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Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment

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Abstract

To investigate the seasonal contamination levels and to evaluate the potential human health risks, ten heavy metals (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn) were measured in ten different marine fish species from the Bay of Bengal during four seasons. The metal concentrations varied significantly ($p < 0.05$) among the fish species with maximum and minimum accumulation of Zn (46.47 µg/g) and Cd (0.25 µg/g), respectively. The fishes captured during summer accumulated higher amount of metals relative to other seasons attributed to washing down the agricultural waste, sewage water and sludge by heavy rainfall and floods. According to estimated daily intake (EDI), target hazard quotient (THQ), total target hazard quotient (TTHQ), and the permissible safety limits prescribed by various agencies, consumption of the examined fishes should be considered as safe for human health. However, the estimation of carcinogenic risk (CR > 10^{-5}) due to exposure to arsenic indicated that consumers remain at risk of cancer.

Keywords: Fish; Bay of Bengal; Seasonal fluctuations of heavy metals; Carcinogenic and non-carcinogenic human health risk.

1. Introduction

Aquatic environments worldwide are being heavily polluted by various contaminants, such as heavy metals (Rahman, Molla, Saha, & Rahman, 2012; and reference therein), polycyclic aromatic hydrocarbons (PAHs) (Y. Li, Li, Ma, Song, Zhou, Han, et al., 2015; and reference therein), polychlorinated biphenyls (PCBs) (Zhang, Li, Wang, Zhang, Zhang, Zhang, et al., 2014, Hosseini, et al., 2008; and reference therein) with rapid economic development and population growth. Pollution from the heavy metals (e.g., Cd, Cr, Pb etc.) has become a serious issue due to their stable, persistent, and nonbiodegradable nature (Saha & Zaman, 2013). Although the metals are naturally occurring constituents in the environment, their concentrations can be exacerbated by anthropogenic activities such as rapid industrialization and urbanization, massive land use changes and associated enhanced terrestrial runoff (Rahman, Molla, Saha, & Rahman, 2012). Another important source of anthropogenic pollution in Chittagong coastal area, Bangladesh is ship breaking activities began since 1969. Various refuse and disposable materials are being discharged and spilled from scrapped ships, and often get mixed with the beach soil and sea water. Under certain conditions, the heavy metals can be bioaccumulated in aquatic organisms (e.g., fish) from the surrounding environments and/or bioamplified to hazardous levels via dietary exposure. Subsequently, these contaminants migrate to human body through diet and results in various adverse health effects such as impaired kidney function, poor reproductive capacity, liver damage, skin and bladder cancer, and even death (Wei, Zhang, Zhang, Tu, & Luo, 2014). However, there are some essential heavy metals, such as Fe, Zn, and Cu required for both aquatic organisms and human body. But prolonged exposure to the excess level of these metals could be toxic. On the contrary, there are some nonessential toxic metals such as As, Cd, and Pb with no known potential benefits for a human being (Wei, Zhang, Zhang, Tu, & Luo, 2014).

Food consumption is the main pathway (accounting for > 90%) for human exposure to heavy metals compared to other routes such as inhalation and dermal contact. Among various foodstuffs, fish is widely consumed and a main source of nutrition in many coastal communities. It contributes to a healthy diet by providing high-value amino acids and nutrients and is an excellent source of essential omega-3 fatty acids. Although fish is highly nutritious, higher consumption rate can have significant deleterious effects on human health because of bioaccumulated toxic metals beyond the safe limits. To date, the balance between benefits and risks has been poorly known. However, fishes are often treated as the most suitable bioindicator of aquatic ecosystem since they occupy high trophic level (Rahman, Molla, Saha, & Rahman, 2012). Many researchers globally have made efforts to determine the concentrations of heavy metals in fishes to investigate the eco-environments and potential health risks to the consumers (Ahmed, Shaheen, Islam, Habibullah-al-Mamun, Islam, Mohiduzzaman, et al., 2015; Copat, Arena, Fiore, Ledda, Fallico, Sciacca, et al., 2013; P. Li, Zhang, Xie, Liu, Liang, Ren, et al., 2015; Mendil, Demirci, Tuzen, & Soylak, 2010; Saha & Zaman, 2013; Wei, Zhang, Zhang, Tu, & Luo, 2014).

In recent years, the quantification of risk has become important due to the fact that exceedance of recommended levels of contaminants prescribed by various regulatory bodies does not always represent a risk for the human health. The target hazard quotient (THQ) and/or total target hazard quotient (TTHQ) set by US Environmental Protection Agency (USEPA, 2000) are commonly used to evaluate the potential non-carcinogenic health risks associated with variety of metals through fish consumption (Copat, et al., 2013; Saha & Zaman, 2013). USEPA also provided cancer slope factor for arsenic, to determine the carcinogenic risk (CR) over a lifetime exposure to arsenic.

Bangladesh is one of the coastal countries of the Bay of Bengal, where 37 to 38 million peoples live in the coastal zone. The country is located between 20^{o}34´ to 26^{o}38´ north latitude and 88^{o}01´ to 92^{o}42´ east longitude. Fisheries resources play a vital role in the economy of Bangladesh (4.57% of GDP in 2008-09) and it is the second most important source of the foreign exchange earnings (5.71%). The national and international demand of Bangladeshi fishes is increasing gradually. Recently, CBI (CBI, 2012) reported that in the period of 2002-2011, import of marine fishes by the European Union (EU) has been increased from US$ 150 m to US$ 360 m. Thus, the concentrations of heavy metals in the seafood deserves farther investigation not only for their potential ecological impacts but also due to the health risks for the people in Bangladesh and other importer countries.

The objectives of this study are (i) to report on the fluctuation of concentrations of heavy metals (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn) in fishes captured from Chittagong coastal zone with respect to seasonal variation, and (ii) to quantify the non-carcinogenic and carcinogenic human health risks associated with these metals.

2. Materials and Methods

2.1. Sample collection and preservation

Ten fish species of *Lates calcarifer* (Bhetki), *Pangasius pangasius* (Pangas), *Polynemus indicus* (Lakhua), *Ilisha megaloptera (*Choukya), *Arius cruciger* (Rita), *Pampus chinensis* (Rupchanda), *Setipinna phasa* (Phasa), *Scomberomorus guttatum* (Maitya), *Cirrhina reba* (Beta) and *Arius arius* (Kata mach) were procured from fishermen while they were fishing nearshore sites at Cox’s Bazar, Chittagong (Fig. 1) during spring, summer, autumn and winter. Samples were collected in triplicate and in total, 120 individuals (i.e., 30 samples in each season) representing 10 different species were collected. Immediately after collection, fishes were washed with fresh water to remove the mud or other fouling substances, and were wrapped in polyethylene bags to transport into the analytical chemistry laboratory of Institute of Nuclear Science and Technology, Bangladesh Atomic Energy Commission (BAEC). After transportation to the laboratory, the muscle tissue of each sample was removed and chopped into pieces with the aid of a steam cleaned stainless steel knife. The samples were then air dried to remove the extra water and subsequently, ovendried to a constant weight. Finally, the dried samples were ground, sieved, and stored in clean and dry airtight plastic vials inside desiccators for succeeding uses.

