NUCLEARSCIENCEANDAPPLICATIONS Vol.29. No. 1&2 2020

Metals in Fish and Shell Fishes Collected from the Major Watercourse of the Chittagong Region, Bangladesh and their Average Daily Intake

M.S. Rahman[1,2], S. Akther[3], M.K. Ahmed[3], Y.N. Jolly[4], S. Akhter[4], M.J. Kabir[4], T.R. Choudhury[5], M.Hasan[1,2], M.A.M. Sarkar[1,2] and B.A. Begum[1,2*]

[1]*Water Quality Research Laboratory, Chemistry Division, Atomic Energy Centre, Bangladesh Atomic Energy Commission 4-Kazi Nazrul Islam Avenue, Shahbag, Dhaka 1000, Bangladesh*
[2]*Air Particulate Research Laboratory, Chemistry Division, Bangladesh Atomic Energy Commission, Shahbag, Dhaka-1000 Bangladesh*
[3]*Department of Oceanography,* Faculty*of Earth and Environmental Science, University of Dhaka, Dhaka 1000, Bangladesh*
[4]*Atmospheric and Environmental Chemistry Laboratory, Atomic Energy Centre, 4-Kazi Nazrul Islam Avenue, Shahbag Dhaka 1000, Bangladesh*
[5]*Analytical Chemistry Laboratory, Atomic Energy Centre, 4-Kazi Nazrul Islam Avenue, Shahbag, Dhaka 1000, Bangladesh*

Abstract

Current study was undertaken to investigate the toxic metal concentration in the pre-selected fish and shell fishes of major watercourse of the Chittagong region(Karnafuli River Estuary) and the measurement of average daily intake of metal through fish fillet ingestion. The concentration of metals namely Cr, Mn, Cu, Zn, Pb, Co, Fe, Br, Rb, K and Ca were quantified in 10 fish samples namely *Labeobata, Tenualosa toil,Latescalcarifer, Polynemusparadiseus, Pampus argenteus, Penaeus monodon, Harpadonnehereus, Mystusbleekeri, mpokpabda, Taenioidescirratus* using X-ray Fluorescence (XRF) technique at Chemistry Division of Bangladesh Atomic Energy Commission. From this study, iron (Fe) was found in high amount in fish samples and the sample *Taenioidescirratus* (65.88 mg/kg) have the highest value. On the other hand, low amount of Ni and K among those elements were found in the studied fish samples. Metal concentration in the fish samples were found in sequence of Fe>Zn>Br>Mn>Rb> Cu>Pb>Cr>Co>Ca> K respectively. The estimated daily intakes (*EDI*) of the studied metals are also calculated. The mean values of *EDI* for the fish samples were found to be the following descending order of Fe>Zn>Br>Mn>Rb>Cu>Pb>Cr>Co>Ca>K respectively. It was found that the *EDI* values for the examined fish samples were below than that of the recommended values set by WHO, MOFL and EU.

Keywords: Fish, Toxic element, major water course, Karnafuli river, estimated daily intakes

1. Introduction

Bangladesh is a riverine country. A large number of rivers are flowing through Bangladesh, which are originated from the outside of the country and those rivers waters have heavy loads of sediments and other debris including domestic wastes, agro-chemical and industrial wastes. Therefore, the water bodies of the rivers are polluted with organic and inorganic pollutants, which might be affected the environment. Therefore, river pollution becomes a serious problem in Bangladesh [1].

Heavy metals are a global concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems [2-4]. Pb, Ba, Cd, Hg, Cr, and As are classified as toxic heavy metalsand which have no role in biological systems [5-8]. On the other hand, Cu, Na, K, Ca, Mn, Se, Fe, and Zn are essential metals for fish metabolism but may also bioaccumulate and reach toxic levels that can potentially destroy the ecological environment [9-11]. The increasing usage of heavy metals in industry has led to serious environmental pollution through effluents and emanations during the past several decades [12]. Subsequently, these activities have increased the release of harmful heavy metals into the aquatic environment [9-11], which are well known environmental pollutants [13].

Toxic heavy metals, such as arsenic (As), zinc (Zn), copper (Cu), cadmium (Cd), and lead (Pb) are harmful to eco-environments and human beings. Cd and Pb are non-essential metals that exhibit extreme toxicity, even at trace levels [14]. Arsenic (As) is one of the most toxic elements for human and animal health, which can cause toxic and detrimental biological effects such as liver, skin, and bladder cancer [15]. Although copper (Cu) and zinc (Zn) are essential minerals for humans, but excessive exposure of these beneficial elements can also cause health problems [16]. Heavy metals cannot undergo degradation process, and they are continuously deposited and incorporated into water, sediments and aquatic organisms [17, 18]. Anthropogenic activities, such as increased population, urbanization, industrialization, and agricultural practices, have further aggravated this problem [19].

Fishes are an important source of protein for humans. Additionally, they are low in cholesterol and contain beneficial poly-unsaturated fatty acids [20]. However, contaminant levels in fish present a considerable concern. Heavy metals are known to accumulate in various aquatic organisms and can be bioaccumulated via the food chain to levels that are hazardous to health [21]. The general population is most commonly exposed to Hg through the consumption of fish [22]. Methyl-mercury (Me-Hg) concentrations, in particular, are typically high in some fish species and account for most of the total Hg found in fish.

---

*Corresponding author:*bilkisab@dhaka.net*

Me-Hg is known to cause adverse health effects in people who consume large quantities of fish [23]. So, the determination of metal concentrations in fish and the evaluation of possible risks to human health after taking the fish as a food become the urgent need. Not surprisingly, numerous previous studies have focused on metal accumulation in various fish species [24-27].

Fish are widely used as bio-indicators of heavy metal contamination [28-30] because they occupy different tropic levels and are of different sizes and ages [31]. Relationship between metal concentrations in fish and in the water has been studied in both field and laboratories [32-34]. Fishes are well-known for their ability to concentrate heavy metals in their muscles [23]. Therefore, the fish muscles were selected as a primary site of metal uptake and since fishes are integral component of human diet, they need to be carefully screened so that unnecessary harmful amount of heavy metals do not stored in human body after consumption of fish.

Karnafuli River is the largest river and the most important river in major watercourses of the Chittagong region and the Chittagong Hill Tracts, which is originating in the Lushai hills in Mizoram State of India. Because of the industrially development, the heavy metal pollution of the Karnafuli river is increasing day by day. The contamination of the heavy metals in river water directly affects the fish physiology and by the consumption ultimately affects the human health [35]. For the increasing level of pollution, the availability of fish is decreasing rapidly, as a result the socio-economic condition of the fishing community is affected [36]. About 800 industrial units are located on and adjacent to the banks of the Karnafuli in different industrial areas such as Kalurghat, Nashirabad, Sagarica, and Anawara industrial zone as well as oil refinery, oil companies depot, ship breaking activities, etc. [37]. In addition, Chittagong port handles about 1700 ships and 70-80 oil tankers annually.

Fish and shellfish are considered as an important dietary food, especially in seaboard areas and smaller islands, which supplies all essential elements required for life processes in a balanced manner [38]. Hence, it need to study the levels of heavy metals in these living being to evaluate these metals whether is in permissible limit or not and to find out that these heavy metals do not cause any hazard to the consumers [39-42].Considering the above facts, the aim of this study is to monitor the level of metals in fishes collected from a major watercourse of the Chittagong region (KarnafuliRiver estuary) and to determine whether these heavy metals are within permissible limits for human consumption. Average daily intake for the studied metals through intake of the fishes and shell fishes are also targeted to determine.

