

Determination of Toxic Metal Accumulation in Shrimps by Atomic Absorption Spectrometry (AAS)

Fatema K¹, Naher K², Choudhury TR^{3*}, Islam MA², Tamim U², Hossain SM², Islam SMA¹ and MP Ali^{4*}

¹Department of Physics, Jahangirnagar University, Savar, Dhaka, Bangladesh

²Institute of Nuclear Science and Technology, Atomic Energy Research Establishment, GPO Box No. 3787, Savar, Dhaka-1000, Bangladesh

³Atomic Energy centre, 4 Kazi Nazrul Islam Avenue, P.O. Box No. 164, Ramna, Dhaka, Bangladesh

⁴Laboratory of Biotechnology, Graduate School of Science and Technology, Shizuoka University, Japan

Abstract

The study was carried out to assess the levels of lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), and mercury (Hg) in different shrimp species. Atomic absorption spectrometry was used to estimate and evaluate the levels of these metals from 11 shrimp samples. The results of this study showed that the average concentrations detected were ranged from <0.1 to 0.202, <0.1 to 0.416, <0.1 to 0.156, <0.1 to 0.21 and <0.1 to 0.28 mg/kg for Pb, Cd, As, Cr and Hg, respectively in river shrimps. In case of Gher, it was ranged from <0.1 to 0.267, <0.1 to 0.166, <0.1 to 0.156, <0.1 to 0.21 and <0.1 to 0.228 mg/kg for Pb, Cd, As, Cr and Hg, respectively. The results show that Pb concentration for both river and Gher samples lies between EU and IAEA limits, Cd concentration in river samples are higher than both EU and IAEA limits. Arsenic is observed only in one river sample that was also below the recommended level. Hg concentration for both Gher and river samples are less than the EU permissible limit. Our study highlights those toxic metals are found to be below the recommended tolerable levels in shrimp of Bangladesh and may not constitute a health hazards for consumers.

Keywords: Heavy metals; Fish; Contaminants; Shrimps

Introduction

Heavy metals are hazardous contaminants in food and environment. These are non-biodegradable with long biological half-lives [1]. According to the World Health Organization [2] heavy metals must be controlled in food sources in order to assure public safety. Excessive concentration of food heavy metals is associated with the etiology of a number of diseases, especially cardiovascular, renal, neurological, and bone diseases [3]. A major reason to monitor levels of toxic metals in foods follows from the fact that contamination of the general environment has increased. The contamination of fresh and marine waters with a wide range of pollutants has become a matter of concerned over the last few decades [4-5]. The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other anthropogenic activities [6-7]. Heavy metal contaminations could have devastating effects on the ecological balance of the recipient environment via altering the diversity of aquatic organisms [8-10] especially the fish community [11]. These metals could reach food chain through various biochemical process and ultimately biomagnified in various trophic levels and eventually threaten the health of human by aquatic/seafood consumption [12-14].

The natural concentration of metals in sea waters is very low and hence the risk of contamination in living tissue is high, when organism started accumulating more amounts of metals than the level of its excretion [15]. These metals, being conservative in nature has the maximum probability of biomagnification, when they are transferred to human beings through different trophic levels in marine/aquatic food chains [16-17]. Fisheries are one of the most important food sectors for supplying protein to human population [15]. Due to increasing health consciousness of the consumers and to meet the food requirements of rapidly increasing population, demand for sea foods have been increasing drastically during the past decades.

Currently, fisheries is an important leading sector feeding huge additional population in Bangladesh. During the last few years, it is has been widely expanded and transformed into a big industry. Among the fisheries sector, shrimp growing is the most profitable and economical in Bangladesh as it has great export value. The second largest export

industry in Bangladesh is the shrimp industry and its contribution is about 4.7% to GDP and 9.38% of total exports. At present Bangladesh is the seventh largest country of shrimp exporting to USA and Japan market. Almost 600,000 people of our country are directly and indirectly related with the activities of shrimp cultivation and shrimp export [18-19].

