Heavy metals, trace elements and biochemical composition of Asian seabass (*Lates calcarifer*) in coastal areas of Bangladesh

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Abstract. Asian seabass (*Lates calcarifer*) is considered as a nutritionally and commercially important fish species all over the world because of its high-quality dietary protein, omega-3-polyunsaturated fatty acids, and minerals. Nutritional value of this species may be impaired by the environment in which it exists. Toxic level of heavy metals along with trace element in the environment has deleterious effects on human health through consumption of heavy metals contaminated fish. This study aimed to estimate the heavy metal, trace element and biochemical composition in Asian seabass collected from six different coastal areas of Bangladesh namely Satkhira, Khulna, Bagerhat, Patuakhali, Bhola and Cox's Bazar. The heavy metal Cr (0.757 ppm) was the most abundant metal followed by Pb (0.118 ppm) and As (0.00255 ppm). On the other hand, trace element Fe (3.578 ppm) was the most abundant mineral followed by Zn (0.864 ppm) and Cu (0.0036 ppm). The highest amount of Fe, Zn, Cu and Cr were found in Patuakhali sub population whereas As and Pb were found to be most abundant in Khulna sub population. Concentration of heavy metals in all fish population was below the Food Safety Guideline (FSG) prescribed by WHO/FAO. Proximate analysis revealed that the range of crude protein, lipid, ash, and moisture content were 15.85-18.76%, 0.71-1.74%, 0.64-1.12% and 75.92-81.13%, respectively. The maximum content of protein, lipid and ash was found in Patuakhali sub population, whereas the lowest amount of protein was in Satkhira sub population. In addition, the lowest amount of lipid and ash were observed in Bhola sub population. The study suggests that Asian seabass population from Patuakhali sub population had a better biochemical composition with substantial amount of nutrients and select for future culture, and study showed that negligible heavy metal found in the studied fish. Therefore, Asian seabass from different coastal regions of Bangladesh is safe for human consumption and free from health hazard.
Key Words: Asian seabass, biochemical composition, heavy metal, human health, trace element.

Introduction. Toxic materials are introduced into the aquatic environment by both natural and anthropogenic processes. Heavy metals are accumulated in fish tissue and used as a bio-indicator of aquatic pollution (Sabbir et al 2018a, b). Fish species have the capacity to absorb a significant amount of heavy metals from water through ingesting food and suspended particulate matter and by using their gills, body surface, and digestive tract (Mansour & Sidky 2002). Due to their durability and harmful effects, they can bioaccumulate and biomagnify in the food chain, posing a major risk to both ecological stability and human health (Rahman et al 2013; Passarelli et al 2018).

Asian seabass (*Lates calcarifer*) is an important coastal, estuarine, and freshwater fish (Griffin 1987; Nelson 2006). It is one of the species of the family Latidae and is found in many parts of the world as well as on the coast of the Bay of Bengal, Bangladesh (Hanif et al 2015; Ghosh et al 2022). The species is a very popular and highly valued fish throughout the Indo-Pacific region which has a high market price (Griffin 1987; Ghosh et al 2022). Few investigations have been done on the proximate composition and mineral content of the Asian seabass to date. The proximate composition and mineral content of aquaculture Asian seabass has been reported by Erkan & Özden (2007). The proximate composition and mineral contents in captive reared European seabass (*Dicentrarchus labrax*) have been documented by Raso & Anderson (2003). In this context, measurements of some biochemical profiles including protein, lipids, ash, and moisture levels, are frequently required to make sure that they adhere to commercial criteria and food laws (Waterman 2000; Tawfik 2009; Sutharshiny & Sivashanthini 2011).

However, currently the information regarding on the heavy metal, trace element and biochemical composition of Asian seabass globally is still scarce. The aim of this study was to estimate the heavy metal, trace element content and to carry out the biochemical composition in the muscle of Asian seabass in the six different coastal areas in Bangladesh (Satkhira, Khulna, Bagerhat, Patuakhali, Bhola and Cox's Bazar).