2. Materials and Method

2.1 Study area

The major watercourse of the Chittagong region Karnafuli river Estuary is one of the most important estuaries in Bangladesh and hydro-biologically it is the meeting place where fresh water from upstream in continuously mixing with salt water of Bay of Bengal. Combinations of diverse fluctuating parameters are responsible for occurrences and distribution of different micro-organisms in estuarine environment. It is also blessed with estuarine water, soil, marine resources and varieties of fish species. Besides, many industries, fishing boats, vessels, trawlers and container ships are not uncommon on the river water, which hampers the status of water, soil and fish species of this river. The lower area of the river Karnafuli as brackish water is used as naturalfeeding, breeding and nursery ground for marine and coast living fish species.The river Halda, another unique and important natural breeding ground ofindigenous carp species of our country, which have a direct connection with the river Karnafuli. So, to know the level of heavy metal pollution of the river is very much important. The study area was Karnafuli river estuary, Bangladesh. The study area is shown in the **Fig. 1**.

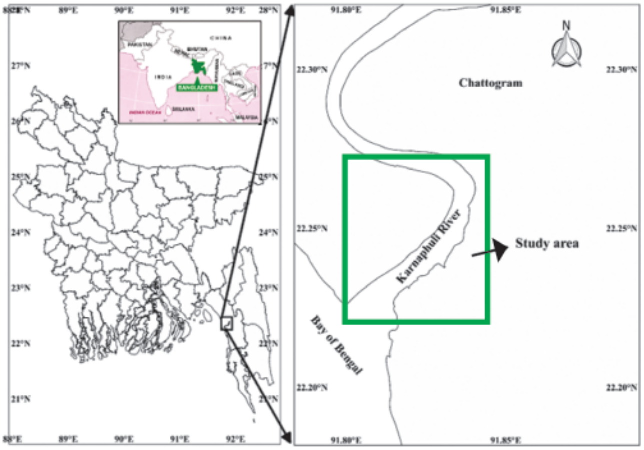


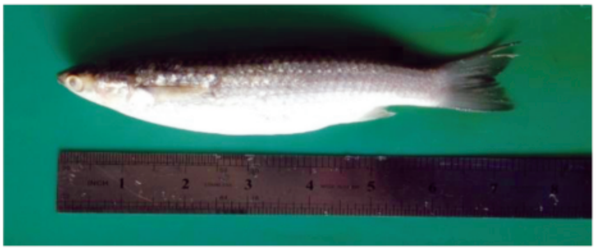
Fig. 1: Geographic location of the major watercourse of the Chittagong region (Karnaphuli river estuary) considering the study area

2.2 Sample collection and preparation

The experimental fresh fish were procured in September, 2015. Different types of fishes are found in the estuary. The local name, scientific nameand the general characteristics of the fishes for this study are given in Table 1.

Table 1: Sample code, local name, scientific name and general characteristics of the studied fish sample

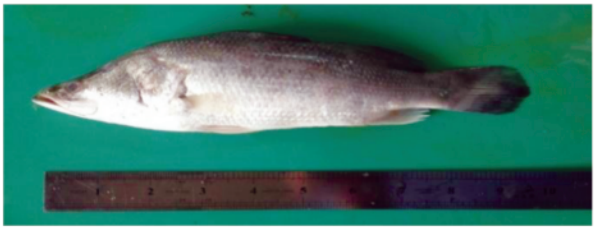
Sample Code	Local Name	Scientific Name	General characteristics
F-1	Bata	*Labeobata*	Dorsal profile more convex than that of abdomen. Head 3.8-4.2" standard and 4.8-5.5" total length.
F-2	Fali chanda	*Pampus argenteus*	Ovate, strongly compressed fish. Dorsal and ventral profile about equally convex. Height 1.3-1.5" standard,2.3- 2.5" total length.
F-3	Chingri	*Penaeus monodon*	Shrimp have an exoskeleton (the "shell") that is periodically shed (moulted) to allow further growth. Largest commercially available shrimp, reaching 330 mm or more.
F-4	Chandanailish	*Tenualosa toil*	Dorsal surface of head thickly covered by skin. Height 2.8-3.0" standard, 3.7-4.0" total length.
F-5	Bhetki, Koral	*Latescalcarifer*	Dorsal profile more complex than that of abdominal. Height 2.9-3.1 in standard, 3.6-3.8 in total length.
F-6	Rishi	*Polynemusparadiseus*	Dorsal profile more complex than that of abdominal. Height 4.0-4.6" standard, 5.6-6.3" total length.
F-7	Loitta	*Harpadonnehereus*	Head thick with very short snout. Height 5.0-7.2 in standard, 6.2-8.7 in total length. Scales cycloid**.**
F-8	Chewa	*Taenioidescirratus*	Body elongate, sub-cylindrical anteriorly, compressed posteriorly. Dorsal and anal fins enveloped in skin, separated from caudal by a notch; pelvic rounded. Height 15.3-16.0 in standard, 17.4-18.0 in total length.
F-9	Gulsatengra	*Mystusbleekeri*	Median longitudinal groove on head shallow, reaches base of occipital process. Height 3.8-4.3 in standard, 5.5-5.8 in total length. A dark shoulder spot behind head.
F-10	Madhupabda	*Ompokpabda*	Head depressed, snout rounded. Mouth superior, ends in front of eye;2 pairs of barbels; maxillary pair reaches to end of pectoral fin. Height 4.1-4.5 in standard, 4.6-5.0 in total length.