Shrimp has great importance for its taste and food value. Shrimp contains high protein content, calories, enzymes, vitamins, minerals and toxic elements. Recently, toxic metals are detected from different sea/aquatic foods especially shrimp and their accumulated trends increasing [1,20]. Many elements, in trace amounts, play a vital role in metabolic processes and are essential for the general well being of humans. It is now well established that their deficiency or excess may cause disease and be deleterious to health. In Bangladesh, there are two system shrimps culture; one is river and another is Gher which is made by farmers. They can control water level and some other factors in the Gher. Therefore, determination of toxic element composition of foods/fish like shrimp is essential for understanding their nutritive importance.

The toxic element in food items like shrimp can be determined by different analytical techniques, such as inductively-coupled plasma atomic emission spectrometry (ICP-AES), inductively-coupled plasma mass spectrometry (ICP-MS), charged particle activation analysis (CPAA), cathode stripping voltammetry, total reflection X-ray

***Corresponding authors:** MP Ali, Laboratory of Biotechnology, Graduate School of Science and Technology, Shizuoka University Japan, Tel: 08036504222; E-mail: panna_ali@yahoo.com

Choudhury TR, Atomic Energy centre, 4 Kazi Nazrul Islam Avenue, P.O. Box No. 164, Ramna, Dhaka, Bangladesh

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fluorescence, EDXRF and ion chromatography, neutron activation analysis (NAA), atomic absorption spectrometry (AAS) etc. Atomic absorption spectrometry (AAS) is one of the most favored techniques for determination of toxic element in biological samples as well as living tissues. The aim of the present work is to determine the toxic element in shrimps using AAS method and to evaluate the level of toxicity.

Materials and Methods

Sample collection

Six shrimp samples were collected from Kobadak River and five shrimp samples were collected from local Gher of Tala upazila at Satkhira (Figure 1). The famous local name of these shrimps is Bagda, Golda, Chali, Rosnai and Harina. At first eleven shrimp samples were individually cleaned by de-ionized water and the head was removed from the flesh with a stainless steel knife. Collected shrimp samples were allowed to dry in an electric oven at 80°C until having constant weight. After drying the samples were crushed into fine powder with an agate mortar and pestles. After crashing the sample into fine powder, samples were transferred into ultra clean small polyethylene packet.

Sample preparation

Samples were prepared for the purpose of:

- Determination of the toxic element (Hg) in the shrimp samples by cold vapor atomic absorption spectroscopy (CV-AAS).
- Determination of the toxic element (As) in the shrimp samples by hydride generation atomic absorption spectroscopy (HG-AAS).
- Determination of the toxic elements (Pb, Cd, Cr) in the shrimp samples by graphite furnace atomic absorption spectroscopy (GF-AAS).

Microwave digestion

Analytical conditions for determination of trace amounts of As, Pb, Cd, Cr and Hg by AAS have been optimized in our previous study [4,5]. For present study to determine Cr, Cd, Pb, As and Hg, micro wave digestion was used for sample preparation. A portion of 0.5 g of ground sample was transferred directly to the flasks of the microwave oven (CEM MARS 5) and 6 ml of supra pure nitric acid was added. There is no heating procedure for CVAAS and HFAAS. But there is a heating program for GFAAS.

The heating program employed was the one proposed in the user's manual. The following three steps temperature program

