. Results and discussions

All the analyzed heavy metals were found in all the species at different levels. The metal concentration varies not only with the species but also with the study sites. Concentrations (mean concentrations ±SD) are represented in Table 1.

Here, concentrations were determined based on the sample dry weight in mg/kg. Mean metal concentration follows the order Zn > Cu > Cr > As > Cd. Graphical representation of mean concentrations is shown in Fig. 2.

Zinc is an essential heavy metal and the cells of living organisms contain Zn as one of the main components of various enzymes. It is involved in numerous aspects of cellular metabolism [27]. It is also essential for a healthy immune system, cell division, synthesis of protein and collagen etc. [28]. However, a higher amount of Zn becomes toxic for human health [29, 30]. In the present study, the maximum concentration of Zn was found in *L. rohita* among the fish species. The concentration of Zn follows the order: *L. rohita* > *P. pangasius* > *T. nilotica* with values of 22.27 ± 0.745 > 20.32 ± 0.697 > 16.20 ± 0.303 (mg/kg dry weight) which are below the permissible limit of Zn in fish 30 mg/kg [25]. In the literature [10], Zn concentrations in the fishes of Rajshahi City were found as 38.01 ± 2.28 (mg/kg dry weight) for *T. nilotica* and 71.22 ± 5.32(mg/kg dry weight) for *L. rohita*, which is higher than the present study (see Table 2). Such an elevated concentration of Zn might be linked with the pollution of the Padma River. In another study on cultured fishes of Bangladesh conducted by Ahmed et al. [31]; Zn concentration was found as 3.37 ± 0.17, 3.20 ± 0.33 and 1.8 ± 0.2 mg/kg wet weight in *L. rohita, P. pangasius* and *T. nilotica* respectively, show lower values than the present study. In a study of heavy metal analysis in some commercially important fishes of Kathmandu Valley, Nepal performed by Paudel et al. [7]; Zn was found as 46.68 ± 25.51 μg/g in dry weight. Elnabris et al. [12] reported 3.705–20.535 μg/g (in wet weight) of Zn in some commercially important fishes of Gaza strip, Palestine.

Copper is also an essential heavy metal and an important constituent of a living organism. Cu plays a role in the production of hemoglobin, myelin, melanin, and it also helps in the normal functioning of the thyroid gland. As this mineral is involved in numerous functions of the body, copper deficiency can produce an extensive range of symptoms like hernias, aneurysms, blood vessel breakage manifesting as bruising or nosebleeds [27]. On the other hand, excessive exposure to Cu has been linked to cellular damage in humans. Copper has been reported to cause neurotoxicity which is known as Wilson's disease [32]. Cu concentrations were found highest in *P. pangasius* and lowest in *T. nilotica*. Concentration of Cu follows the order: *P. pangasius* > *L. rohita* > *T. nilotica* with values of 1.030 ± 0.050 > 0.95 ± 0.025 > 0.87 ± 0.037 (mg/kg dry weight), which are below the permissible limit of 30 mg/kg [25]. Mortuza and Misned [10], assessed the concentration of Cu (mg/kg dry weight) in cultured fishes of Rajshshi as 3.48 ± 0.56 in *P. pangasius* and 4.480 ± 0.71 in *L. rohita* which is higher than the current study. Ahmed et al. [31] reported Cu concentration in cultured fishes of Bangladesh as 0.658 ± 0.007, 3.45 ± 0.04 and 1.138 ± 0.003 (mg/kg wet weight) in *L. rohita, P, pangasius* and *T. nilotica* respectively. Cu concentration was reported as 0.251–0.907 μg/g wet weight by Elnabris et al. [12] in some commercially important fishes of Gaza strip, Palestine. Result of the present study shows that, Cu concentration is low in fishes of Chittagong area as compared to the other areas of Bangladesh. Since, the Cu is an essential element for metabolism so lower levels of Cu is an issue of concern. Low Cu concentration may result from the less supplement of Cu in artificial feeds that are used in Bangladesh.