Material and Method

Sample collection. The study period of the research was from July 2020 to December 2021. Six coastal rivers were used to collect a total of 60 Asian seabass samples (10 samples from each subpopulation), ranging in length from 24.8 to 38.9 cm and weight from 160 to 630 g. These rivers include the Kholpetua River in Satkhira, the Vodra River in Khulna, the Boleshwar River in Bagerhat, the Meghna River in Bhola, the Andharmanik River in Patuakhali, and the Moheshkhali River in Cox's Bazar (Figure 1). Samples were cleaned with clean water at the time of collection, kept in ice boxes, transferred to the lab as soon as possible and then frozen at -20°C until dissection.

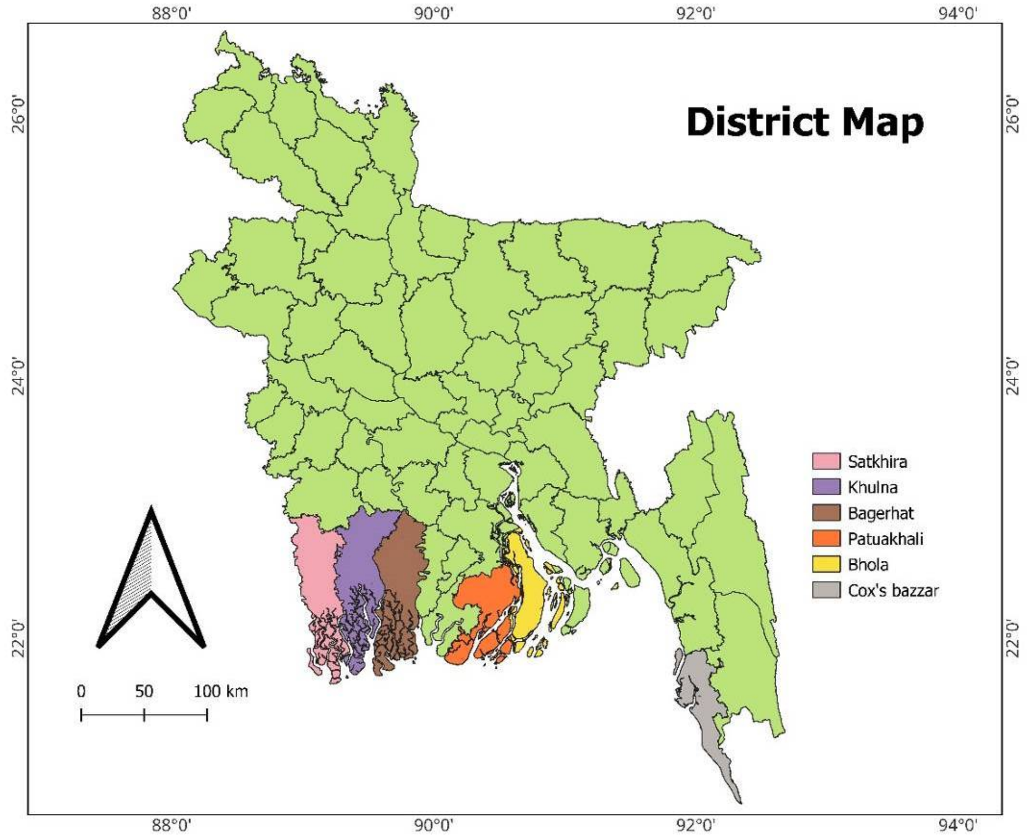

Figure 1. Geographic map of the coastal areas in Bangladesh depicting sampling locations. The map was generated with the available country level GIS data using Q-GIS version 3.26.3.