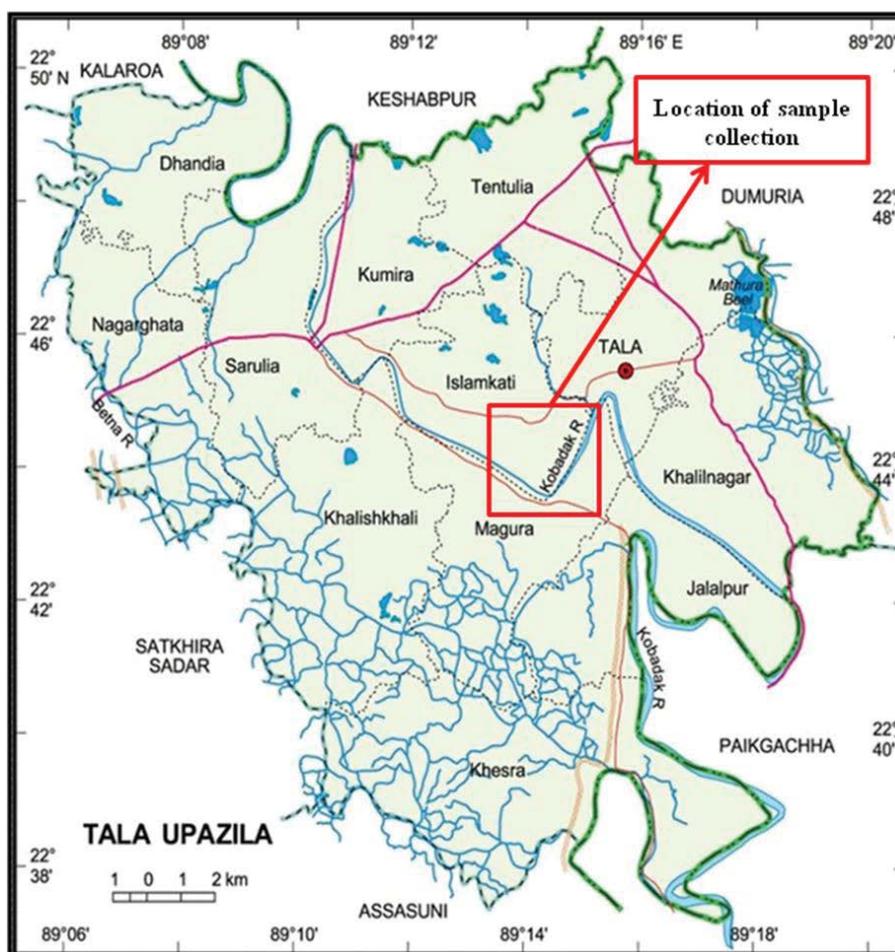


Figure 1: Geographical location of sample collecting area in Tala upazila of Satkhira district.

was applied: 180°C with a ramping time 10 min; and cooling time 10 min. For 6 or more vessels 1200W can be used. The resulting extracts were redissolved in 10 ml of ultra pure water for subsequent analysis by GFAAS. For certified reference material (Oyster tissue) an amount of 0.5 g of sample was digested in microwave as described for the shrimp samples.

Construction of calibration curve

Calibration curve was made for each element and concentration of the element in each sample was determined from the respective calibration curve of the element. The curve was prepared by plotting absorbance of standard versus their concentration. At least three or more separate value of absorption was taken with each solution and an average value was taken. The calibration curve for Pb, Cd, Cr, As and Hg are shown in Figure 2. After the construction of such calibration curves of these elements, the absorbance of sample solutions of unknown concentrations may be measured and the concentrations of these elements were determined from their constructed calibration curve.

Experimental procedure for Pb, Cd, Cr

A Varian (Model no. Varian, DuoAA240FS and AA80Z) GFAAS was used for this experiment. The solutions provided by the sample treatment and those used to construct the calibration curves, made in water containing 1.0% (v/v) HNO₃, were injected in the graphite furnace atomic absorption spectrometer. The temperature program and resonance lines used for measurement are shown in Table S1. Volume

of sample or standard solution (Oyster tissue) of 20 µl, depending on the metal to be analyzed, which was supplied with 5 µl of 1% H₃PO₄ for Pb, 1% NH₄H₂PO₄ for Cd as modifier, was used in each run. For Cr, matrix modifier was not required and the volume of sample or standard solution injected was 15 µl. Determination of Pb, Cd and Cr (mg/kg) in certified reference materials Oyster tissue is shown in Table S2.

Experimental Procedure for Hg

CVAAS technique was used for determining the concentration of Hg in shrimp sample. Using ANALYTIK JENA (novAA350). A volume of digestion sample of 4 ml, depending on the metal to be analyzed, which was supplied with 1 ml HCl and 15 ml DI for Hg as modifier. There were two chamber, Reductant chamber with 0.3% NaBH₄ and 0.1% NaOH and HCl acid chamber with 3% HCl. The wave length of Hg was 253.7 nm. The solutions provided by the sample treatment and those used to construct the calibration curves.

Experimental Procedure for As

HGAAS technique was used for determining the concentration of As in shrimp samples using VarianAA240. A volume of digestion sample of 2 ml, depending on the metal to be analyzed, which was supplied with 3M HCl and 1% KI, 1% of ascorbic acid for Hg as modifier. The total volume of the liquid sample was 20 ml. The wave length of As was 193.7 nm. The solutions provided by the sample treatment and those used to construct the calibration curves.