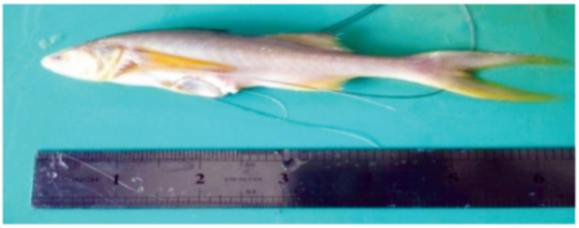

Labeobata

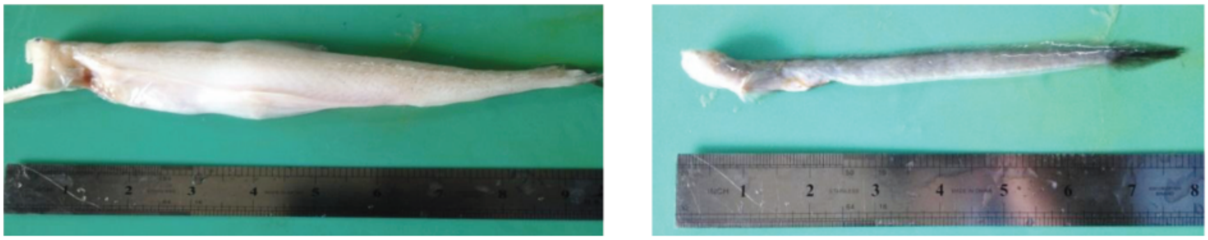

Pampus argenteus


Penaeus monodon


Tenualosa toil

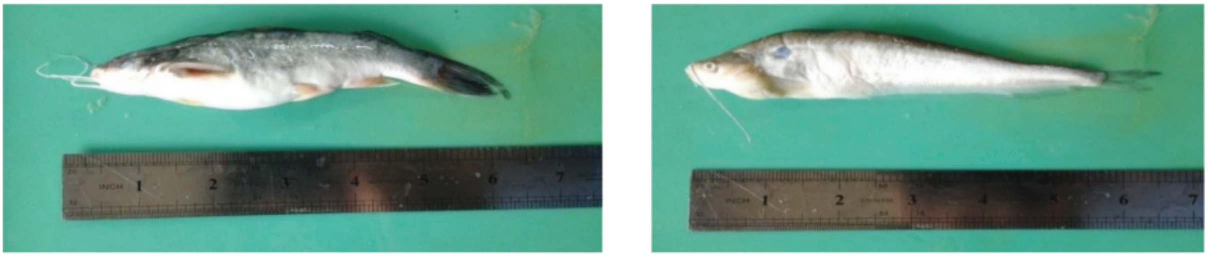

Latescalcarifer


Polynemusparadiseus



Harpadonnehereus

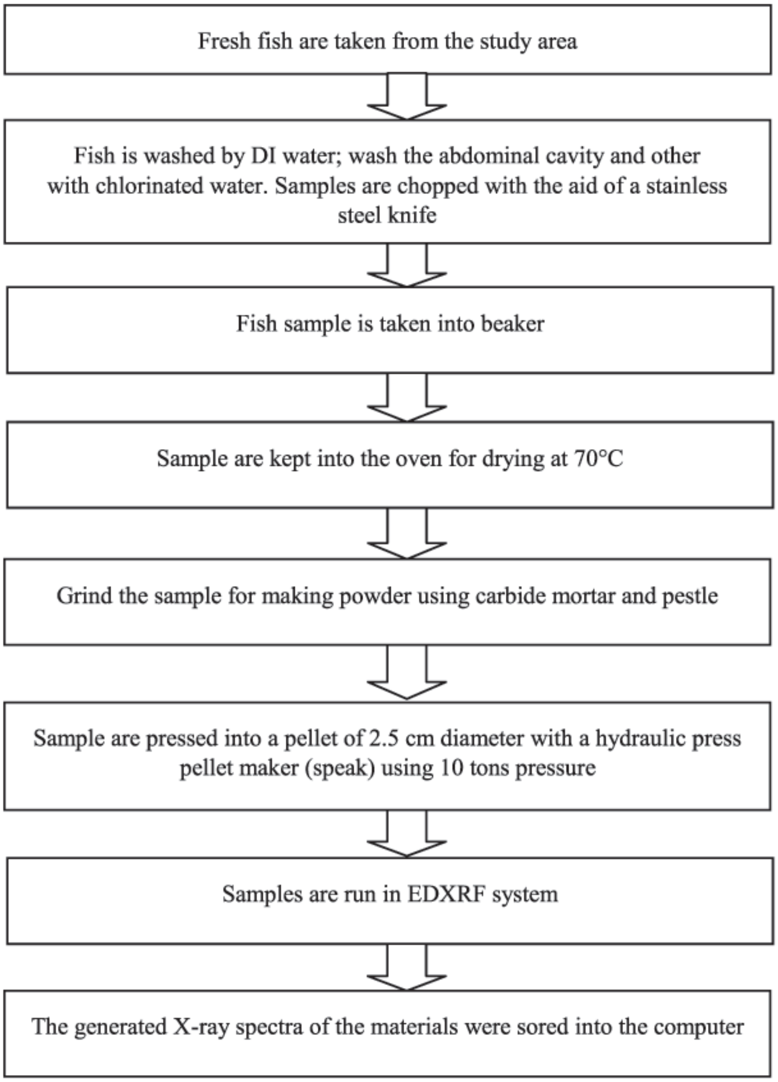
Taenioidescirratus



Mystusbleekeri

Ompokpabda

Fig. 2: Pictures of the studies ten species of commercial coastal fish species in Bangladesh



Fresh fish are taken from the study area

Fish is washed by DI water; wash the abdominal cavity and other with chlorinated water. Samples are chopped with the aid of a stainless steel knife

Fish sample is taken into beaker

Sample are kept into the oven for drying at 70°C

Grind the sample for making powder using carbide mortar and pestle

Sample are pressed into a pellet of 2.5 cm diameter with a hydraulic press pellet maker (speak) using 10 tons pressure

Samples are run in EDXRF system

The generated X-ray spectra of the materials were sored into the computer

Fig. 3: Flow chart showing sample preparation procedure

2.3 Fish sample preparation

The collected fish samples for ten fish species (Fig. 2) were cleaned and washed with deionized water, then it was chopped with the aid of a stainless-steel knife, which was cleaned by acetone and hot distilled water prior to use. Fish flesh was taken to a beaker. These samples were dried in an oven at 70°C for 48 hours. The dried samples were grinding for making powder using carbide mortar and pestle. For EDXRF analysis, each powdered sample was pressed into a pellet of 2.5 cm diameter with a hydraulic press pellet maker (Specac) using 10 (ten) tons pressure. The irradiation of all real samples was performed by assigning a time-based programmed controlled by a software package provided with the EDXRF system. To produce the calibration curves for quantitative elemental analysis for the studied samples, respective standard samples were also irradiated under the same experimental conditions.The generated X-ray spectra of the materials were sored into the computer. The schematic diagram for the preparation of fish samples for elemental analysis was shown in Fig. 3.

2.4 Metals analysis

X-ray Fluorescence analysis is a non-destructive instrumental method of qualitative and quantitative analysis for chemical elements based on the measurement of characteristic fluorescent radiation resulting from the deexcitation of inner shell vacancies produced in the sample elements by means of a suitable source of radiation [43]. The Energy Dispersive X-ray Fluorescence Analysis employs detectors that directly measure the energy of the X-rays by collecting ionization produced in a suitable detecting medium. A wide variety of EDXRF analytical systems using radioisotope sources, X-ray tubes, charged particle accelerators, microprobe electron beams and synchrotron light sources have been developed [43-47].The whole system for the experimental purpose consist of a Cd-109 radioisotope annular source (NEN), a Si(Li) detector (Canberra, Model SL 80175), a fast spectroscopy amplifier (Canberra, Model 2024), a high voltage power supply (Tennelec, Model TC 95OA), and a multi-channel analyser (Canberra, Series 35, Model 3201).The accuracy and precision of the XRF analysis were checked by using SRM 1571, a certified reference material. The results for the recoveries were between 90 and 110% and the accuracy was within 10% (Table 2), which showed good agreement between the measurement and certified values.

Table 2: Analytical results obtained on certified reference materials (µg/g)

Name of Element	Measured value	Certified value	Recovery (%)	Accuracy (%)
Cr	2.57	2.6	98.85	-1.15
Mn	85.28	91	93.71	-6.29
Cu	10.87	12	90.58	-9.42
Zn	25.8	25	103.20	3.20
As	9.06	10	90.60	-9.40
Pb	43.02	45	95.60	-4.40
Co	0.21	0.2	105.00	5.00
K	2090	2079	100.53	0.53
Fe	305.02	300	101.67	1.67
Sr	37.8	37	102.16	2.16

2.5 Estimated daily intake of metals

Metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards [48].

The estimated daily intakes (EDI) of metals through consumption of fish were calculated with the following formula [31]**:**

$$\mathrm{EDI} = (\mathrm{DFC} \times \mathrm{MC})/\mathrm{BW}$$

Where,DFC=daily food consumption, *MC*=mean metal concentration (mg/kg fw) in fish samples and BW = body weight. The daily fish consumption rate for adults' residents was an average of 45.67 g, drawing from the report of the household income and expenditure survey 2010 [49]. The body weight of an adult resident was set to 60 kg in the present study [50].