Heavy metal and trace element determination. Fish samples of 5 g were taken into crucible and dried in the electric oven at 40ºC for 24 hours. Dried samples were crushed by mortar to make powder. Ten mL of HNO_3 was mixed with 0.5 g sample. The solution was kept in the block digester machine at 100ºC for 2 hrs. After cooling the solution, it was filtered by using filter paper. This solution was making a volume of 100 mL by adding distilled water and stored at 2-4ºC until analysis. All minerals were determined using atomic absorption spectrometer (AAS) (Shimadzu, AA-7000) against aqueous standards. Each solution was diluted to the desired concentration and the standard calibration curve for each mineral was prepared according to the procedures described in Analytical Methods for AAS. The mineral concentration was calculated as mg kg^{-1} fish dry weight.

Biochemical composition analysis. Whole bodies and muscle of fish were grinded through an industrial meat grinder then stored at -20°C until further processing. Fish muscle of Asian seabass was taken for analyzing biochemical composition such as crude protein, crude fat, ash, and moisture following the method of AOAC (1990) and Sarower et al (2014). The experiments were performed in triplicate and values were expressed as mean±standard deviation. The protein content was determined by estimation of the total nitrogen by Kjeldahl method and protein content was calculated by multiplying total nitrogen by 6.25 factor (AOAC 2000) while the moisture content of the sample was determined after drying in a thermostat-controlled oven at 105°C for 24 h. The percentage of ash was determined by ignition in a muffle furnace at 550°C for 8 hours as described by Pearson (1976), while lipid content of the sample was determined by extracting a weighted quantity of sample (2-5 g) with methanol and chloroform (1:2) solution which was mixed by using a mixer and filtered by a filter paper.

Statistical analysis. The heavy metals, trace elements and biochemical composition of Asian seabass were analyzed using one-way analysis of variance (ANOVA) and the significant differences between means were determined by post hoc Duncan's multiple range test. Differences were significant when $p < 0.05$. Data were analyzed using SPSS (Version 11, Chicago, IL, USA).

Results

Heavy metal and trace element. The heavy metals of As, Pb, Cr and trace element of Fe, Zn, Cu in Asian seabass were estimated with the help of AAS. There were significant differences among all the sub populations ($F_{(5, 24)} = 1811.88$ where ($p < 0.05$) by one way ANOVA. The highest concentration of Fe was found in Patuakhali sub population and the lowest was found in Bagerhat sub population (Table 1). From one way ANOVA there was significant difference found among Zn content ($F_{(5, 24)} = 79.08$ where ($p < 0.05$). The highest Zn concentration was in Patuakhali sub population but lowest in Bagerhat sub population. There was no significant difference of Cu among the sub populations except Bhola sub population ($F_{(5, 24)} = 7.04$ where ($p < 0.05$) by one way ANOVA. The highest Cu concentration was identified in Patuakhali sub population where the lowest in Bhola sub population. The highest concentration of Cr was found in Patuakhali sub population and lowest in Bhola sub population. There was no significant difference among Satkhira, Bhola and Cox's Bazar sub populations. The permissible limit for Cu is 2 ppm and the result was within the limit (WHO/FAO 2011) (Table 2). Pb was below detection level in Satkhira and Bagerhat while Khulna had the highest and Cox's Bazar had the lowest. As was below detection level in Satkhira, Bagerhat and Cox's Bazar sub populations. Khulna had the highest concentration while Bhola had the lowest. The concentration of heavy metals and trace elements vary from location to location, and it was below the permissible limit suggested by the food safety guideline (FSG) of WHO/FAO (2011). Fe is a crucial micromineral and deficiency of it causes anemia, decreases productivity, and affects both physical and mental development. Fe levels varied greatly.

Table 1 shows the comparison of the concentration of Fe, Zn, Cu, Cr, Pb, and As of 6 different sub populations. Patuakhali sub population had the highest amount of Fe, Zn and Cr concentration but in Khulna sub population, there found highest level of Pb and As. On the other hand, Cu was found highest in Satkhira sub population.