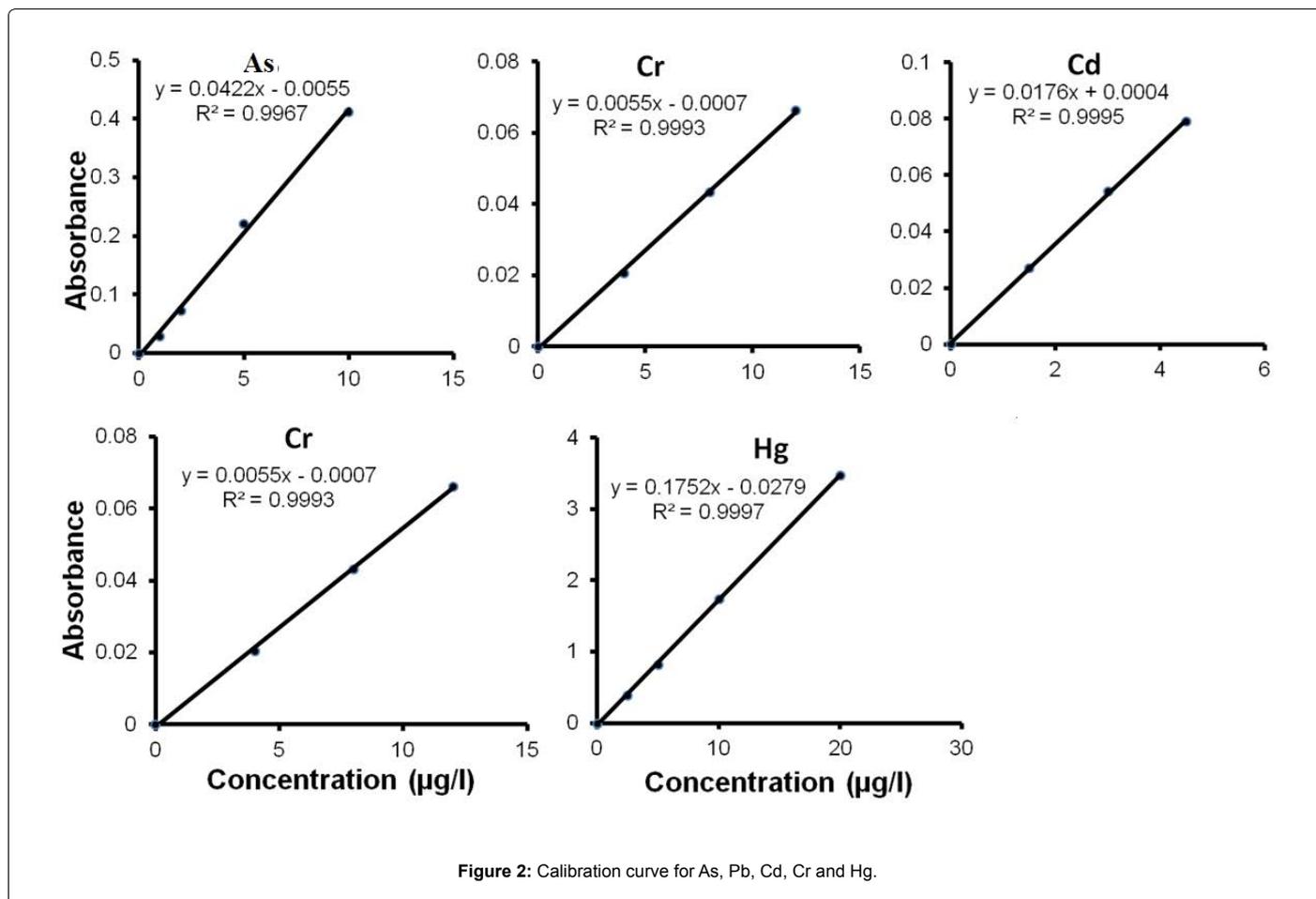


Figure 2: Calibration curve for As, Pb, Cd, Cr and Hg.

Metal	Unit	Certified value	Observed value	CV%	RE%
Cd	mg/kg	4.15 ± 0.38	4.01 ± 0.05	1.25	-3.37
Cr	mg/kg	1.43 ± 0.46	1.41 ± 0.04	2.84	-1.39
Pb	mg/kg	0.37 ± 0.14	0.31 ± 0.01	3.23	-16.4

Table S1: Comparison of observed Pb, Cd and Cr concentration with certified reference materials in oyster tissue.