3. Results and Discussion

3.1 Metal concentration in fish species

The concentrations of the analyzed metals, i.e., Cr, Pb, Cu. Zn, Mn, CoandRbin different ten species of fishes were compared with WHO [51], EU [52] and MOFL. The statistical data (min, max, medium, 25th and 75th percentile) for the studied metals in ten species of fishes were presented in Table 3. The highest concentration in the important commercial fish species was found for Zn (1.38-55.84), followed by Mn (14.01-17.89), Cu (2.69-7.71), Rb (2.69-14.6), Pb (2.68-3.27), Co (0.5-1.81), and Cr (1.44-1.99) (Fig. 4). The hierarchy of the cumulative metal concentration in various species was as follows: *L. bata*(92.68 mg/kg)>P.monodon (91.59 mg/kg)>T. cirratus (90.15 mg/kg)>M. bleekeri (88.29 mg/kg)>O. pabda (83.95 mg/kg)>H. nehereus (82.67 mg/kg)>L. calcarifer (81.5 mg/kg)> P. argenteus (81.33 mg/kg)>P. paradiseus (80.58 mg/kg)>T. toli (79.62 mg/kg) (Table 3). Subsequently, two-way ANOVA test revealed that the metal concentration is different fish species were not statistically different ($F_{cal}<F_{critic}$= 0.253<1.976, p = 0.985) at a 95% confidence level but the concentration for different metals are statistically ($F_{cal}>F_{critic}$ = 47.506>1.87, p = 1.54E-34) different (Table 4). However, specific individual elemental concentrations in ten species of fishes with the comparison of the recommended and the literature values are discussed below.

Table 3: Average concentration (mg/kg) of metals in fish

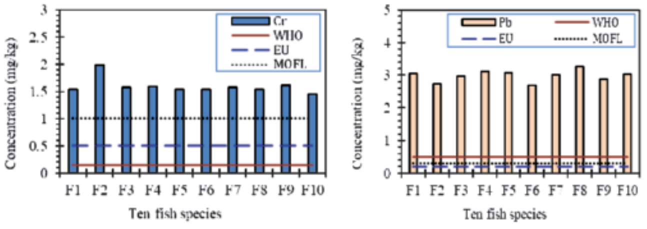
Fish	Metal Concentration										
sample	Cr	Mn	Cu	Zn	Pb	Co	Fe	Br	Rb	K	Ca
F- 1	1.53	17.69	4.88	38.23	3.05	1.81	59.06	28.78	14.60	0.04	1.43
F- 2	1.99	17.90	2.77	38.00	2.73	1.50	40.49	17.96	3.67	0.03	0.94
F- 3	1.57	14.59	4.03	35.85	2.97	1.00	46.59	40.96	9.20	0.05	0.51
F- 4	1.59	17.88	2.97	35.97	3.12	1.01	51.27	35.22	7.40	0.04	1.73
F- 5	1.54	17.80	4.86	38.92	3.07	0.99	44.77	29.14	3.27	0.04	0.41
F- 6	1.53	14.71	7.71	47.12	2.68	1.71	51.64	14.62	5.23	0.02	1.85
F- 7	1.57	15.06	4.16	42.86	3.01	1.02	49.90	38.31	3.87	0.02	0.84
F- 8	1.53	16.51	4.96	47.91	3.27	0.50	65.20	29.18	BDL	0.03	1.22
F- 9	1.61	15.03	2.69	44.78	2.89	1.21	58.78	25.83	BDL	0.04	0.93
F- 10	1.44	14.01	5.84	1.38	3.03	0.95	65.88	2.87	BDL	0.01	5.88
Minimum	1.44	14.01	2.69	1.38	2.68	0.50	40.49	2.87	3.27	0.01	0.41
Maximum	1.99	17.90	7.71	47.91	3.27	1.81	65.88	40.96	14.60	0.05	5.88
Average	1.59	16.12	9.49	37.10	2.98	1.17	53.36	26.29	6.29	0.03	1.58
Stdev	0.15	1.59	16.35	13.31	0.18	0.40	8.58	11.62	3.47	0.01	1.59
25thPercentile	1.53	14.68	2.92	35.94	2.85	0.98	46.14	17.12	3.81	0.02	0.76
75thPercentile	1.59	17.82	5.64	45.36	3.08	1.28	60.38	35.99	6.64	0.04	1.76

Table 4: Two-way ANOVA test for metal concentration in different fish species

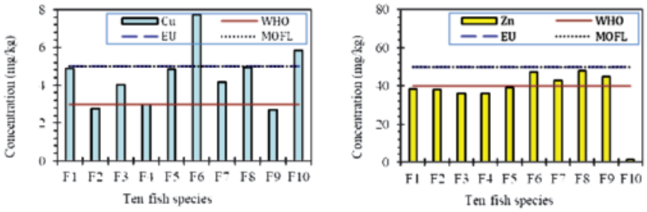
Source of Variation	SS	df	MS	F	P-value	F crit
Between metals	31154.9	11	2832.26	47.500	1.54E-34	1.887
Between fish species	135.92	9	15.10	0.253	0.9851	1.976
Error	5902.99	99	59.63	-	-	-
Total	37193.81	119	-	-	-	-

3.1.1 Chromium (Cr)

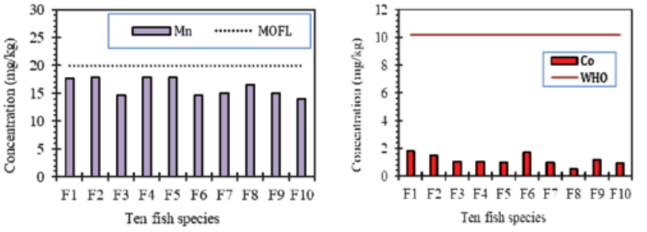
Generally, chromium does not build up in fish, that's why a very low amount of concentration was found in fish even in the most industrialized area in the world. The rate of uptake was higher in young fish but the body burden of Cr was declined with age due to rapid elimination [53]. Our results also showed low concentrations range of 1.44 to 1.99 mg/kgand it was several folds lower than that of fish muscle (4.41 to 8.03 mg/kg) collected from the red sea [54]. In the present study (Table 3) the highest level of Cr was detected in Tenualosatoli (1.99 mg/kg) and the lowest in Taenioidescirratus (1.44 mg/kg). The Western Australian Food and Drug regulations stated concentration of 5.5 mg/kg for Cr which was higher than our values [55]. The average Cr levels found in all fish species are lower compared to the literature values for Cr in fish from the Southeast coast (India) [56], Meghna estuary [57], Bangladesh coastal areas [58] and estuary of Bay of Bengal [59]. Furthermore, the current level was also found to be 2.11, 2.13, and 1.57 times lower than the fish species in local areas including Karnaphuli estuary (3.36 mg/kg), [60], Cox'sbazar coastal area (3.39 mg/kg) [61] and Patagonia (2.5 mg/kg) [62], respectively. The concentration of Cr can be declined due to the body burden when the species grow to adults [53]. However, access concentration of the element can lead to pulmonary disease along with liver, lung, and kidney damage after a long time of exposure [63].The observed concentrations of Cr in the fish samples of Karnafuli river might be due to the wastewater coming from various industries such as dying and tanning industries, photography, garment industries, ceramics industries, leather products manufacturing industries and various coloring agent such as additives, binder, filler, pigments producing industries around KEPZ, and river water flow from upstream which carries various chemicals used in plant cultivation purposes in agricultural fields.