2.2. Sample Digestion

For metal analysis, 0.5 g of each powdered sample was digested with 2.5 ml of conc. H_2SO_4 and 4.0 ml conc. HNO_3. When initial vigorous reaction subsided, the mixture was heated slowly on an oil bath by the addition of 3/4 drops of H_2O_2. This step was repeated till the solution became clear. Subsequently, the solution was heated for additional 20 min. at about 150 °C and allowed to cool to room temperature. The digested fish sample was then diluted to a total volume of 50 ml with double distilled water. Thereafter, the diluted solution was filtered and stored in 50-ml polypropylene tubes.

2.3. Analytical methods and quality control

The concentration of Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn in digested solutions was analyzed by atomic absorption spectrophotometer (AAS) (Model AA-6800, Shimadzu Corporation, Japan) using air-acetylene flame with digital read out system. However, hydride vapor generators (HVG) were used along with the flame AAS (FAAS) systems to determine As and Se concentrations. The instrument calibration standards were made by diluting standards (1000 mg/l) supplied by Wako Pure Chemical Industry Ltd., Japan.

The accuracy of the method was evaluated by analyzing blanks and a certified reference material MA-A-2 (fish-flesh standard from International Atomic Energy Agency (IAEA), Vienna) by the same procedure used for fish samples. Mean recoveries of the analyzed metals were between 95-104 %, indicating a good agreement between the certified and measured values (Table S1).

2.4. Statistical analysis

ANOVA test was conducted to investigate the effect of different fish species and seasons on the variation in metal concentrations. In order to quantify the seasonal effects on the variability of metals, regression analyses were performed using dummy variables for the seasons (winter as reference). In all cases, the level of significance was set at a 95 % (i.e., $\alpha = 0.05$). The data were analyzed statistically using IBM-SPSS Statistics (Version 21) for windows (IBM, USA).

2.5. Human health risk assessment

2.5.1. Estimated daily intake (EDI)

The estimated daily intake (EDI) for each analyzed heavy metal was calculated in the following way (Saha & Zaman, 2013):

$$\mathrm{EDI} = \frac{E_F \times E_D \times F_{IR} \times C_f \times C_m}{W_{AB} \times T_A} \times 10^{-3} \qquad \text{(i)}$$

Where E_F is the exposure frequency (365 days/year). E_D is the exposure duration, equivalent to an average life time of the Bangladeshi (i.e., 60 years). F_{IR} is the ingestion rate (g/person/day) of fish tissue. The fish production in Bangladesh was unable to keep patch with high population growth and as a result, per capita consumption of fish was decreased from 33 g in 1963-64 to 20.5 g in 1989-90 against the recommended level of 38 g per capita per day. Recently, the per capita fish consumption has increased to 26 g (M. M. Hossain, 2010) and used in the EDI calculations. C_f is the conversion factor ($C_f = 0.208$) to convert fresh weight to dry weight considering 79% of moisture content of the fish fillet (Rahman, Molla, Saha, & Rahman, 2012). C_m is the metal concentration in fish fillet (µg/g - dry weight basis). Since most As present in foodstuffs as less harmful organic forms, the EDI for this metal was calculated assuming 3 % toxic inorganic As in total (Copat, et al., 2013; P. Li, et al., 2015). W_{AB} is the average body weight of an adult (60 kg), and T_A (equal to $E_F \times E_D$) is the average exposure time for non-carcinogens (Saha & Zaman, 2013).

2.5.2. Non-carcinogenic risk

The target hazard quotient (THQ), which is used to express the risk of non-carcinogenic effects, is the ratio between the estimated exposure (EDI) and the oral reference dose (RfD, mg/kg bw/day) (USEPA, 2000). The RfD represents an estimate of the daily exposure to which the human population may be continually exposed over a lifetime without an appreciable risk of deleterious effects. The RfDs are based on 0.0003, 0.001, 0.0003, 1.5, 0.04, 0.14, 0.02, 0.004, 0.005, and 0.3 (mg/kg bw/day) for As (inorganic), Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn, respectively. The THQ was calculated based on the following equation (USEPA, 2000):

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}} \qquad \text{(ii)}$$

If the THQ value is less than 1, the exposed population should not experience any adverse health hazard. Conversely, the receptors of concern may experience non-carcinogenic health risks if the THQ is equal 1, with an increasing probability as the value increases. The method for the determination of THQ was provided in US EPA Region III risk-based concentration table (USEPA, 2000). The THQ calculations were made based on the facts that the ingestion dose is equal to the absorbed contaminant dose, cooking has no effect on the contaminants (USEPA, 1989) and average body weight of an adult is 60 kg (Saha & Zaman, 2013).

It has been reported that exposure to two or more pollutants may result in additive and/or interactive effects. Thus, in this study, cumulative health risk was evaluated by summing of the individual metal THQ value and expressed as total THQ (TTHQ) (also called hazard index, HI) as follows:

$$\mathrm{TTHQ} = \mathrm{THQ}\ (\text{toxicant } 1) + \mathrm{THQ}\ (\text{toxicant } 2) + \cdots + \mathrm{THQ}\ (\text{toxicant } n) \qquad \text{(iii)}$$

The greater the value of TTHQ the greater the level of concern. The TTHQ value above 1 generally indicates the potential for adverse human health effects and suggests the need for undertaking a farther level of investigation or possibly remedial action.

2.5.3. Carcinogenic risk

Carcinogenic risk (CR) indicates the incremental probability of an individual developing cancer over a lifetime due to exposure to a potential carcinogen. The cancer risk over a lifetime exposure to As was obtained using cancer slope factor (CSF), provided by USEPA (USEPA, 2000). The equation used for estimating the cancer risk is as follow (USEPA, 2000):

$$\mathrm{CR} = \mathrm{CSF} \times \mathrm{EDI} \qquad \text{(iv)}$$

Where CSF is the carcinogenic slope factor of 1.5 $(\mathrm{mg/kg/day})^{-1}$ set by USEPA only for inorganic As. EDI is the estimated daily intake of heavy metals. The acceptable lifetime carcinogenic risk considered by USEPA is 10^{-5} (risk of developing cancer over a human lifetime is 1 in 100000) and applied in this study.