Arsenic has been classified as a human carcinogen by the International Agency for Research on Cancer [33]. It can exist in both organic and inorganic forms. Inorganic As is significantly more toxic than organic As compound [34]. Long-term exposure to As can cause cancer of the urinary bladder, lung, kidney, skin etc. [29]. Black foot disease is common in Bangladesh due to As poisoning. There are no significant differences in As concentrations among the selected fish species. The concentration of As in fishes follows the order: *P. pangasius* > *T. nilotica* > *L. rohita* with values 0.045 ± 0.005 > 0.042 ± 0.003 > 0.035 ± 0.002 (mg/kg dry weight) which are well below the permissible limit of As in fish 1 mg/kg [35]. Arsenic concentrations in fishes (mg/kg dry weight) of Rajshahi city reported by Mortuza and Misned [10], were 3.61 ± 1.59 and 3.06 ± 1.93 (mg/kg) in *P. pangasius* and *L. rohita* respectively. Concentration of As was reported by Paudel et al. [7] as 0.69 ± 0.17 μg/g dry weight in economically important fishes Kathmandu Valley, Nepal. Qin et al. [36] also reported As level as 0.096 mg/kg in farmed cyprinid fish species from Northeast China. Since, Chittagong district is in the safe zone of arsenic contamination in Bangladesh, so fishes of this area contain a relatively lower level of As in comparison to other areas of Bangladesh.

Cr is also a non-essential heavy metal. IARC [33], has determined that Cr compounds are carcinogenic to human health. Long-term exposure can cause damage to the liver, kidney, circulatory, and nerve tissues as well as skin irritation [37,38]. A high concentration of Cr (III) in cells can lead to DNA damages [39]. There are no significant variations of Cr concentration among the fish species. The highest Cr concentration was found in *L. rohita* and lowest in *P. pangasius*. Concentration follows the order: *L. rohita* > *T. nilotica* > *P. pangasius* with values 0.623 ± 0.06 > 0.590 ± 0.05 > 0.577 ± 0.07 (mg/kg dry weight) which are below the

Table 1
Measured concentrations (Mean ± SD) of heavy metals (in mg/kg dw) in the studied species of farmed fishes together with the recommended limit provided by the regulatory bodies.

Metal	Species	Determined metal concentrations (in mg/kg) in fishes collected from various study sites				Average con. in (mg/kg)	Permissible limit (mg/kg)
		Fatickchari	Hathazari	Patiya	Raozan		
Zn	*T. nilotica*	16.506 ± 0.372	15.482 ± 0.253	17.744 ± 0.665	15.090 ± 0.274	16.205 ± 0.303	30 [25]
	P. pangasius	22.833 ± 0.724	20.732 ± 0.794	18.504 ± 0.850	19.204 ± 0.430	20.324 ± 0.697	
	L. rohita	23.702 ± 0.855	22.045 ± 0.581	23.256 ± 0.571	20.010 ± 0.966	22.270 ± 0.745	
Cu	*T. nilotica*	0.765 ± 0.041	0.964 ± 0.050	0.924 ± 0.040	0.845 ± 0.025	0.874 ± 0.037	30 [51]
	P. pangasius	1.440 ± 0.060	0.992 ± 0.061	0.976 ± 0.032	0.724 ± 0.050	1.035 ± 0.050	
	L. rohita	0.922 ± 0.054	1.365 ± 0.074	0.684 ± 0.045	0.834 ± 0.053	0.953 ± 0.052	
As	*T. nilitica*	0.065 ± 0.002	0.058 ± 0.002	0.022 ± 0.005	0.023 ± 0.001	0.042 ± 0.003	1 [35]
	P. pangasius	0.044 ± 0.004	0.077 ± 0.005	0.037 ± 0.007	0.023 ± 0.002	0.045 ± 0.005	
	L. rohita	0.038 ± 0.001	0.069 ± 0.003	0.023 ± 0.001	0.021 ± 0.002	0.035 ± 0.002	
Cr	*T. nilotica*	0.597 ± 0.052	0.464 ± 0.033	0.561 ± 0.085	0.736 ± 0.044	0.590 ± 0.052	1 [40]
	P. pangasius	0.587 ± 0.091	0.532 ± 0.074	0.564 ± 0.033	0.624 ± 0.077	0.577 ± 0.074	
	L. rohita	0.735 ± 0.083	0.572 ± 0.042	0.478 ± 0.040	0.707 ± 0.071	0.623 ± 0.060	
Cd	*T. nilotica*	0.004 ± 0.000	0.002 ± 0.000	0.004 ± 0.000	0.005 ± 0.000	0.004 ± 0.000	0.05 [26]
	P. pangasius	0.005 ± 0.000	0.006 ± 0.000	0.005 ± 0.000	0.006 ± 0.000	0.006 ± 0.000	
	L. rohita	0.004 ± 0.000	0.005 ± 0.000	0.002 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	