(a) Chromium (Cr) **(b)** (Lead (Pb)



(c) Copper (Cu) **(d)** Zinc (Zn)



(e) Manganese (Mn) **(f)** Cobalt (Co)

Fig. 4: Concentration of metals (Cr, Pb, Cu, Zn, Mn and Co) in ten species of fishes in comparison with the recommended value of WHO (1989), EU (2006) and MOFL (2014)

3.1.2 Lead (Pb)

Lead is a non-essential element and it is well documented that Pb can cause neurotoxicity, nephrotoxicity, and many others adverse health effects [64]. The highest concentration of Pb for this study was detected in *M. bleekeri* (3.27 mg/kg), followed by, *P. paradiseus* (3.12 mg/kg), *P. argenteus* (3.07 mg/kg), *L. bata* (3.05 mg/kg), *T. cirratus* (3.03 mg/kg), *H. nehereus* (3.01 mg/kg), *L. calcarifer* (2.97 mg/kg), *O. pabda* (2.89 mg/kg), *T. toil* (2.73 mg/kg), and *P. monodon* (2.68 mg/kg), respectively (Table 3). Among all the specimens, *M. bleekeri*contributed 11% of total Pb concentration. On the other hand, the least concentration confined at 9% in *P. monodon.* The details of lead concentration detected for individual fish were given in Table 3. Maximum allowable level of Pb contaminants in fish, and shellfish are 0.5 and 1 mg/kg, respectively, set by Food Standards Australia and New Zealand [65]. The maximum permissible doses for an adult are 3 mg Pb per week, but the recommended doses are only one-fifth of those quantities [66]. This study revealed that Pb concentration in the studied ten species fishes was higher (Fig. 4b) than the maximum permissible limit set by the world health organization (0.5 mg/kg) [67] and European Union (0.2 mg/kg) [52]. In addition, lead is a ubiquitous pollutant, which could finds its way into the major watercourse of the Chittagong region through discharge of industrial effluents from various industries such as printing, dyeing, oil refineries, and textile around the Dhaka export Processing Zone (DEPZ) and other sources.

3.1.3 Copper (Cu)

Copper is an essential part of several enzymes and is necessary for the synthesis of hemoglobin [40]. However, high intake of Cu has been recognized to cause adverse health problem [68]. Copper was detected in all examined fish samples and the highest content found in *Taenioidescirratus* (7.71 mg/kg) and the lowest was in *Ompokpabda* (2.69 mg/kg). The permissible limit of Cu proposed by ANHMRC and FAO, was 30 mg/kg fresh weight [69, 70]. According to UK Food Standards Committee Report, Cu concentration in food should not exceed the value of 20 mg/kg as wet weight [71]. Many countries of the world also maintain a regulatory guideline for the use of Cu in meals to protect people from health hazards. For example, Turkish legislation established the level of Cu at 5 mg/kg, whereas Spanish legislation proposed the level at 20 mg/kg as wet weight [72]. The Australian Food Standard Code established the maximum concentration for Cu at 10 mg/kg wet weight [73]. From this study we see that *Taenioidescirratus* exceeded the permissible limits prescribed by various agencies. Cu is an essential element that is carefully regulated by physiological mechanisms in most organisms [74]. Though Cu play an important role in body functioning, yet it has some negative impact on living being especially on animal and human being. Cu can be found in a natural environment and is essential for the normal growth and metabolism of all living organisms [75]. However, it becomes toxic at high concentrations. Contaminated fishes can increase Cu content in human body when it is taken as dietary food.

Miramand and Bentley [76] stated that Cu accumulated in the species due to the industrial activities in that area.

The concentration of Cu in fish species was observed in the following increasing order: *P. monodon* (7.71 mg/kg) >*T. cirratus* (4.96mg/kg)>*M. bleekeri* (4.94mg/kg)>*L. bata* (4.88 mg/kg)>*P. argenteus* (4.86 mg/kg)>*H. nehereus* (4.16 mg/kg) >*L. calcarifer* (4.03 mg/kg)>*P. paradiseus* (2.97 mg/kg) >*T. toil* (2.77 mg/kg)>*O. pabda* (2.69 mg/kg) (Table 3). It was observed that the *P. monodon*species accumulated 59% of total Cu content, whereas the least amount was accumulated at 3% in *O. pabda*. The mean concentration of Cu in the studied fish species was lower than the Meghna estuary (13.88 mg/kg) [60] and Bangladesh coastal areas (5.7 mg/kg) [58]. Conversely, some studies like Bahía Blanca estuary (124.5 mg/kg) [77] and Karnaphuli estuary (12.1 mg/kg) [60] were higher than our observation. The concentration of Cu at the Coxbazar coastal (8.54 mg/kg) area [61] was mostly in line with this study (Table 3). On the other hand, the average Cu concentration (4.49 mg/kg) in all fish species was lowerthan the recommended values (5 and 20 mg/kg) set by the EU (2006) [52] and MAFF (2000) [78] respectively, while 10% fish species contained higher Cu level (Fig. 4c) compared to the world health organization [67] and the Ministry of Agriculture, Forestry and Fisheries, UK [78].

3.1.4. Zinc (Zn)

Zinc is a heavy metal, which has a possibility to get bio-accumulated in the tissues such as fatty tissues, muscles tissuesof marine or aquatic animal including fish and can affect reproductive physiology in fishes.Some authors reported that chronic exposure to Cu and Zn is associated with Parkinson's disease and these elements might act alone or together over time to induce the disease [68]. Generally, Zn content in fish are found highest in amount among other heavy metal. There was a great variation in Zn concentrations among the studied fish muscles (Fig. 4d). The highest amount of zinc was found in the fish sample of *Mystusbleekeri* (47.91 mg/kg) and the lowest was in *Latescalcarifer* 35.85 mg/kg) among the ten species of fish and crustaceans in Karnafuli river. The amount of Zn determined in all the fish samples were far below the standard of 1000 mg/kg set by the Australian National Health and Medical Research Council(ANHMRC) [69].

The hierarchy of Zn concentration in the fish species was as follows: *M. bleekeri* (47.91 mg/kg)>*P. monodon* (47.12 mg/kg)> *O. pabda* (44.78 mg/kg)>*H. nehereus* (42.86 mg/kg)>*P. argenteus* (38.92 mg/kg)>*L.bata* (38.23 mg/kg)>*T. toil* (38.00 mg/kg)>*P. paradiseus* (35.97 mg/kg)>*L. calcarifer* (35.85 mg/kg)>*T. cirratus* (1.38 mg/kg) (Table 3). It was noticed that 13% of the total concentration of Zn was attributed to *M. bleekeri*. However, the mean concentration of Zn in our study was 17, 742 and 16 times higher than the results of the Southeast Coast (2.25 mg/kg) [56], Daya Bay (47.74 mg/kg) [25], and Estuary of Bay of Bengal (2.25 mg/kg) [59], respectively. On the contrary, Zn concentration in both Bangladesh coastal areas (64.1 mg/kg) [58] and Patagonia (86.89 mg/kg) [62] surpassed at 2 folds higher than this study.

3.1.5 Manganese (Mn)

Manganese is a heavy metal which has a low toxicity to the human healthand has a considerable biological significance. No maximum is specified for manganese in fish samples. The concentration of Mn in the analyzed samples ranged from 14.01 to 17.89 mg/kg. *Tenualosa toil* exhibited the maximum Mn concentration of 17.89 mg/kg and *Taenioidescirratus* showed the minimum of 14.01 mg/kg. Normally, water contain low level (0.05 mg/kg) of Mn [79]. Manganese is found to use in producing a variety of important alloys and to deoxidize steel and desulfurize and it is also used in dry cell batteries, paint, steel, glass, ceramics, and electric coils etc. that might be regarded as an important sources of pollution due to Mn uses.

However, this result was in good agreement with the value found in fish species from Gumti River, Bangladesh [80].

This study revealed that manganese (Mn) concentration was observed at a high level of 11% in *T. toli* (17.89 mg/kg), followed by *P. paradiseus* (17.88 mg/kg), *P. argenteus* (17.8 mg/kg*), L. bata* (17.69 mg/kg), *M. bleekeri* (16.51 mg/kg), *H. nehereus* (15.06 mg/kg), *O. pabda* (15.03 mg/kg), *P. monodon* (14.71 mg/kg), *L. calcarifer* (14.59 mg/kg) and *T. cirratus* (14.01 mg/kg) (Table 3). Comparatively, some international studies like the Southeast coast (1.2 mg/kg) [56], Estuary of Bay of Bengal (0.9 mg/kg) [59] and Cox'sbazar coastal area (10.71 mg/kg) [61] showed that the element was 13, 18, and 1.5 times, respectively, below our investigation. Meanwhile, Bahía Blanca estuary in South America (57.72 mg/kg) [77] was 3.5 times higher than our findings (Table 3). The high quantity ingestion of Mn may cause neurologic and mental disorders [61].