3. Result

3.1. Levels of metals accumulation in fish fillets

Mean concentrations and standard deviations of As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn obtained in the edible portions of ten fish species during spring, summer, autumn, and winter are presented in Table S2 (also see Fig. 2). Although some investigators prefer to determine heavy metals in liver, kidney, and gills, the present investigation was carried out on the edible flesh of the marine fish species, because the dietary habit of the people of Bangladesh excludes other parts of fish. Our results revealed that the ranking order of mean metal concentrations were, Zn (46.47) > Mn (10.61) > Cu (5.21) > Ni (4.85) > Pb (3.57) > Cr (3.06) > As (2.79) > Se (2.66) > Co (0.38) > Cd (0.25) (mean concentration; µg/g - dry weight basis). The heavy metal concentrations detected in this study were also compared with other published data in Fig. 3 as an effort to determine the degree of contamination in the present study area.

3.1.1. As

Arsenic is ubiquitous and a potential toxic trace metal in the environment and it originates from both the natural and anthropogenic processes. It has been reported by US Food and Drug Administration (USFDA) that fish and other seafood account for 90% of total human exposure to As (USFDA, 1993). In this study, the highest concentration (4.43 µg/g) of As was found in *S. guttatus* during summer, while the lowest concentration (1.15 µg/g) was in *L. calcarifer* during winter season (Table S2). The maximum permitted concentration of As set by Australia New Zealand Food Standards Code is 2.0 µg/g on a wet weight basis, i.e., 9.6 µg/g on a dry wet basis assuming 79% of moisture content in fish muscles (ANZFA, 2011). As a criterion for human health protection, USEPA has set arsenic tissue residues of 1.3 µg/g on a wet weight basis, i.e., 6.24 µg/g on a dry wet basis (Burger & Gochfeld, 2005). Thus, the concentrations of As recorded in this study were well below the recommended values by ANZFA and USEPA. In addition, Fig. 3 shows that our values were in good agreement with the As levels found in fishes from the Bay of Bengal (Sharif, Alamgir, Krishnamoorthy, & Mustafa, 1993), the Paira River (Islam, Ahmed, Raknuzzaman, Habibullah-Al-Mamun, & Masunaga, 2015), the Bangshi River (Rahman, Molla, Saha, & Rahman, 2012) and various markets throughout Bangladesh (Ahmed, et al., 2015).

3.1.2. Cd

Cadmium is a nonessential element that causes severe toxic effects even at a concentration of ~ 1 µg/g. Its accumulation in the human body may lead to the occurrence of renal, pulmonary, hepatic, skeletal, reproductive effects and even cancer (Ahmed, et al., 2015). The highest (0.47 µg/g) and lowest (0.02 µg/g) concentrations of Cd were found in *P. indicus* and *P. chinensis*, respectively (Table S2). Maximum Cd level permitted by Spanish legislation is 1.0 µg/g. Australian National Health and Medical Research Council (ANHMRC) standard for Cd in seafood is 2.0 µg/g while Western Australian Authorities has proposed concentration of 5.5 µg/g for Cd (Plaskett & Potter, 1979). Our Cd results, however, were below the standard limits. The measured mean Cd concentration (0.25 µg/g) was similar to the mean values found in fishes from the Bay of Bengal (M. S. Hossain & Khan, 2001), the Paira River (Islam, Ahmed, Raknuzzaman, Habibullah-Al-Mamun, & Masunaga, 2015) and the Bangshi River (Rahman, Molla, Saha, & Rahman, 2012) (Fig. 3). However, mean Cd concentration measured by Sharif, Alamgir, Mustafa, Hossain, and Amin (1993), Sharif, Mustafa, Amin, and Safiullah (1993), and Ahmed, et al. (2015) were lower than our mean value, while Ahmad, Islam, Rahman, Haque, and Islam (2010) found higher mean value relative to us in fishes from the Buriganga River which is highly polluted due to municipal sewage discharge (Fig. 3).

3.1.3. Co

Cobalt is an essential transition element in metabolism of many different organisms. Muscle is the main site of accumulation of Co in fish, and the accumulated concentration is low, perhaps due to its higher affinity with organic matter in the water column (Andreji, Stránai, Massányi, & Valent, 2005). Muscular contents of Co in this study were also low with the highest recorded value of 0.56 µg/g in *P. indicus* and the lowest value (0.21 µg/g) in *C. reba* (Table S2). Unfortunately, the safe level for Co in fish is not defined.

3.1.4. Cr

The presence of Cr in the diet is essential due to its active involvement in insulin function and lipid metabolism (Ahmed, et al., 2015). According to Western Australian Food and Drug regulations, the recommended maximum permitted value of Cr is 5.5 µg/g (Plaskett & Potter, 1979). In this study, *A. cruciger* accumulated the highest amount (4.66 µg/g) of Cr during summer, while *C. reba* accumulated the lowest amount (1.27 µg/g) during winter (Table S2). A comparison of Cr accumulation in fishes from various regions of Bangladesh is presented in Fig. 3.

3.1.5. Cu

Copper (Cu) is an essential element due to its presence in several enzymes and its necessity for hemoglobin synthesis (Sivaperumal, Sankar, & Nair, 2007). However, very high levels of Cu can cause acute toxicity. Human deaths have been known to occur from deliberate ingestion of large quantities of copper sulfate.

Different regulatory body established a safe limit for Cu concentration. For instance, ANHMRC and FAO prescribed permissible limit of 30 µg/g on a wet weight basis (Bebbington, Mackay, & Chvojka, 1977). According to UK Food Standards Committee Report, Cu levels in food should not exceed 20 µg/g on a wet weight basis (Cronin, Davies, Newton, Pirie, Topping, & Swan, 1998). Australian Food Standard Code established maximum concentration for Cu at 10 µg/g (wet weight basis). There is also legislation in other countries regulating maximum metal concentration. For example, Spanish legislation proposed the level at 20 µg/g (wet weight basis). Our recorded maximum value (8.54 µg/g) in *I. megaloptera* during summer was lower than the recommended concentrations by various authorities and the variability of Cu concentrations was also similar to other published studies (Fig. 3).

3.1.6. Mn

Manganese is an essential element and its deficiency might lead to severe skeletal and reproductive abnormalities in mammals (Ahmed, et al., 2015). No maximum limit is specified for Mn in fish samples. In this study, the highest (17.80 µg/g) Mn content was found in *A. cruciger* during summer and the lowest (3.63 µg/g) was in *P. pangasius* during winter (Table S2). Similar ranges of Mn concentrations were reported in fishes from the Bay of Bengal (M. S. Hossain & Khan, 2001; Sharif, Mustafa, Amin, & Safiullah, 1993), and various markets throughout Bangladesh (Ahmed, et al., 2015) (Fig. 3).

