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Regular Article

Trace Elements and Heavy metals in Five Cultured and Captured Fishes from Rajshahi City, Bangladesh

M. Golam. Mortuza[1, 2*] and Fahad A. Al-Misned[1]
[1]Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[2]Department of Zoology, Faculty of Life and Earth Science, Rajshahi University, Rajshahi 6205, Bangladesh
[*]Correspondence Email: mmortuza@KSU.EDU.SA, Tel: +966-46-97797; Fax: +966-46-78514

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Abstract
Concentrations of 15 trace elements and heavy metals (Beryllium (Be), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Strontium (Sr), Molybdenum (Mo), Cadmium (Cd), Antimony (Sb) and Lead (Pb)) in the muscles of five fish species (*Pangasius pangasius*, *Anabas testudineus*, *Labeo rohita*, *Catla catla*, and *Tenualosa ilisha*), collected from Rajshahi, Bangladesh, were detected, using inductively coupled plasma mass spectrometry (ICP-MS). The risk to humans from these trace elements, through fish consumption, was then assessed. The average trace element concentration in fish muscles varied in a decreasing order of Zn> Fe> As> Cu> Sr> Mn> Cr> Se> Ni> Co> Pb> Mo> Cd> Sb> Be. This study also showed that Zinc was predominant, while Beryllium (Be) was the least accumulated metal in the muscles of the studied fish. Data demonstrated that there was significant variation in the heavy metal concentrations in the fish species of the Rajshahi area. The estimated daily intake (EDI) was below the Reference Dose of daily intake (RfD) established by the United States Environmental Protection Agency (USEPA), and the Hazard Quotient (HQ) values indicated that there was no carcinogenic risk for consumers on account of the intake of the studied fishes, under the current consumption rate.

Key word: Trace elements, heavy metals, culture, capture, risk assessment and human health

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1. Introduction

An aquatic environment is of great interest at present, owing to the increase in pollutants caused by anthropogenic activities [1-3]. The growing agriculture in many developing countries and the expansion of urban areas close to the natural aquatic environments cause negative impacts on the environmental quality of the surrounding ecosystems. Among the bioindicators of the aquatic ecosystem, fishes are often considered as the most suitable objects, as they occupy a high trophic level and are an important food source for the human population [4-5]. Metal content in the tissues and organs of fishes indicate the concentrations of metals in the water and their accumulation in the food chain [6]. Fishes are known for their ability to accumulate heavy metals in their muscles.

Freshwater fish culture in Bangladesh has been experiencing a dramatic growth over the past few decades, as a result of increasing domestic fish consumption and foreign exports. On account of low investment and the easy and routine management required, freshwater fish culture has grown tremendously in recent years in the north-western part of Bangladesh [7-8]. To evaluate the implications of heavy metal pollution, five fish species (*P. pangasius*, *A. testudineus*, *L. rohita*, *C. catla*, and *T. ilisha*) have been collected from different fish culture ponds and from the Padma River in Rajshahi City, Bangladesh. These fish species are very common and popular among the consumers for their taste and flavor. The cultured fishes have different feeding habits and are exclusively taken from freshwater habitats; however, *T. ilisha* is a diadromous fish. In the present study, the concentrations of 15 trace elements and heavy metals have been determined in five fish species. On the basis of the measurements of these trace element concentrations, a human risk assessment was performed for the average Bangladeshi people, who consumed freshwater fish.

2. Materials and Methods

2.1 Sampling Site

The Rajshahi city is located on the north-west part of Bangladesh, on the bank of the river Padma. The fishes, *P. pangasius* (Thai Pangus); *A. testudineus* (Thai Koi); *L. rohita* (Rui); *C. Catla* (Katla), and *T. ilisha* (Ilish) were collected from mono- and mixed-fish culture ponds in Boalia and Matihar Thana, in Rajshahi City. The *T. ilisha* was collected directly from the fishermen of the Padma River, near the city of Rajshahi. The sampling sites were located between $24^{0}25$ N and $46^{0}48$ E and $24^{0}34$ N and $46^{0}40$ E (Figure 1). A total of seven fish of each species, of marketable size, were sampled from each pond and the Padma River fishing spot. No gender difference was considered in this study. Similar-sized fish were collected, to minimize any differences in trace element concentrations resulting from size. The length and weight of the fishes are presented in the Table 1.