3.1.6 Cobalt (Co)

This study showed that the highest Co was accumulated in *L. bata* at the rate of 16% of the total Co content, in where the minimum amount was observed at 4% in *M. bleekerI* (Table 3). The rank of Co concentration in the organisms was as follows: *L. bata* (1.81 mg/kg)>*P. monodon* (1.71 mg/kg)>*T. toli* (1.50 mg/kg)>*O. pabda* (1.21 mg/kg)>*L. calcarifer* (1.04 mg/kg)>*P. paradiseus* (1.02 mg/kg)>*H. nehereus* (1.01 mg/kg)>*P. argenteus* (0.99 mg/kg)>*T. cirratus* (0.95 mg/kg)>*M. bleekeri* (0.50 mg/kg). The finding of this study was found to be lower than the recommended guideline value (10.2 mg/kg) suggested by WHO [61]. However, the mean content crossed the values of the Southeast coast (0.1 mg/kg) [56], Estuary of Bay of Bengal (0.008 mg/kg) [59], and Cox'sbazar coastal area (0.39 mg/kg) in Bangladesh [61]. Moreover, our mean concentration of the element was 146 times higher than the record of the Estuary of the Bay of Bengal [59]. This study revealed that Co concentration in different fish species were below (Fig. 4f) that the recommended value set by WHO [51].

3.2 Estimated daily intake (EDI)

The EDI of metals (Cr, Mn, Cu, Zn, Pb, Co, Fe, Br, Rb, K and Ca) were evaluated according tothe average concentration of each metal in each food and the respective consumption rate [61]. The EDI of the studied metals from consumption of fish are shown in Table 3. In fish samples, mean values of EDI showed the descending order of Fe>Zn>Br>Mn>Rb>Cu>Pb>Cr>Co>Ca>K. The EDI (estimated daily intake) values presented in Table 3 were estimated by assuming that a 60 kg person will consume 45.67 g fish per day. The result shown in Table 3 revealed that the EDI values for the examined fish samples were below the recommended values set by WHO [67], JECFA [81] andNRC [82], indicating that health risk associated with the intake studied heavy metals through the consumptionofexamined fish sampleswere absent. Therefore, we can say that these metals at these levelsshould not pose any health threat to the consumers resulting from the consumption of studied fish.

Table 5: Estimated dietary intake (EDI) (mg/day) of metals due to consumption of fish

Time	F- 1	F- 2	F- 3	F- 4	F- 5	F- 6	F- 7	F- 8	F- 9	F- 10
Cr	6.99E-02	9.09E-02	7.17E-02	7.26E-02	7.03E-02	6.99E-02	7.17E-02	6.99E-02	7.35E-02	6.58E-02
Mn	8.08E-01	8.17E-01	6.66E-01	8.17E-01	8.13E-01	6.72E-01	6.88E-01	7.54E-01	6.86E-01	6.39E-01
Cu	2.23E-01	1.27E-01	1.84E-01	1.36E-01	2.22E-01	3.52E-01	1.89E-01	2.27E-01	1.23E-01	2.55
Zn	1.75	1.74	1.64	1.64	1.78	2.15	1.96	2.19	2.05	6.30E-02
Pb	1.39E-01	1.25E-01	1.36E-01	1.42E-01	1.40E-01	1.22E-01	1.37E-01	1.49E-01	1.32E-01	1.38E-01
Co	8.27E-02	6.85E-02	4.57E-02	4.57E-02	4.52E-02	7.81E-02	4.66E-02	2.28E-02	5.53E-02	4.34E-02
Fe	2.69	1.85	2.13	2.34	2.04	2.36	2.28	2.98	2.68	3.00
Br	1.31	8.20E-01	1.87	1.61	1.33	6.68E-01	1.74	1.33	1.17	1.31E-01
Rb	6.67E-01	1.68E-01	4.20E-01	3.38E-01	1.49E-01	2.39E-01	1.77E-01	-	-	-
K	1.83E-03	1.37E-03	2.28E-03	1.83E-03	1.83E-03	9.13E-04	9.13E-04	1.37E-03	1.83E-03	4.57E-04
Ca	6.53E-02	4.29E-02	2.33E-02	7.90E-02	1.87E-02	8.45E-02	3.84E-02	5.57E-02	4.25E-02	2.69E-01

4. Conclusion

Accumulation of metals by different fish and shrimp species from Karnafuli river estuary assumes importance due to its flowing from large-scale industrial and mining related areas. The concentration of metals in the fishes and shrimps do not exceed the limits for the recommendation values for food. They are within the standard limits proposed by various agencies, i.e., ANHMRC, ANZFA, Western Australian Food and Drug Regulations etc. The study also suggests that, irrespective of the locations, the shrimps accumulated more metals than the fish species. In the fish samples metal concentration were found in sequence ofFe>Zn>Br>Mn>Rb>Cu>Pb>Cr>Co>Ca>K. The present study showed that the contributions of estimate daily intake (*EDI*) showed the descending order of Fe>Zn>Br>Mn>Rb>Cu>Pb>Cr>Co>Ca>K. The EDI value indicating that health risk associated with the intake of studied metals through the consumption of examined fish samples was absent.

Acknowledgement

The authors gratefully acknowledge Ministry of Science and Technology (MOST), Government of the People's Republic of Bangladesh for some chemicals through the special allocation grant project (Grant No. 39.009.002.01.00.057.2015-2016/ES-307).