3.1.7. Ni

The concentration of Ni in the environment is normally very low, but can cause a variety of adverse health effects including lung inflammation, fibrosis, emphysema, and tumours. The highest concentration (7.56 µg/g) of Ni was measured in *C. reba* during summer and the lowest concentration (1.88 µg/g) was found in *P. indicus* during winter. Our values were lower than the established safe level of 5.5 µg/g on a wet weight basis (i.e., 26.4 µg/g on a dry weight basis assuming 79% moisture content in fish muscles) by Western Australian Food and Drug Regulations (Plaskett & Potter, 1979). Figure 3 shows that Ni concentrations found in this study were similar to the values in literature. However, the mean Ni content found in the fishes from the Buriganga River (Ahmad, Islam, Rahman, Haque, & Islam, 2010) was two-fold higher than this study, while the values reported by Ahmed, et al. (2015) were several fold lower than us.

3.1.8. Pb

Lead is a nonessential trace metal and the adverse health effects from Pb are well known. Among the individual fish species, *A. arius* accumulated the highest Pb concentration of 6.23 µg/g during summer, while *I. megaloptera* accumulated the lowest of 0.80 µg/g during winter (Table S2). ANHMRC has set the maximum permitted concentration of Pb as 2.0 µg/g (wet weight basis, i.e., 9.6 µg/g on a dry weight basis assuming 79 % moisture content) (Plaskett & Potter, 1979). According to UK and Spanish legislation, Pb in fish should not exceed 9.6 µg/g on a dry weight basis (Demirak, Yilmaz, Tuna, & Ozdemir, 2006). The present observation showed that Pb level in the all the analyzed fish species was well within the acceptable limit for human consumption.

3.1.9. Se

Selenium is an essential element in some oxidation-reduction processes and is a component of a cellular enzyme called glutathione peroxidase, which is responsible for converting H_2O_2 to CO_2 and H_2O (Sivaperumal, Sankar, & Nair, 2007). Selenium was found to be a significant element in the prevention of Hg toxicity due to a strong binding affinity between Se and Hg that protects direct sequestration of Hg by Se. However, excessive intake of Se can cause chronic toxicity in human, resulting in a condition termed selenosis, characterized by hair and nail loss and brittleness, gastrointestinal problems, skin lesions, tooth decay, garlic breath odor, and nervous system abnormalities (Yang, Wang, Zhou, & Sun, 1983). Clinical symptoms of Se toxicity may also include severe irritations of the respiratory system, lung edema, rhinitis, and broncho-pneumonia. Due to the narrow margin between beneficial and harmful effects of Se (as little as a factor of two), accurate and precise data on selenium intake are needed and thus, the researchers are being interested in the distribution and speciation of Se in fish samples. The details of the mean Se levels detected in individual fish species during four seasons are given in Table S2.

Among individual fish species minimum (1.23 µg/g) Se concentration was observed in *I. megaloptera* during winter and maximum (4.00 µg/g) was in *P. pangasius* during summer. The recorded selenium concentrations were significantly lower than FAO/WHO (FAO/WHO, 1984, 2009) and ASTDR (ATSDR, 2003) recommended permissible limits of 9.6 µg/g on a dry weight basis, suggesting that exposure to Se was not a problem. The Se concentrations recorded in this study were also on the low end of the published Se values (Fig. 3).

3.1.10. Zn

Zinc is present in all organisms and is an essential trace element for metabolic processes. However, prolonged excessive dietary intake of Zn can lead to the deficiencies in iron and copper, nausea, vomiting, fever, headache, tiredness, and abdominal pain. It is also a human skin irritant. However, in this study, the highest and lowest Zn concentrations were found as 74.36 µg/g in *C. reba* during summer and as 13.22 µg/g in *P. chinensis* during winter, respectively. WHO and ANHMRC (Bebbington, Mackay, & Chvojka, 1977) recommended safe level for Zn is 1000 mg/kg, which is much higher than our determined values, indicating no adverse health effects. The ranges of Zn in different fish species found in this study were within the range of variability reported for various fish species in different parts of Bangladesh (Fig. 3).

4. Discussion

The variation of every metal concentration in ten different fish species was significantly different at 95% confidence interval (Table S3). A large number of studies worldwide have also reported interspecific differences in metal concentrations (e.g., Burger & Gochfeld, 2005; Merciai, Guasch, Kumar, Sabater, & García-Berthou, 2014; Rahman, Molla, Saha, & Rahman, 2012). The observed differences were might be due to differences in trophic level (P. Li, et al., 2015), variances in size of fishes (Merciai, Guasch, Kumar, Sabater, & García-Berthou, 2014), differences in seasons (Mendil, Demirci, Tuzen, & Soylak, 2010), and tendency of heavy metals to undergo bioamplification in the food chain (Burger & Gochfeld, 2005). However, this study revealed a similar trend in changes of the metal concentrations in all examined fish species (Fig. 2, inset).

4.1. Seasonal investigation of heavy metals

Figure 2 displays the variation of metal levels in the fish muscles with respect to the seasonal (spring, summer, autumn and winter) fluctuation. The metal concentrations during summer were higher followed by spring, autumn and winter. Similar seasonal effect on metal accumulation was reported in fish species from the Nile River (Zayed, Eldien, & Rabie, 1994) and recently from the Black sea (Mendil, Demirci, Tuzen, & Soylak, 2010). This observed summer maximum accumulation may be attributed to heavy rainfall and flood events during summer in Bangladesh, which increases metal loads in water bodies by washing down agricultural waste, sewage water, and sludge. Several other factors may be responsible for driving the metal loads toward higher values during summer. For example, cyclonic activity, which is quite common in Bangladesh at the end of spring and summer, may lead to wind associated resuspension of settled sediments, resulting in a higher amount of metals into the water column. Moreover, delivery of higher nutrients to nearshore marine environments during summer can cause a large phytoplankton bloom. Subsequent bacterial decomposition of this organic matters may release metals into the water which in turn accumulate in fish. In addition, the observed maxima may be related to higher fish respiratory rates during summer when they require increased volume of water due to low dissolved oxygen content, and also to a higher feeding rate on plants and grasses during the summer (Zayed, Eldien, & Rabie, 1994). The relative decrease in metal accumulation during autumn and winter months could be attributed to stable hydraulic condition due to low flows or cease-to-flows of terrestrial runoff into the nearshore coastal environments. Suspended particles (major trace metal carrier) also settle down with clear water conditions during dry winters.