Table 1

Trace elements and heavy metals of Asian seabass from six different coastal areas

Sub population	*Trace element*				*Heavy metal*	
	Fe (ppm)	*Zn (ppm)*	*Cu (ppm)*	*Cr (ppm)*	*Pb (ppm)*	*As (ppm)*
Satkhira	0.643±0.04[c]	0.377±0.03[b]	0.0038±0.002[a]	0.077±0.01[d]	BDL	BDL
Khulna	1.181±0.02[b]	0.406±0.08[b]	0.0034±0.001[a]	0.380±0.03[b]	0.118±0.01	0.00255±0.07
Bagerhat	0.319±0.07[d]	0.198±0.03[d]	0.0027±0.001[a]	0.133±0.01[c]	BDL	BDL
Patuakhali	3.578±0.03[a]	0.864±0.07[a]	0.0036±0.001[a]	0.757±0.07[a]	0.035±0.01	0.000228±0.05
Bhola	0.657±0.13[c]	0.266±0.06[cd]	0.0008±0.0005[b]	0.037±0.01[d]	0.024±0.01	0.000145±0.008
Cox's Bazar	0.387±0.002[d]	0.282±0.07[c]	0.0008±0.0004[a]	0.064±0.01[d]	0.021±0.007	BDL

BDL = below detection level. Values are shown as mean±SD; values with different superscripts in the same column are significantly different ($p < 0.05$).

Table 2

Range of heavy metals and trace elements of Asian seabass with permissible limit

Serial no of element	*Heavy metal/ trace element*	*Concentration (ppm)*	*Permissible limit (ppm) by WHO/FAO (2011)*
1	Fe	0.32-3.58	100
2	Zn	0.86-0.198	5
3	Cu	0.0036-0.0008	2
4	Cr	0.037-0.757	0.05
5	Pb	0.021-0.118	0.5
6	As	0.00015-0.00255	0.01

***Biochemical composition**.* Biochemical composition of Asian seabass included protein, lipid, ash, and moisture and is presented in Figure 2. The crude protein content ranged from 15.85±1.45 (Satkhira) to 18.76±1.67% (Patuakhali). The lipid content was found in between 0.71±0.19% (Bhola) to 1.74±0.47% (Patuakhali) in this study. The moisture content ranged from 76.32±1.97% (Patuakhali) to 81.13±1.16% (Satkhira) and ash ranged from 0.64±0.24% (Bhola) to 1.12±0.29% (Patuakhali). The result indicates significant differences among the six different sub populations of Asian seabass from one way ANOVA at 5% significance level ($p < 0.05$).