Instrumental condition	Pb	Cd	Cr
Wave length (nm)	283.3	228.8	357.9
Slit width (nm)	0.5	0.5	0.2
Lamp current(mA)	10	4	7
Argon flow (L/min)	0.3	0.3	0.3
Sample volume(μL)	15	15	15
Modifier(μL)	5	5	-
Heating program temperature (°C)			
Drying	85-120 (Time: 55 sec)	85-120 (Time: 55sec)	85-120 (Time: 55sec)
Ashing	400 (Time: 8 sec)	250 (Time: 8 sec)	1000 (Time: 8 sec)
Atomization	2100 (Time: 4.9 sec)	1800 (Time: 4.8sec)	2600 (Time: 4.8 sec)
Cleaning	2800	2800	2800

Table S2: Analytical conditions for determination of Pb, Cd and Cr.

The analytical methods combining closed digestion, followed by determination with GF-AAS, CV-AAS and HG-AAS was developed for study of arsenic, cadmium, lead, chromium and mercury at very low levels in shrimp sample. The detection limits for are Pb, Cd, Cr and As are <0.1 mg/kg and for Hg <0.3 mg/kg under the study experimental conditions.

Results and Discussion

The heavy metals not only affect the nutritive values of fish, grains, fruits and vegetables but also have deleterious effect on human beings using these food items. National and international regulations on food quality have lowered the maximum permissible levels of toxic metals in human food; hence, an increasingly important aspect of food quality should be to control the concentrations of trace metals in food [21]. Five heavy toxic metals (Pb, Cd, As, Cr and Hg) were detected from 11 shrimp sampled of two different sources. The mean concentrations and range of heavy metals found in fresh shrimp sampled from the Gher and river in Bangladesh are summarized in Table 1. The heavy metal concentrations determined were based on sample dry weight.

Lead (Pb)

The results showed that the levels of Pb in all shrimp species were between 0.108 mg/kg in Harina and 0.87 mg/kg in Chali. Higher amount of Pb was detected in river sample than that of Gher (Table 2) but there was no significant difference between two sources sampled. It was detected in Chali and Bagda shrimp and concentrations of Pb in rest of the shrimp samples are found below the detection limit (detection limit of Pb is <0.1 mg/kg). This trend is similar to those reported in a previous study in Bangladesh [22]. The level of Pb in all of the shrimp samples was found to be below also below permissible limits (Table 2). EU has set the maximum limit for Pb in shrimp at 0.5 mg/kg. Lead can be adsorbed from air, food and water. Food and water account for approximately 70% of the total lead intake in adults. Data showed that in all shrimps from both Gher and river, lead concentration is less than permitted level, so they are suitable for consumption. Lead is a toxic element that can be harmful to aquatic organisms, although they usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many fishes, Pb accumulation can exceed several times the threshold of maximum level permissible for human. The introduction of Pb into the food chain may affect human health and

thus, studies concerning Pb accumulation in shrimp have increasing importance. Unfortunately, all shrimps that were studied in this study were less contaminated by Pb and they were still non-toxic to consumer.

Cadmium (Cd)

Cadmium contents varied from no detectable amount in Harina, Chali and Bagda to 0.4 mg/kg in Dhola. Among the study shrimps, higher amount of Cd was found in river sampled than that of Gher. Cd content in Dhola from river exceeded the threshold of maximum level permissible for human. In addition, it exceeded both EU and IAEA limit (Figure 3) but rest of the tested shrimps showed lower level. Similar result was found in previous study [22] except Dhola in river. The reason for remarkable increased of Cd content in Dhola is unknown at this stage. Cadmium is one of the contaminants that health authorities are most concerned about, due to the long biological half-life in humans. A high concentration of cadmium causes several health problems to humans. Cadmium and its compound along with mercury and some other dangerous metals are included in the blacklist [23]. Ingestion of cadmium produces shock acute renal failure when the amount exceeds 350 mg [24]. The introduction of Cd into the food chain will affect human health and thus, studies concerning Cd accumulation in shrimp have increasing importance.