The variation of metal concentrations in the edible fish fillets in four different seasons were used for ANOVA to know if the observed variances were significant (Table S3). The data obtained from ANOVA clearly demonstrated that seasonal effects on the variability of Cd, Cr, Pb, and Se were significant at 95 % confidence interval while rest of the metals variability were not statistically significant. Moreover, to quantify the seasonal effects on the metals variability found in the studied fish species, three dummy variables were taken for the seasons (winter as reference) when the fish samples were collected (Table S4). The coefficients of dummy variables revealed that on average As concentrations in summer were ~ 0.41 µg/g higher than those in winter while its concentrations in spring and autumn were ~0.24 and ~0.11 µg/g higher relative to winter. Likewise, Cd accumulation during summer, spring, and autumn were ~0.11, ~ 0.05, and ~0.03 µg/g, respectively, higher relative to winter. See Table S4 for rest of the metals.

4.2. Risk to humans by consumption of sea-fish

Even though the concentrations of ten analyzed metals in the tested fish samples did not exceed the legal limits set by various agencies (as we discussed in the Result section), the toxic potency of these analytes depends on exposure doses. Thus, combining the determined concentration of contaminant and the estimated daily fish consumption limit is considered as a great tool for evaluating the balance between benefits and risks (carcinogenic and non-carcinogenic), and applied in this study.

As a first step of estimation of human health risk, daily intake of every metal through ingestion of the fish was estimated using the formula (1). The highest average value of EDI was observed for Zn (7.73×10^{-3} mg/person/day) while the lowest was for As (1.39×10^{-5} mg/person/day). The EDI results were compared with the respective recommended daily dietary allowance of individual metal suggested by various agencies including Joint FAO/WHO Expert Committee on Food Additive (JECFA) for As, Cd, Cu, Pb, and Zn (JECFA, 1982, 1989, 2000), World Health Organization (WHO) for Co and Ni (WHO, 1996, 2006), National Research Council (NRC) for Cr and Mn (NRC, 1989), National Academy of Science (NAS) for Se (ATSDR, 2003) (Fig. 4 and also see Table S5). Figure 4, however, displays that the EDI values for all the metals were far below the respective recommended levels, indicating that adverse health effects from exposure to the examined metals were unlikely.

Figure 5 represents the THQ values of the selected heavy metals due to consumption of the examined ten fish species by average Bangladeshi adults. The highest average value of THQ was observed for Co (4.18×10^{-2}) while the lowest was for Cr (3.39×10^{-4}). None of the metals individually exceeded the hazard quotient threshold of 1. This implies that the level of daily intake of each examined metal by the Bangladeshi adults was lower than that of respective reference dose. Thus, we can suggest that these level of human exposure to the analyzed metals should not cause any deleterious effect during an entire lifetime. Considering that consumption of the fishes involved exposure to a mixture of ten examined metals, we evaluated cumulative health risks by summing the health risks posed by ten studied metals (i.e., total target hazard quotient, TTHQ). The value of TTHQ (i.e., 6.39×10^{-1}) was also less than 1, again indicating no potential significant health risks. In this study, Co and Pb were the two major contributors to TTHQ, accounted for 33.32 % and 23.24 % respectively. Whereas, Cr contributed the least (only 0.05%) to TTHQ.

US Food and Drug Administration (USFDA, 1993) has pointed out that dietary intake of inorganic As is strongly associated with wide spectrum carcinogenesis. The CR values of As obtained in this study were ranged from 1.28×10^{-5} to 2.76×10^{-5} (with a mean value of 2.09×10^{-5}) and exceeded the risk of cancer benchmark (i.e., 10^{-5}) (Fig. 5, inset). This implies that the ingesting of fish from the Bay of Bengal would cause a cancer risk for lifetime consumption. However, in this study, we assumed that ingestion dose of heavy metals is equal to the absorbed dose of contaminants, without considering bioavailability of the metals in human body. This assumption might overestimate the As-carcinogenic risk calculation due to the fact that a part of ingested As may be excreted. Thus for more robust estimation of health risks, future study should focus on biomonitoring of metals in blood, urine and hair samples to determine the actual concentrations of bioavailable metals in the local population.

4.3. Risk management

Risk management is the process of systematic identification of environmental hazards, analyzing the likelihood of occurrence and severity of the potential consequences, weighing the policy alternatives and, if required, selecting and implementing appropriate control options, including regulatory measures and subsequent enforcement. In the least developed countries like Bangladesh, anthropogenic pollution of heavy metals in marine environment is the prime concern. A periodic monitoring program and risk assessment approach are necessary to evaluate the scale of anthropogenic impacts on ecological balance and human health. In this communication, we provided information on As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn levels in commonly consumed fish species from the Bay of Bengal as a function of four seasons. Such local data are required to increase the global data inventory and to inform management agencies to facilitate decision making and improve the management strategies of these metals. Though the present study indicates the low concentation of aforementioned elements in the fish species, we should monitor the metal contamination periodically so that the anthropogenic activates can't disrupt the balance of aquatic ecosystem by polluting the marine organisms and to ensure food safety. The estimation of health risks associated with the dietary intake of heavy metals by the consumers is a vital and integral part of regulatory processes. This study estimated the human health risks associated with ten heavy metals due to consumption of ten fish species, and hence farther study is recommended taking into account other contaminants, for example, Hg, in a broader range of fish species from the Bay of Bengal. This study deals with fish consumption which constitutes only 3% of per capita per day calorie intake by foodstuffs for the coastal population in Bangladesh. Thus, other foodstuffs such as vegetable, fruits, rice, and cereals are required to be estimated to determine the public health risk via consumption of foodstuffs more robustly and to provide more information to allow the public to maximize the health benefits from food consumption while to minimize the risks from dietary intake of contaminants.