References

1. M.A.K. Sarker, study on the accumulation and seasonal variation of trace metals in water, sediment and some fishes of the Buruganga river, M.Sc. thesis, Department of Zoology, Dhaka University, 1-4 (2005).
2. I.G. David, M.L. Matache, A. Tudorache, G. Chisamera, L. Rozylowicz and G.L. Radu, Food chain biomagnification of heavy metals in samples from the lower prut flood plain natural park, Environ Eng. Manag. J, **11**, 69-73 (2012).
3. J.E. Hall, Bioconcentration, bioaccumulation, and the biomagnification in Puget sound biota: assessing the ecological risk of chemical contaminants in Puget sound, Tahoma West Literary Arts Magazine,**8,** 40-51 (2014).
4. B. Batvari, D. Prabhu, S. Kamalakannan and R.R. Krishnamurthy, Heavy metals accumulation in two fish species (*Labeorohita* and *Cirrhinamrigala*) from Pulicat Lake, North of Chennai, Southeast Coast of India, J Chem Pharm Res.,**7,** 951-956 (2015).
5. FAO Compilation of legal limits for hazardous substances in fish and fishery products, FAO Fish Circ., **464**, 5-10 (1983).
6. EC Commission regulation No. 466/2001, Official Journal of European Commission, **1, 77**/1 (2001).
7. FDA Fish and fisheries products hazards and controls guidance (3rd Ed.), Center for Food Safety and Applied Nutrition, US Food and Drug Administration, (2001).
8. M. Canli and G. Atli, The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six mediterranean fish species, Environ Pollut., **121,** 129-136 (2003).
9. T. Agusa, T. Kunito, G. Yasunaga, H. Iwata, A. Subramanian, A. Ismail and S. Tanabe, Concentrations of trace elements in marine fish and its risk assessment in Malaysia, Mar.Pollut. Bull., **5**, 896-911 (2005).
10. T. Agusa, A. Kunito, T. Sudaryanto, S.K. Monirith, A. Klap and H. Iwata, Exposure assessment for trace elements from consumption of marine fish in Southeast Asia, Environ Pollut., **145**, 266-777 (2007).
11. P. Hajeb, S. Jinap, A. Ismail, A.B. Fatimah, J. Jamilah and M.A. Rahim, Assessment of mercury level in commonly consumed marine fishes in Malaysia, Food Control, **20,** 79-84 (2009).
12. J.L. Sericano, T.L. Wadeand T.J. Jackson, Trace organic contamination in the Americas: an overview of the US National Status, Trends and the International Mussel Watch Programmes, Mar.Pollut. Bull., **31,** 214 (1995).
13. C.W. Gulec,C.P. Lol, C.S. Jang, S.W. Wang and Y.M. Hsueh, Bioaccumulation of arsenic compounds in aquaculture clams (*Meretrixlusoria*) and assessment of potential carcinogenic risks to human health by ingestion. Chemosphere, **4,** 38 (2004).
14. E. Merian, Metals and their compounds in the environment, occurrence analysis and biological relevance, UCH, Weinheim-New York-Basel-Cambridge (1991).
15. S. Kapaj, H. Peterson, K. Liber and P. Bhattacharya, Human health effects from chronic arsenic poisoning - a review, J. Environ. Sci. Health, Part **A 42**, 2399-2428 (2006).
16. N.L. Gale, C.D. Adams, B.G. Wixson, K.A. Loftin and Y.W. Huang, Lead, zinc, copper and cadmium in fish and sediments from the big river and flat river creek of missouri's old lead belt, Environ. Geochem, Health, **26**, 37-49 (2004).
17. P.M. Linnik and I.B. Zubenko, Role of bottom sediments in the secondary pollution of aquatic environments by heavy metal compounds, Lakes Reserv: Res. Manag., **5**, 11-21 (2000).
18. N. Malik, A.K. Biswas, T.A. Qureshi, K. Borana and R. Virha, Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal, Environ. Monit. Assess., **160,** 267 (2010).
19. A. Gupta, D.K. Rai, R.S. Pandey and B. Sharma, Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad, Environ. Monit. Assess., **157**, 449-458 (2009).
20. B. Ersoy and M. Celik, The essential and toxic elements in tissues of six commercial demersal fish from Eastern Mediterranean, Sea. Food Chem. Toxicol., **48,** 1377-1382 (2010).
21. T. Mathews and N.S. Fisher, Dominance of dietary intake of metals in marine elasmobranch and teleost fish, Sci. Total Environ., **407**, 5156-5161 (2009).
23. R.A. Hites, J.A. Foran, D.O. Carpenter, M.C. Hamilton, B.A. Knuthand S.J. Schwager, Global assessment of organic contaminants in farmed salmon, Sci., **303**, 226-229 (2004).
24. K.C. Cheung, H.M. Leung and M.H. Wong, Metal concentrations of common freshwater and marine fish from the Pearl River Delta, South China, Arch. Environ. Contam. Toxicol., **54**, 705-715 (2008).
25. Y.W. Qiu, D. Lin, J.Q. Liu and E.Y. Zeng, Bioaccumulation of trace metals in farmed fish from south China and potential risk assessment, Ecotoxicol Environ. Saf., **74**, 284-293 (2011).
26. S.W. Cai, Z.H. Ni, Y.F. Li,Z.W. Shen, Z.T. Xiong, Y. Zhang and Y.T. Zhou, Metals in the tissues in two fish species from the rare and endemic fish nature reserve in the upper reaches of the Yangtze River, China Bull. Environ. Contam. Toxicol., **88**, 922-927 (2012).
27. R.J. Medeiros, L.M.G. Dos Santos, A.S. Freire, R.E. Santelli, A.M.C.B.Braga, T. M. Krauss and S.C. Jacob, Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil, Food Control., **23**, 535-541 (2012).

28. Z. Svobodova, O. Celechovska, J. Kolara, T. Randak and V. Zlabek, Assessment of metal contamination in the upper reaches of the Ticha Orlice River, Czech J Anim Sci., **49,** 458-641 (2004).

29. I. Kuklina, A. Kouba and P. Kozák, Real-time monitoring of water quality using fish and crayfish as bio-indicators: a review, Environ Monit Assess, **185,** 5043-5053 (2013).

30. M.M.N. M.S. Authman, Zaki, E.A. Khallaf and H.H. Abbas, Use of fish as bio-indicator of the effects of heavy metals pollution, J Aquac Res. Dev., **6,** 2 (2015).

31. J. Burger, K.F. Gaines, B.C. Shane, W.L. Stephens, J. Snodgrass and C. Dixon, Metal levels in fish from the Savannah River: potential hazards to fish and other receptors, Environ Res., **89,** 85-97 (2002).

32. A.R. Linde, P. Arribas, S.S. Galanand E.G. Vazquez, Eel (*Anguillaanguilla*) and brown trout (*Salmo trutta*) target species to assess the biological impact of trace metal pollution in freshwater ecosystems. Arch Environ ContamToxicol., **31,** 297-302, (1996).

33. T.I. Moiseenko, L.P. Kudryavtseva, I.V. Rodyushkin, V.A. DauvalterA.A. Lukinand N.A. Kashulin, Airborne contamination by heavy metals and aluminum in the freshwater ecosystems of Kola subarctic region (Russia), Sci. Total Environ., **60,**715-727 (1995).

34. T. Zhou, P. Weisand J.S. Weis, Mercury burden in two populations of Fundulusheteroclitus after sublethal methylmercury exposure,AquatToxicol., **42,** 37-47 (1998).

35. A.U. Ahmedand M.Reazuddin, Industrial pollution of water systems in Bangladesh, University Press Limited, Dhaka, Bangladesh, 175-178 (2000).

36. H. Rashid, M.N. Hasan, M.B. Tanu, R. Parveen, Z.P. Sukhan, M.S. Rahmanand Y. Mahmud, Heavy Metal Pollution and Chemical Profile of Khiru River, Bangladesh,*International Journal of Environment*, **2**(1), 57-63 (2012).

37. ADB Institutional strengthening of Chittagong Port Authority in environmental management, **2**, 177(2004).

38. G.V. Iyengar, Milestones in biological trace elements research, Sci. Total Environ., **100,** 1-15 (1991).

39. J.A. Krishnamurtiand R.V. Nair, Concentration of metals in shrimps and crabs from Thane-Bassein creek system, Maharastra, Indian J of Mar Sci., **28,** 92-95 (1999).

40. P. Sivaperumal, T.V. Sankar and P.G. V. Nair, Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards, Food Chem., **102,** 612-620 (2007).

41. K. Uysal, Y. Emreand E. Köse, The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey), Microchem J, **90,** 67-70 (2008).

42. P.L.R.M. Palaniappanand S.Karthikeyan, Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish Cirrhinusmrigala individually and in binary solutions with nickel, J Environ. Sci., **21,** 229-236 (2009).

43. E.P. Bertin, Principles and Practice of X-ray Spectrometric Analysis, 2nd Ed., Plenum Press, New York, USA (1975).

44. R. Jenkins, An Introduction to X-Ray Spectrometry, Heyden and Son, New York, USA (1974).

45. H.A. Leibhatsky, H.G. Pleiffer, E.H. Winshowand P.O. Zemany, X-Rays, Electrons and Analytical Chemistry, Wiley-interscience, New York, USA (1972).

46. F.S. Gouldingand D.A. Landis, Semiconductor detector electronics in nuclear spectroscopy and reactions, Part A., Academic Press, New York, USA (1974).