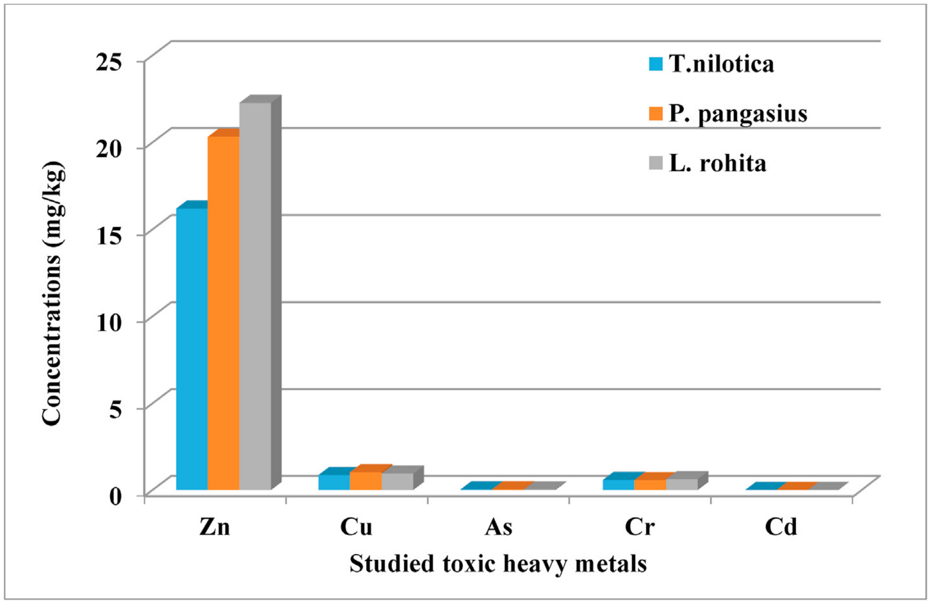

Fig. 2. Average concentration (in mg/kg) of heavy metals in flesh of three species (dry weight).

Table 2
Comparison of the determined concentrations of heavy metals in farmed fishes of Chittagong with other studies in Bangladesh as well as around the world.

Study area	Measured heavy metals concentration (mg/kg) in the present study together with the reported literature					References
	Zn	Cu	As	Cr	Cd	
Chittagong, Bangladesh.	16.205 ± 0.030–22.270 ± 0.745 (dw)	0.874 ± 0.037–1.035 ± 0.050 (dw)	0.035 ± .0002–0.045 ± .005 (dw)	0.577 ± 0.074–0.623 ± 0.060 (dw)	0.0035 ± 0.000–0.0067 ± 0.000 (dw)	Present study
Rajshahi, Bangladesh.	63.78 ± 16.61 (dw)	4.12 ± 0.587 (dw)	4.742 ± 3.329 (dw)	0.795 ± 0.104 (dw)	0.015 ± 0.11 (*dw*)	[10].
Bangladesh.	1.850–3.735 (ww)	0.658–3.459 (ww)	0.077–1.486 (ww)	1.054–1.349 (ww)	0.001–0.003 (ww)	[31]
India	1.112 ± 0.251 (ww)	0.096 ± 0.033 (ww)	_	0.524 ± 0.053 (ww)	0.014 ± 0.000 (ww)	[43].
Kathmundu, Nepal.	46.68 ± 25.51 (dw)	_	0.69 ± 0.17 (dw)	10.32 ± 2.23 (dw)	0.88 ± 0.35 (dw)	[7].
China.	5.907 (dw)	0.437 (dw)	0.113 (dw)	0.173 (dw)	0.009 (dw)	[46].
Gazastrip, Palestine	3.701–20.53 (dw)	0.251–0.907 (dw)	_	_	ND- 0.090 (dw)	[12]