5. Conclusions

Accumulation of heavy metals in fish muscles is a threat to global public health since the consumption of contaminated foods is considered as the primary route of human exposure to toxic heavy metals. This study showed that variations in concentrations of As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn in the fish species from the Bay of Bengal were dependent on the seasonal fluctuation. The fishes captured during summer months exhibited higher metal contents followed by spring, autumn, and winter. This summer maxima can be attributed to heavy rainfall and floods during wet summer months in Bangladesh. The concentration levels of all the examine metals remained well below the acceptable limits for human consumption established by various international authorities such as USEPA, ANHMRC, and WHO. From the human health point of view, the estimated daily intake (EDI) of each element was lower than the respective recommended daily dietary allowance. The THQ values for individual element and the TTHQ values for combined elements were lower than 1, indicating no health risks for consumers due to the intake of either individual element or combined elements. However, the risk of cancer due to exposure to arsenic was of concern since the values of carcinogenic risk (CR) were above the acceptable risk limit of 10^{-5}.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Figure captions

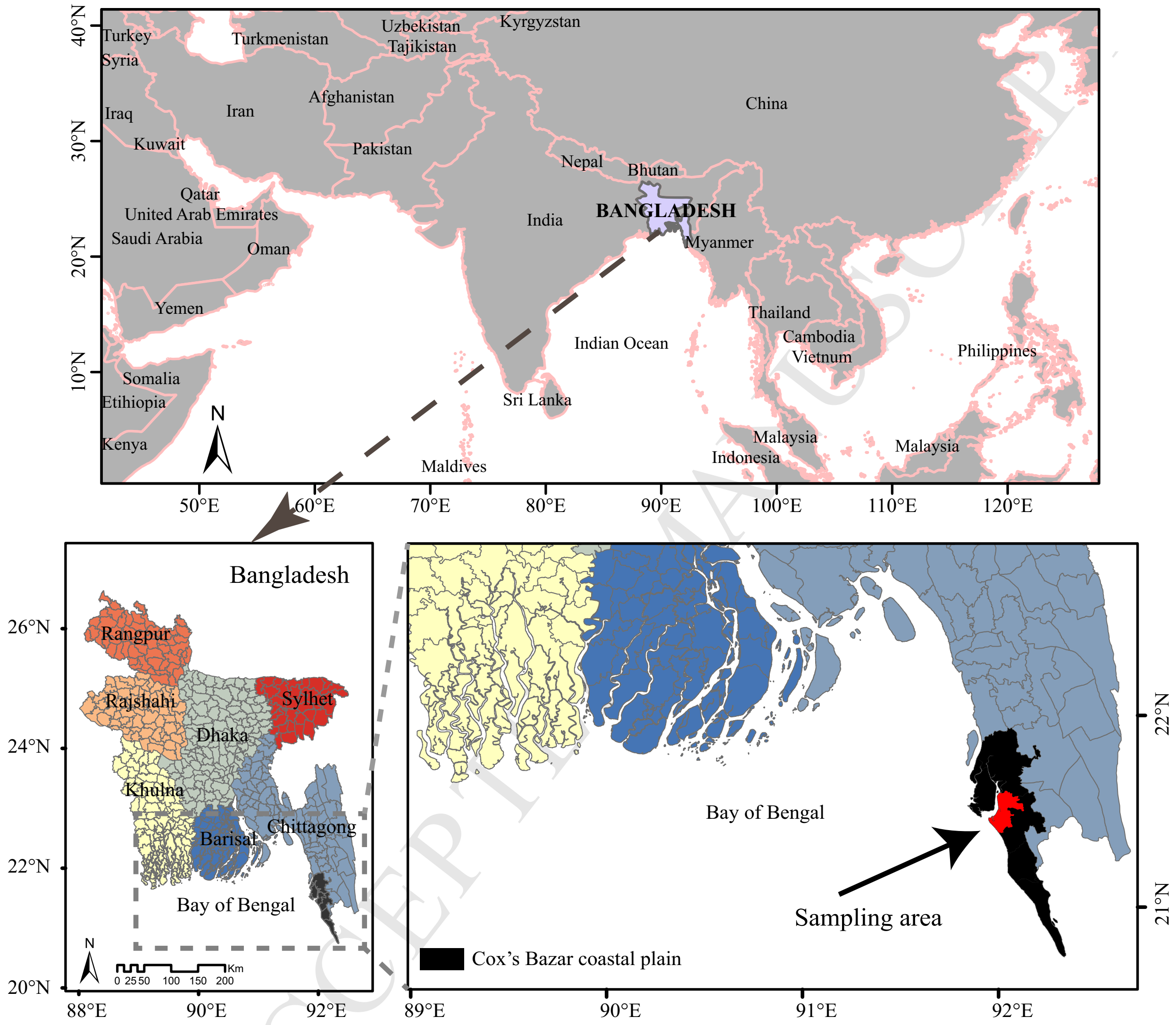
Fig. 1. Photographs of the sampling area, Cox’s Bazar, Chittagong, Bangladesh.

Fig. 2. Box plots show the seasonal variation of metals in the examined fish species. The inset displays the trend in changes of the mean metal concentrations in ten different fish species.

Fig. 3. Comparison of fish metal concentrations (µg/g - dry weight basis) found in this study with published data from the Bay of Bengal (Hossain & Khan, 2001; Sharif, Alamgir, Krishnamoorthy, & Mustafa, 1993 (in Fig. Sharif et al. 1993a)); Sharif, Mustafa, Amin, & Safiullah, 1993 (in Fig. Sharif et al. 1993c)); the Paira River (Islam, Ahmed, Raknuzzaman, Habibullah-Al-Mamun, & Masunaga, 2015); Soari Ghat, the Gumti and Turag Rivers (Sharif, Alamgir, Mustafa, Hossain, & Amin, 1993 (in Fig. Sharif et al. 1993b)); the Bangshi River (Rahman, Molla, Saha, & Rahman, 2012); various markets (Ahmed, Shaheen, Islam, Habibullah-al-Mamun, Islam, Mohiduzzaman, et al., 2015); and the Buriganga River (Ahmad, Islam, Rahman, Haque, & Islam, 2010) of Bangladesh. The concentrations of metals in fishes from the Paira River (Islam, Ahmed, Raknuzzaman, Habibullah-Al-Mamun, & Masunaga, 2015) and various markets (Ahmed, et al., 2015) were wet weight basis and for comparison the values were transformed to dry wet assuming 79% of moisture content.

Fig. 4. Estimated daily dietary intake of the metals and their comparison with the respective recommended daily dietary intake. For more information see the Table S5 provided in supplementary materials.