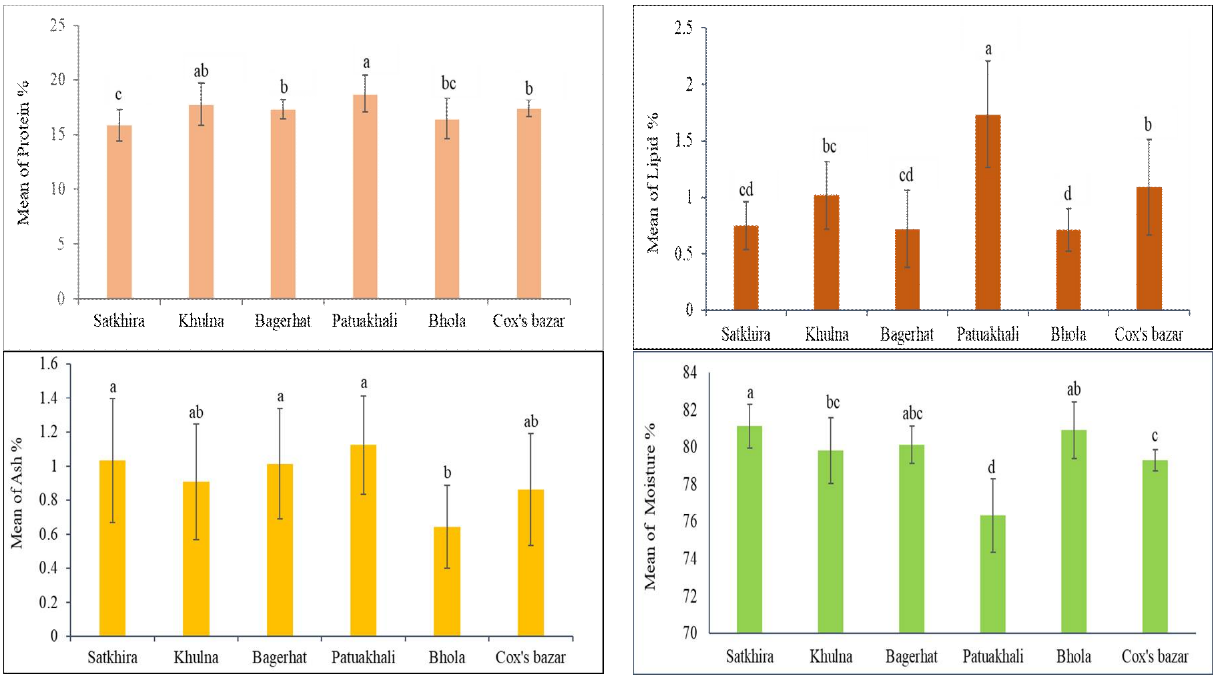

Figure 2. Biochemical composition of Asian seabass collected from six coastal areas of Bangladesh. Values represent the mean±SD. Same letters on the bars indicate that the means are not significantly different.

Discussion. In this experiment, there were also observed some trace element concentration like Fe, Zn, Cu in fish muscles where some heavy metals (Pb, Cr, As) were found but except Cr, all were in under the acceptable limit set by WHO/FAO (2011) (Table 2). The highest level of Pb and As were observed in Khulna while the others (Fe, Zn, Cr) level was highest in Patuakhali (Table 1). Some microelements like Fe, Zn and Cu which are important for body when their concentrations in the tissues do not exceed the metabolic demands (Hogstrand & Wood 1996; Ako & Salihu 2004). Zn is essential element which is required by a large number of enzymes for the synthesis and dissolution of carbohydrates, proteins, lipids, nucleic acids and other micronutrients as well as in the metabolism of other nutrients (FAO/WHO 1998). Zn is necessary for optimal health, but excessive use can result in health issues like damage to the liver and kidneys (Pervin et al 2012). According to WHO/FAO (2011), Zn concentration of more than 5 ppm is hazardous to human health, yet as it is, the Zn in six different sub populations were below the safety level for ingestion. The highest Zn concentration (0.864±0.07 ppm) was found in Patuakhali sub population which supports the result of Marimuthu et al (2014). However, Cu is necessary to produce haemoglobin and is an essential part of a number of enzymes (Malik et al 2010). Cu content peaked at 0.0038±0.002 ppm in Satkhira (Table 1). Fe is an important micro mineral, as its deficiency lead to anemia and thus with reduced working capacity and impaired intellectual development. The range of Fe content was considerably varied from 0.32 to 3.58 ppm. The highest Fe content was found in Patuakhali (3.58±0.03 ppm), which was significantly lower than that was found by Rejomon et al (2010). Fe content of Asian seabass (3.578±0.03 ppm) was found similar as reported by Marimuthu et al (2014). Fe is also present in organisms in substances like haemoglobin or myoglobin (Sures et al 1999). Cr content was observed highest (0.757±0.07 ppm) in Patuakhali sub population. This result is well supported by Marimuthu et al (2014). More Cr content indicates environmental pollution, and its less content indicates the environmental condition of the area of fishing is amicable.