permissible limit 1 mg/kg [40]. Hasan et al. [41] reported the concentration of Cr in market fish from Dhaka city as 0.75 ± 0.02 mg/kg dw, and Ahmed et al. [31] found the level of Cr in cultured fish of Bangladesh within the range of 1.054–1.349 mg/kg ww, both are higher than the present study. Chatta et al. [42] determined the level of Cr as 0.049 ± 0.006 μg/g in farmed fishes of Head Qadirabad Area of Turkey, meanwhile concentration of Cr was recorded as 0.524 ± 0.053 μg/g in *Pangasianodon hypothalamus* by Srivastava et al. [43] in India. Both studies show somewhat lower values than the present study. The higher concentration of Cr in fishes of Bangladesh most probably results from the use of tannery waste in the artificial feed.

Cd is an extremely toxic pollutant classified as a human carcinogen (Group-1) according to the IARC, [33]. Research on chronic exposure to Cd in a rat model showed that liver and kidney toxicity is induced via inhibition of Cd components [37]. Cd interacts with essential nutrients and disrupts their function [44]. Among the three kinds of fish species, *P. pangasius* contains the maximum Cd level. The order of concentration is *P. pangasius* > *T. nilotica* > *L. rohita* with values 0.006 ± 0.000> 0.004 ± 0.000> 0.0035 ± 0.000 (mg/kg dry weight) which are also below the permissible limit of Cd in fish 0.05 mg/kg [26]. Ahmed et al. [31] reported Cd concentration in cultured fish of Bangladesh as 0.003 mg/kg ww in *T. nilotica*, and 0.001 mg/kg wet weight for *L. rohita* and *P. pangasius*. An overall highest concentration of Cd was found as 0.775 mg/kg in the flesh of *A. testudeneus* in the market fish of Dhaka city [45]. Paudel et al. [7] found Cd in fishes of Kathmandu Valley, Nepal as 0.88 ± 0.35 μg/g dry weight. Both of these literature data show higher concentration than the present study.

Table 3 shows the daily intake of metal (DIM) values of the selected

Table 3
Concentration of heavy metals (mg/kg) in wet weight and Daily intake of metal (DIM) of Zn, Cu, As, Cr and Cd from fish. A conversion factor (0.208) was used to convert dry weight (D_W) to wet weight (W_W) considering 79 % of moisture content in fish [21].

Heavy Metals	Mean concentration of metals (mg/kg wet weight)			Daily intake of metal (DIM) in mg/kg (bw/day)			Maximum tolerable daily intake (MTDI) mg/day
	T. nilotica	P. pangasius	L. rohita	T. nilotica	P. pangasius	L. rohita	
Zn	3.337	4.225	4.632	2.911	3.732	4.091	60 [47]
Cu	0.181	0.214	0.198	0.160	0.189	0.175	30 [25]
As	0.009	0.010	0.007	0.008	0.008	0.006	0.13 [48]
Cr	0.123	0.120	0.130	0.108	0.106	0.115	0.20 [49]
Cd	0.001	0.002	0.001	0.001	0.001	0.001	0.06 [48]

Table 4
Target hazard quotient (THQ) and target cancer risk (TR) for different heavy metals and their hazard index (HI) through consumption of *T. nilotica, P. pangasius and L. rohita*.