Fig. 5. Non-carcinogenic risks due to intake of ten examined metals. The inset shows the carcinogenic risk of As. For more information see the Table S5 provided in supplementary materials.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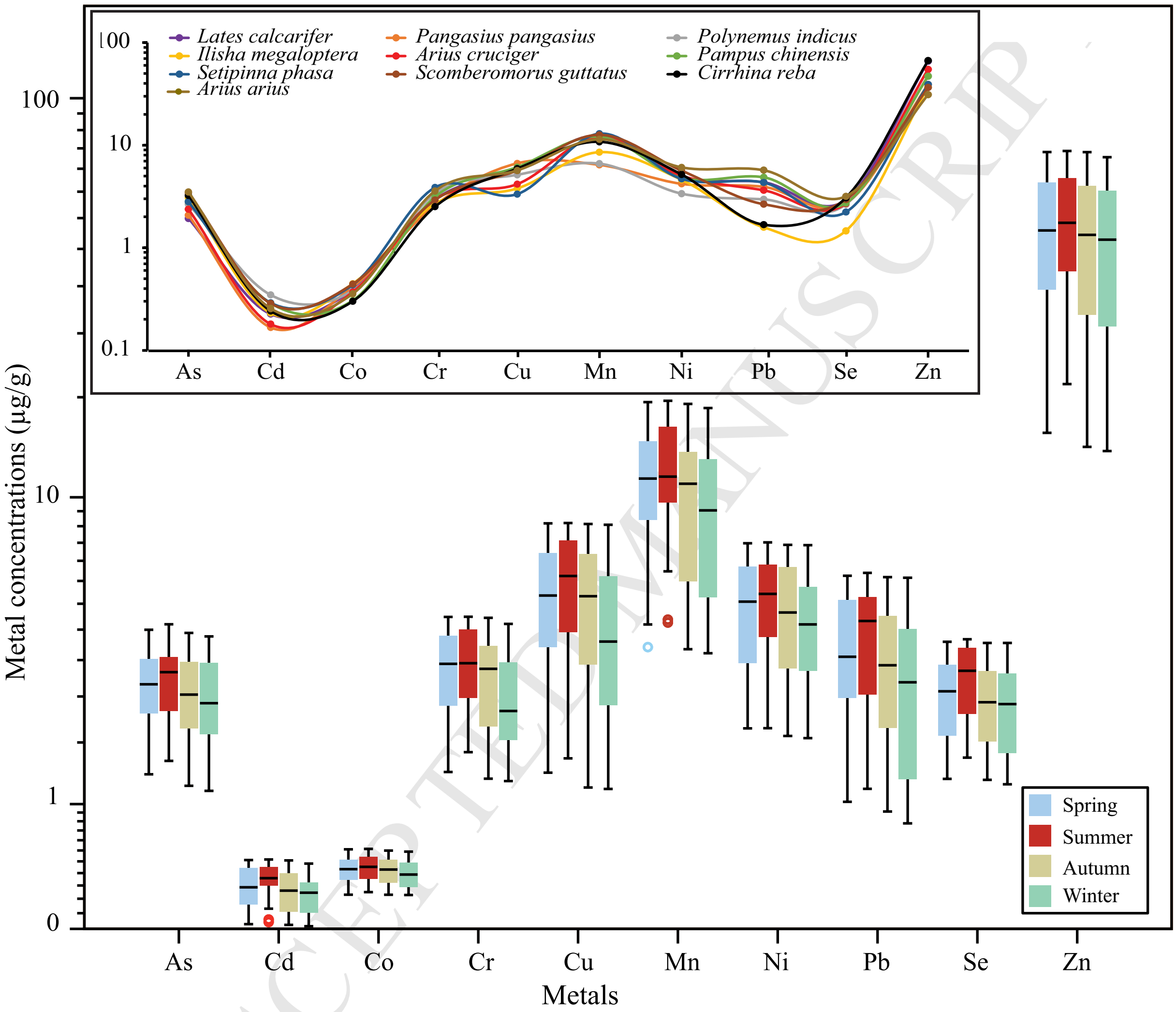
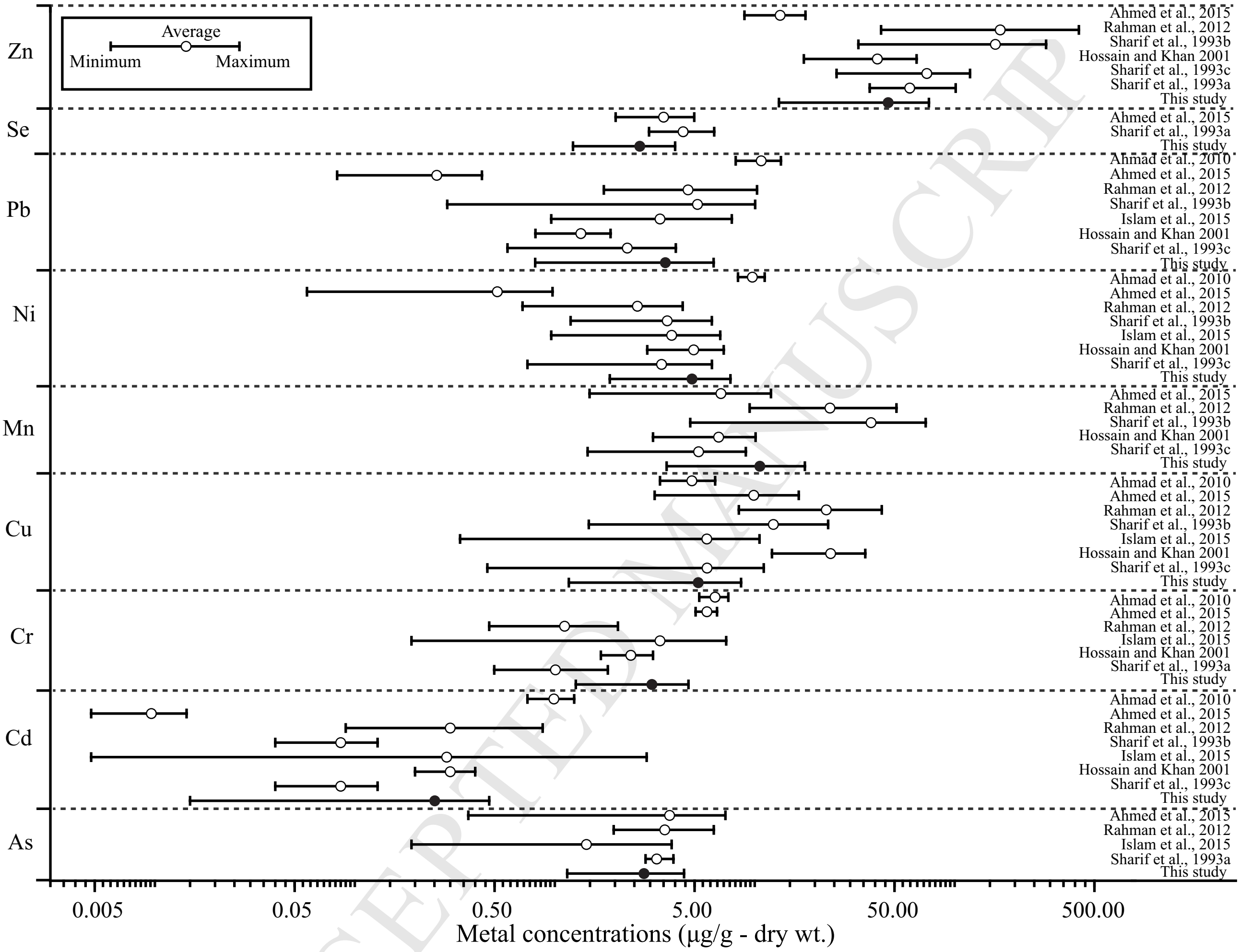
Bhutan
Qatar
United Arab Emirates
India
BANGLADESH
Saudi Arabia
Oman
Myanmer
Yemen
Thailand
Indian Ocean
Cambodia
Vietnum
Philippines
Somalia
Etihiopia
Sri Lanka
N
Kenya
Maldives
Malaysia
Indonesia
Malaysia
40°N
30°N
20°N
10°N
50°E
60°E
70°E
80°E
90°E
100°E
110°E
120°E
Bangladesh
Rangpur
Rajshahi
Sylhet
Dhaka
Khulna
Barisal
Chittagong
Bay of Bengal
N
0 25 50 100 150 200 Km
26°N
24°N
22°N
20°N
88°E
90°E
92°E
Bay of Bengal
Sampling area
■ Cox's Bazar coastal plain
22°N
21°N
89°E
90°E
91°E
92°E