Arsenic (As)

Among different chemical forms, both inorganic and organic, inorganic arsenic compounds are the most toxic, particularly As. The mean arsenic content in all tested shrimp was below the detection limit except only Chali in Gher (Table 1 and 2). But the level of As content of Chila didn't exceed the European Union (EU) and IAEA limit (Figure 3). Since toxic metals may accumulate in shrimp through feed, levels of toxic metals in seafood is a great concern [22]. From this point of view, some farm shrimp are checked periodically for their toxic. Humans may be exposed to arsenic through food, water and air. If a large amount of arsenic is swallowed by humans, in a form that is readily absorbed, it can cause rapid poisoning and death. The gut, the heart and the nervous system are affected. So it is important to evaluate the food like shrimp periodically whether this food contains larger amount of As or not. Unfortunately, the food tested in this study has no toxic level of As that may cause health hazard of human if these shrimps are eaten.

Type of shrimp	Element concentration (mg/kg)				
	Pb	Cd	Cr	As	Hg
Harina	<0.1	<0.1	0.338 ± 0.010	<0.1	0.186 ± 0.010
Rosnai	<0.1	0.100 ± 0.010	<0.1	<0.1	<0.03
Chali/Chapda	0.177 ± 0.010	<0.1	<0.1	<0.1	<0.03
Bagda	0.202 ± 0.010	<0.1	0.156 ± 0.010	<0.1	<0.03
Golda	<0.1	0.186 ± 0.010	<0.1	<0.1	<0.03
Dhola	<0.1	0.416 ± 0.010	<0.1	<0.1	<0.03

Table 1: Concentrations of Pb, Cr, Cd, As and Hg in six types of shrimps collected from river of Kobadak.

Type of shrimp	Element concentration (mg/kg)				
	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Arsenic (As)	Mercury (Hg)
Harina	0.108 ± 0.010	<0.1	<0.1	<0.1	<0.03
Chali/Chapda	0.267 ± 0.010	<0.1	0.21 ± 0.01	0.156 ± 0.01	<0.03
Rosnai	0.133 ± 0.010	<0.1	<0.1	<0.1	<0.03
Bagda	<0.1	<0.1	<0.1	<0.1	0.228 ± 0.010
Dhola	<0.1	0.166 ± 0.01	<0.1	<0.1	<0.03

Table 2: Concentrations of Pb, Cr, Cd, As and Hg in five types of shrimp samples collected from Gher.

Chromium (Cr)