Target Hazard Quotient (THQ)				Target Cancer Risk (TR)		
Heavy metals	*T. nilotica*	*P. pangasius*	*L. rohita*	*T. nilotica*	*P. pangasius*	*L. rohita*
Zn	0.001	0.012	0.014	–	–	–
Cu	0.004	0.005	0.004	–	–	–
As	0.026	0.028	0.021	1.1E-05	1.2E-05	1.0E-05
Cr	0.036	0.035	0.038	5.4 E−05	5.3E-05	5.7E-05
Cd	0.001	0.001	0.001	4.8E-06	8.0E-06	4.1E-06
Hazard index (HI)	∑THQs = 0.076	∑THQs = 0.081	∑THQs = 0.078			

heavy metals (see Table 2). Here, mean concentrations were used to calculate the DIM for the estimation of health risk of the consumers through consumption of the studied fishes. The result shows that the DIM values of all metals are below the maximum tolerable daily intake (MTDI) recommended by various organizations in all fishes.

The THQ's and TR values of selected toxic heavy metals from fish consumption by an average individual (adults) are presented in Table 4, and the calculations were made using the standard procedure of US-EPA, [21]. The THQ's of each metal through consumption of fish followed the descending order Cr > As > Zn > Cu > Cd. THQ's of all metals in each species are less than 1, which indicates that the fishes under study do not pose any non-carcinogenic health hazard. In addition, the HI's of the selected elements were also calculated. There is no significant difference in HI's among the fish species. HI's for all metals were found much less than 1. So it can be said that the combined effect of all selected metals poses no health hazard at all.

The TR (Target cancer risk) values were calculated for As, Cr and Cd because these three heavy metals are classified as a group-1 carcinogen by the IARC, [33]. In general, the TR values lower than E−06 is considered to be negligible for carcinogenic risk. Values above E−04 are considered unacceptable and values lying between E−06 to E−04 are generally considered an acceptable range [50]. Table 4 represents the TR values for the studied carcinogenic heavy metals which lie within the range of E−06 to E−05. Note that the US-EPA referenced acceptable range of TR (E−06 - E−05) is due to the dietary intake of all food. Since, fish consumption forms a minor part of the total diet for human beings, the calculated TR range (4.1E-06-5.7E-05) obtained for the studied fishes cannot be totally neglected. Therefore, considering the non-biodegradability of toxic metals and their potential uptake in fish tissues via the artificial feed, necessary measures should be taken to reduce the metal supplementation in fish feed, and periodic monitoring/measurement of heavy metals in fish may help to mitigate non-essential metal toxicity to the consumer.

4. Conclusion

The present study concludes that all the analyzed heavy metals (Zn, Cu, As, Cr and Cd) were found in all samples at different levels, and the degree of accumulation varies among different species as well as different study sites. Maximum concentrations of Zn and Cr were recorded in the flesh of *L. rohita*, but *P. pangasius* contains the highest levels of Cu, As and Cd. Minimum levels of Zn and Cu were found in *T. nilotica*. On the other hand, the *L. rohita* species contains minimum levels of As and Cd. It is noteworthy that the studied fishes accumulated essential metals higher than the non-essential ones. Point be noted that the concentration of the essential nutrient Zn is considerably higher among the studied heavy metals in all examined species. However, another essential nutrient, Cu concentration was found to be extremely low in comparison with Zn. Concentrations of all studied metals especially non-essential heavy metals (As, Cr, Cd) are far below the permissible limit recommended by the FAO/WHO and other international organizations. The results revealed that the metals under study do not pose any non-carcinogenic risk to the consumers, if they consume the fishes following the present consumption characteristics. The obtained hazard index of <1 for all metals discards any serious health risk via combined effects of these metals. Target cancer risk (TR) values suggest a negligible carcinogenic risk from As, Cr and Cd, if the studied farm fishes are consumed at the current rate.

CRediT authorship contribution statement

Nighat Sultana Resma: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Funding acquisition. **AKM Moinul Haque Meaze:** Conceptualization, Supervision. **Shahadat Hossain:** Methodology, Software, Resources, Data curation, Project administration. **Mayeen Uddin Khandaker:** Validation, Writing - review & editing, Visualization. **Masud Kamal:** Software, Resources, Project administration. **Nipa Deb:** Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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