Lates calcarifer
Ilisha megaloptera
Setipinna phasa
Arius arius
Pangasius pangasius
Arius cruciger
Scomberomorus guttatus
Polynemus indicus
Pampus chinensis
Cirrhina reba
100
10
1
0.1
As
Cd
Co
Cr
Cu
Mn
Ni
Pb
Se
Zn
Metal concentrations (μg/g)
100
10
1
0
Spring
Summer
Autumn
Winter
As
Cd
Co
Cr
Cu
Mn
Ni
Pb
Se
Zn
Metals


Average
Minimum
Maximum
Zn
Ahmed et al., 2015
Rahman et al., 2012
Sharif et al., 1993b
Hossain and Khan 2001
Sharif et al., 1993c
Sharif et al., 1993a
This study
Se
Ahmed et al., 2015
Sharif et al., 1993a
This study
Pb
Ahmad et al., 2010
Ahmed et al., 2015
Rahman et al., 2012
Sharif et al., 1993b
Islam et al., 2015
Hossain and Khan 2001
Sharif et al., 1993c
This study
Ni
Ahmad et al., 2010
Ahmed et al., 2015
Rahman et al., 2012
Sharif et al., 1993b
Islam et al., 2015
Hossain and Khan 2001
Sharif et al., 1993c
This study
Mn
Ahmed et al., 2015
Rahman et al., 2012
Sharif et al., 1993b
Hossain and Khan 2001
Sharif et al., 1993c
This study
Cu
Ahmad et al., 2010
Ahmed et al., 2015
Rahman et al., 2012
Sharif et al., 1993b
Islam et al., 2015
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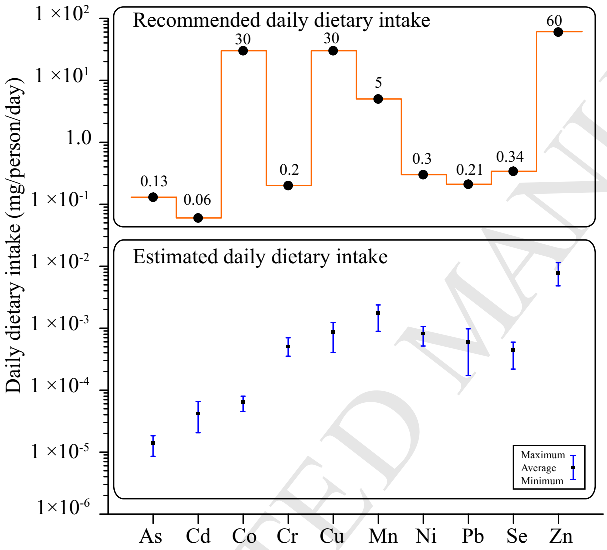
This study
Cr
Ahmad et al., 2010
Ahmed et al., 2015
Rahman et al., 2012
Islam et al., 2015
Hossain and Khan 2001
Sharif et al., 1993a
This study
Cd
Ahmad et al., 2010
Ahmed et al., 2015
Rahman et al., 2012
Sharif et al., 1993b
Islam et al., 2015
Hossain and Khan 2001
Sharif et al., 1993c
This study
As
Ahmed et al., 2015
Rahman et al., 2012
Islam et al., 2015
Sharif et al., 1993a
This study
0.005
0.05
0.50
5.00
50.00
500.00
Metal concentrations (μg/g - dry wt.)


Recommended daily dietary intake
Estimated daily dietary intake
Daily dietary intake (mg/person/day)
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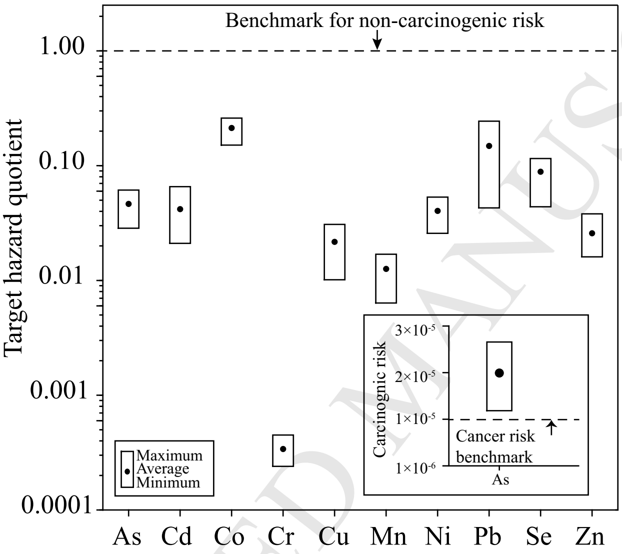
1 ×10^1
1.0
1 ×10^-1
1 ×10^-2
1 ×10^-3
1 ×10^-4
1 ×10^-5
1×10^-6
0.13
0.06
30
0.2
30
5
0.3
0.21
0.34
60
Maximum
Average
Minimum
As
Cd
Co
Cr
Cu
Mn
Ni
Pb
Se
Zn


Benchmark for non-carcinogenic risk
Target hazard quotient
1.00
0.10
0.01
0.001
0.0001
As
Cd
Co
Cr
Cu
Mn
Ni
Pb
Se
Zn
Maximum
Average
Minimum
Carcinogenic risk
3×10-5
2×10-5
1×10-5
1×10-6
Cancer risk benchmark
As

Highlights

- Ten metals in 10 fish species from the Bay of Bengal were estimated in 4 seasons.
- Significant interspecies variations were observed for all metals.
- Metal accumulation was higher during wet summer season.
- Consumption of fish was safe for human health.
- Consumers were exposed to arsenic with carcinogenic risks.