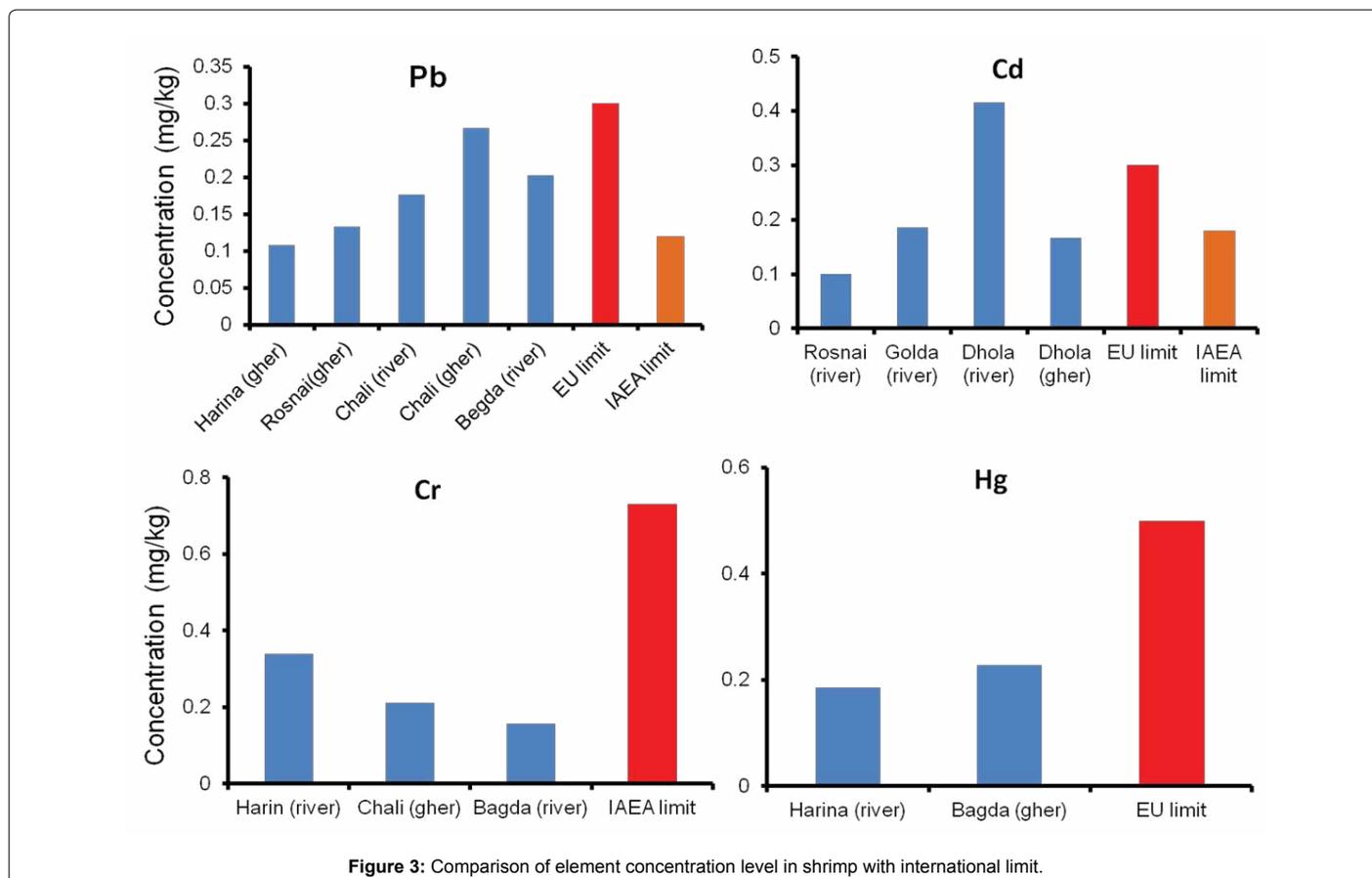
The results showed that the levels of Cr in shrimp species were between 0.156 mg/kg in Bagda and 0.338 mg/kg in Harina. Higher amount of Cr was detected in river sample than that of Gher (Table

2) but there was no significant different between two sources sampled. It was detected in Harina, Bagda and Chali and the rest of the shrimp samples are found below the detection limit (detection limit of Pb is <0.1 mg/kg). This trend is similar to those reported in a previous study in Bangladesh [22]. The level of Cr in all of the shrimp samples was found to be below also below permissible limits (Figure 2).

Data showed that in all shrimps from both Gher and river, lead concentration is less than permitted level, so they are suitable for consumption. Chromium, one of the most common elements in the earth's crust and seawater, exists in our environment in several oxidation states, principally as metallic (Cr0), trivalent (+3), and hexavalent (+6) chromium [25]. The latter is largely synthesized by the oxidation of the more common and naturally occurring trivalent chromium and is highly toxic. Trivalent chromium, found in most foods and nutrient supplements, is an essential nutrient with very low toxicity. Chromium VI is the most dangerous form of chromium and may cause health problems including: allergic reactions, skin rash, nose irritations and nosebleed, ulcers, weakened immune system, genetic material alteration, kidney and liver damage, and may even go as far as death of the individual [26]. Unfortunately, all shrimps that were studied in this study were less contaminated by Cr and they were still non-toxic to consumer.

Mercury (Hg)

Mercury occurs naturally in the marine food chain, both as organically bound mercury (e.g. methyl mercury) and inorganic bound mercury [22]. It is the organic form that is most toxic and gives rise to concern. The mercury concentration in Shrimp is less than 0.03 mg/



kg in most cases, with the exception of one case of Harina shrimp (0.2 mg/kg). The EU has a maximum level for mercury in feed of 0.1 mg/kg (Figure 3).