The result indicates that heavy metals and trace elements concentration varied among different sub populations. According to Tiquio et al (2017), there is an estimation that land-based activities including agriculture, industry, urban/rural, and others account for 80% of the pollution load in coastal and marine habitats. Effluents from sewage treatment facilities, wastewater treatment plants, household, industrial, mining, and aquaculture facilities may be discharged directly or indirectly (Nugegoda & Kibria 2017). As urbanization spreads around the globe, human activities that harm the environment are also increasing. Around the various sample locations, there were also agricultural fields and wastewater treatment facilities. This might be the cause of variations in the levels of heavy metals and trace elements at different sub populations. Though there were found heavy metals and trace elements in sampled fish body, the levels were all under the permissible limit of WHO/FAO (2011) (Table 2) only Cr slightly crossed the limit.

Various factors, such as age, fishing areas, fishing season, fish sex, feed intake, and migratory swimming affect the biochemical composition of different fish species (Lall 1995). According to FAO (1986), the five usual constituents measured for compositional analysis of fish are moisture, protein, fat, ash, and carbohydrate which is obtained by subtraction according to the AOAC (1990) and Sarower et al (2014). Proteins, lipids, and moisture contents as well as ash were the major constituents, which had been considered in evaluating the nutritional value of the species studied. The nutritional elements showed variable values in the species among different sub populations. Crude protein and lipid recorded the highest values and moisture recorded the lowest in the samples of Patuakhali sub population (Table 1). The high moisture content, according to Oluwaniyi & Dosumu (2009), is a drawback since it makes fish more susceptible to microbial spoilage and the oxidative degradation of polyunsaturated fatty acids, which decreases the fish's quality for longer periods of time during preservation. This result supports the finding that high lipid fishes had less water and more protein than low-lipid fishes (Mohamed et al 2010). Moisture percentage of Asian seabass in Patuakhali sub population was 76.32±1.97% which was found similar as reported in different studies (Hui et al 2001; Pervin et al 2012; Rahman et al 2018). The percentage of water is a good indicator of its relative contents of energy, proteins, and lipids.

The amount of proteins and lipids and energy density of the fish increase with decreasing water content (Dempson et al 2004). The two main nutrients in fish, protein, and fat, are used to determine an organism's nutritional condition. Highest protein and lipid content of Asian seabass were found 18.76±1.67 % and 1.74±0.47 % respectively. Protein and lipid content of Asian seabass agree with the results of Pervin et al (2012) and Rahman et al (2018). The mean protein, lipid, ash, and moisture contents of Asian seabass are similar to the results reported by Marimuthu et al (2014). The protein content of Asian seabass was found to be higher than that of gray eel-catfish (*Plotosus canius*) and cuttlefish (*Sepia apama*) and comparable to that of long-tailed butterfly ray (*Gymnura poecilura*) (Nurnadia et al 2011). In this study, highest protein and micronutrients content were found in Patuakhali sub population. So, it can be concluded that the muscle quality of the Asian seabass from Patuakhali was better than the other five sub populations.

Conclusions. Heavy metals are non-essential elements causing deleterious effects on human health. Almost all the heavy metals (except gold) are toxic in very low concentration, but trace elements are essential elements required in trace amount for growth and development of our body and they are not toxic at low concentration but toxic at very high concentration. In the study, the result shows that the populations of Asian seabass carry heavy metals under the permissible limit and contains trace elements as a mineral which is needed for development and survival in the nature. In another part biochemical composition is rich in content of protein and lipid. Asian seabass population from Patuakhali had a better biochemical composition with high nutrients and thus Patuakhali sub population is the best source of seed of Asian seabass for cultivation. So, from this study it can be concluded that trace elements accumulation in the muscle of Asian seabass is not making any hazard to human health till now and it is safe for human to consume Asian seabass from different coastal regions of Bangladesh.

Data availability. The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Conflict of interest. The authors declare that there is no conflict of interest.

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