47. G.F. Knoll, Radiation Detection and Measurement, John Wiley and Sons, New York, USA (1971).

48. A.G. Bravo, J.L. Loizeau, S. Bouchet, A. Richard, J.F. Rubin and V.G. Ungureanu, Mercury human exposure through fish consumption in reservoir contaminated by a chlor-alkali plant: Babeni reservoir (Romania), Environ. Sci. and Pollut,Res. Int., **17**, 1422-1432 (2010).

49. HIES Household Income and Expenditure Survey, Preliminary report on household income and expenditure survey-2010, Bangladesh Bureau of statistics, statistics division, Ministry of Planning, Dhaka, Bangladesh (2011).

50. Food and Agriculture Organization, Arsenic contamination of irrigation water, soil and crops in Bangladesh: Risk implication for sustainable agriculture and food safety in Asia, FAO Regional Office for the Asia and Pacific, Bangkok, Thailand (2006).

51. WHO Guidelines for drinking water quality (2nded., **2**), World Health Organization, Geneva (1996).

52. EU Maximum levels for certain contaminants in foodstuffs, official J of the European Union, L. 364/5 (2006).

53. S.S. Dara, Environmental chemistry and pollution control, New Delhi, India: S. Chand and Company Ltd., 191-1912 (1995).

54. H.A.H. Ahmad and S.I. Naim, Heavy metals in eleven common species of fish from the gulf of Aqaba, Red sea. Jordan J Bio. Sci., **1**, 13-18 (2008).

55. D. Plaskett and I.C. Potter, Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia, Australian JMarine and Freshwater Res., **30**, 607-616 (1979).

56. S.L. Senthil, T.A. Kumar, T.M. Pandi, K.V. Dhaneesh, J.B. Murugan and T.B. Subramanian, Metal contagion in ecologically important estuary located in Bay of Bengal. Water Quality, Exposure and Health, **4(3),** 137-142(2012).

57. A.S.S. Ahmed, S. Sultana, A. Habib, H. Ullah, N. Musa, M.B. Hossain and M.S.I. Sarker, Bioaccumulation of heavy metals in some commercially important fishes from a tropical river estuary suggests higher potential health risk in children than adults,PloS one, **14**(10) (2019a).

58. M. Raknuzzaman, M.K. Ahmed, M.S. Islam, M.H.A. Mamun, M. Tokumura, M. Sekine and S. Masunaga, Trace metal contamination in commercial fish and crustaceans collected from coastal area of Bangladesh and health risk assessment, Environ. Sci. and Pollu. Res., **23(17),** 17298-17310 (2016).

59. S.L. Asenthil, T.V. Kumar, T.T.A. Kumar, T. Marudhupandi, D.K. Veettil, R. Ganeshamurthy and T. Balasubramanian, Harmful metals concentration in sediments and fishes of biologically important estuary, Bay of Bengal, JEnviron. Health Sci. and Engin., **11(1),** 33 (2013).

60. A.S. Ahmed, M. Rahman, S. Sultana, S.O.F. Babu and M.S.I. Sarker, Bioaccumulation and heavy metal concentration in tissues of some commercial fishes from the Meghna River Estuary in Bangladesh and human health implications, Marine pollution bulletin, **145,** 436-447 (2019).

61. N. Saha, M.Z.I. Mollah, M.F. Alam and M.S. Rahman, Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control, **70**, 110-118, (2016).

62. D.F. Bubach, S.P. Catán, V.H. Baez and M.A. Arribére, Elemental composition in rainbow trout tissues from a fish farm from Patagonia, Argentina, Environ. Sci. and Pollu. Res., **25(7),** 6340-6351 (2018).

63. H. Alipour A. Pourkhabbaz and M. Hassanpour, Estimation of potential health risks for some metallic elements by consumption of fish, Water Quality, Exposure and Health, **7(2),** 179-185 (2015).

64. J. Garcia-Leston, J. Mendez, E. Pasaro and B. Laffon, Genotoxic effects of lead: an updated review, Environ. Inter., **36**, 623-636 (2010).

65. FSANZ Food Standards Australia New Zealand. Australia New Zealand Food Standards Code (Incorporating amendments up to and including Amendment 97), Anstat Pty Ltd., Melbourne (2008).

66. FAO/WHO List of maximum levels recommended for contaminants by the Joint FAO/W H O codex alimentariuscommission, Second Series CAC/FAL Rome, **3,** 1-8 (1976).

67. WHO Evaluation of certain food additives and the contaminants mercury lead and cadmium, WHO technical report series, 505 (1989).

68. J.M. Gorell, C.C. Johnson, B.A. Rybicki, E.L. Peterson, G.X. Kortsha, G.G. Brown and R.J. Richardson, Occupational exposures to metals as risk factors for Parkinson’s disease, Neurology, **48**, 650-658 (1997).

69. G.N. Bebbington, N.J. Mackay, R. Chvojka, R.J. Williams, A. Dunn and E. H. Auty, Heavy metals, selenium and arsenic in nine species of Australian commercial fish, Australian JMarine and Freshwater Res., **28**, 277-286 (1977).

70. M. Dural, M.Z.L. Goksu and A.A.Ozak, Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon, Food Chemistry, **102**, 415-421 (2007).

71. M. Cronin, I.M. Davies, A. Newton, J.M. Pirie, G. Topping and S. Swan, Trace metal concentrations in deep sea fish from the North Atlantic, Marine Environ. Res., **45,** 225-238 (1998).

72. A. Demirak, F. Yilmaz, A. L. Tuna and N. Ozdemir, Heavy metals in water, sediment and tissues of Leciscuscephalus from a stream in southwestern Turkey, Chemosphere, **63,** 1451-1458 (2016).

73. M.G.M. Alam, A. Tanaka, G. Allinson, L.J.B. Laurenson, F. Stagnitti and E.T. Snow, A comparison of trace element concentrations in cultured and wild carp (*Cyprinuscarpio*) of LakeKasumigaura, Japan, Ecotoxicology and Environ, Safety, **53**, 348-354 (2002).

74. O.Erdogrul and D.A. Ates,Determination of Cadmium And Copper in fish samples from Sir And Menzelet Dam Lake Kahramanmaras, Turkey Environ. Monitoring and Assessment, **117**,281-290, (2006).

75. R. Eisler, Copper hazards to fish, wildlife and invertebrates: a synoptic review. Biol Sci. Report, **33**,100, (1998).

76. P. Miramand and D. Bentley, Heavy metal concentrations in two biological indicators (Patella vulgata and Fucusserratus) collected near the French nuclear fuel reprocessing plant of La Hague, Sci. Total Environ., **111,** 135-149 (1992).

77. P. Simonetti, S.E. Botté and J.E. Marcovecchio, Heavy metal bioconcentration factors in the burrowing crab Neohelicegranulata of a temperate ecosystem in South America: Bahía Blanca estuary, Argentina, Environ. Sci. and Pollu. Res., **25(34),** 34652-34660 (2018).

78. MAFF Monitoring and Surveillance of Non-radioactive Contaminants in the Aquatic Environment and Activities Regulating the Disposal of Wastes at Sea, 1997, Aquatic Environment Monitoring Report No. 52. Center for Environment, Fisheries and Aquaculture Sci., Lowestoft, UK (2000).

79. H.J.M. Bowen, Trace Elements in Biochemistry, New York: Academic Press (1966).

80. M.N. Amin, A. Begum and M.G.K. Mondal, Trace element concentrations present in five species of freshwater fish of Bangladesh, Bangladesh j Sci. and Indus. Res., **46**, 27-32 (2011).

81. JECFA Evaluation of certain food additives and contaminants, Fifty-third report of the joint FAO/WHO Expert Committee on Food Additives, (WHO technical report series, No. 896), World Health Organization, Geneva (2000).

82. NRC National Research Council Recommended Dietary Allowances (10thed., 241-243). Washington, DC: National Academy of Sciences (1989).