Toxic effects include damage to the brain, kidney, and lungs [27]. Mercury poisoning can result in several diseases, including acrodynia (pink disease) [28], Hunter-Russell syndrome, and Minamata disease [29]. Mercury in its various forms is particularly harmful to fetuses as an environmental toxin in pregnancy, as well as to infants. Women who have been exposed to mercury in substantial excess of dietary selenium intakes during pregnancy are at risk of giving birth to children with serious birth defects. Mercury exposures in excess of dietary selenium intakes in young children can have severe neurological consequences, preventing nerve sheaths from forming properly. The consumption of fish is by far the most significant source of ingestion-related mercury exposure in humans and animals, although plants and livestock also contain mercury due to bioconcentration of mercury from seawater, freshwater, marine and lacustrine sediments, soils, and atmosphere, and due to biomagnification by ingesting other mercury-containing organisms [30]. Humans risk ingesting dangerous levels of mercury when they eat contaminated fish. Since mercury is odorless, invisible and accumulates in the meat of the fish, it is not easy to detect and can't be avoided by trimming off the skin or other parts. This study showed that tested shrimp fishes have no toxic level that cause human health problem.

It is most important step now to reveal the secrets whether the toxic elemental status of shrimp which cultivated in Gher is comparable with other shrimp which found in river. The concentrations of toxic elements in shrimp collected from Gher and river are compared to the international permissible values in fish set by EU and IAEA. Comparing obtained concentrations with international permissible limit the overall condition of Bangladeshi shrimp samples should be observed. Figure 3 shows the comparison of observed lead concentration in shrimp of Gher and river with EU and IAEA limits. EU set the maximum level for Pb in fish is 0.3 mg/kg [31] and IAEA set the maximum level for Pb in fish is 0.12 mg/kg [32]. From the Figure 3 the highest value of Pb in shrimp of Gher is 0.267 mg/kg which is nearly as the permissible value of Pb in shrimp set by European Union (EU) of 3 mg/kg. In most of the cases the concentrations of Pb in shrimp samples are higher than the permissible limit of IAEA for lead. So lead concentrations of all samples are in safe level with respect to EU value and are very harmful for our health with respect to IAEA value.

Figure 3 shows the comparison of observed Cadmium (Cd) concentration in shrimp of Gher and river with EU and IAEA limit. EU set the maximum level for Cd in fish is 0.3 mg/kg [31] and IAEA set the maximum level for Cd in fish is 0.18 mg/kg [32]. Here the concentration of Cd in shrimp is higher than the shrimp of Gher. Cd is found in Dhola shrimp of Gher in which the concentration is below the permissible limit of both EU and IAEA. So it is not harmful for our health. The concentration of Cd in Dhola shrimp is very high than both EU and IAEA limit. Again the concentration of Cd in Golda shrimp of river is below than the EU limit but higher than the IAEA limit. The Concentration of Cd in shrimp which is higher than the permissible limit is very harmful for our health.

The comparison of observed Cr concentration in shrimp of Gher and river with IAEA limit is shown Figure 3. IAEA set the maximum level for Cr in fish is 0.73 mg/kg [32]. Chromium is found in Harina and Bagda shrimps of river and in Chali shrimp of Gher. The concentration of Cr in both river and Gher sample is below the IAEA permissible value in fish. So observed Cr level in shrimp is not harmful for our health.

Figure 3 shows the comparison of observed mercury concentration in shrimp of Gher and river with EU limit. In river Hg is found in only Harina shrimp and in Gher. Hg is found in only in Bagda shrimp. The concentration of Hg in both shrimp is less than the EU permissible limit. EU set the maximum level for Hg in shrimp is 1.0 mg/kg [21].

From the above discussion, contamination of river's shrimps is greater than the Gher's shrimps. So it is comparatively safe for us to eat those shrimp which produced in Gher than in river. However, further systematic studies with large number of shrimp samples from river and Gher are needed to confirm this observation. In Gher the probability of contamination with toxic element is lower than river, because in a Gher shrimp produces in a controlled way. However, a little contamination that occurs in Gher's shrimp may be from the fish feed and the water.

Conclusion

The result shows that Bangladeshi shrimp contained low concentrations levels of these toxic elements. The results show that Pb concentration for both river and Gher samples lies between EU and IAEA limits, Cd concentration in river samples are higher than both EU and IAEA limits. Arsenic is observed only in one river sample. Finally, Hg concentration for both Gher and river samples are less than the EU permissible limit. These indicate that the probability of contamination with toxic elements in Gher is lower than river. So, it is comparatively safe for us to eat those shrimp which produced in Gher than in river.

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