Figure 1. Location of sampling areas in Rajshahi City, Bangladesh.

2.2 Sample Collection and Preparation

The fishes were killed with percussive stunning [9]. A total of 35 fish were used in the assessment. The fishes were put on ice immediately in order to maintain the freshness and later brought to the laboratory and their total length (cm) and total weight (g) measured (Table 1). To prevent metal contamination, special care was taken and tissues were dissected with special ceramic knife, scissors and plastic forceps (Miyako, California, USA). Samples were thawed, cleaned and scaled, then filleted. The fillets were dried at 65 ^{0}C then round to a fine powder as described by Hoo Fung [10].

2.3 Digestion

The dried fish samples were digested according to the method of Hanson [11] as described by Rahman [5]. 0.5 g of dried powdered fish tissues (three replications) was taken in a digestion apparatus and 2.5 ml conc. H_2SO_4 and 4ml conc. HNO_3 were taken. The mixture was slowly heated using a hotplate for 20 min. at 100^{o}C and allow to cool at room temperature [12]. The content was diluted with deionized water and filtered quantitatively into a 50 ml volumetric flask.

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2.4 Analytical Methods

After acid digestion, concentrations were determined according to APHA [13] through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, NexION 300D). Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of mono element certified reference solution ICP Standard (Merck). All laboratory plastic and glassware was cleaned by soaking overnight in a 10% nitric acid solution and then rinsed with deionized water.

2.5 Risk Assessment

The risk for human health as a result of eating these species was evaluated by calculating Estimated Daily Intake (EDI) was estimated using the following equation [14]

$$EDI = C_{fish} \times [D_{fish} / BW] \quad (1)$$

Where C_{fish} = average trace element concentration in fish muscle (µg/g wet weight), D_{fish} = the average daily fish consumption (g/day) per person was estimated by assuming that a 60 kg Bangladeshi will eat 21 g wet wt/ person/ day [5, 15], and BW = the average body weight (kg) of target population. The hazard quotient (HQ) was calculated by dividing the estimated daily intake (EDI) by the established Rfd to assess the health risk from fish consumption. There would be no obvious risk if the HQ were less than 1[14].

Table 1. Length and weight of collected fish species.

Species	English name	Total length (cm)	Total weight (g)	Food and Artificial Food	Collected from
Pangasius pangasius	Pangus catfish	46.5±2.66	1179±96.39	Fish meal, oil cake, rice bran, wheat bran, poultry viscera etc.	Mono culture pond
Anabus testudineus	Climbing perch	17.65±1.53	133.6±29.4	Artificial feed: Fish meal, oil cake, rice bran, wheat bran, pellets etc	Mono culture pond
Labeo rohita	Rui, Roh	48.62±3.16	986.03±162.77	fish meal, mustard cake, rice bran, wheat bran, pellets etc	Mixed culture pond
Catla Catla	Catla	43.12±2.17	1176.1±153.65	fish meal, mustard cake, rice bran, wheat bran, pellets etc	Mixed culture pond
Tenualosa ilisha	Hilsa shad	41.93±2.24	875.77±52.80	Phytoplankton, algae, diatoms, copepods, rotifers, etc	Padma river

2.6 Statistical analysis

Two-Sample Assuming Unequal Variances tests with significance levels of 5% were conducted on each metal to test for significant differences between species tissues. Differences between level means per factor were treated using Excel student's t-test. All values are expressed in mean ± standard deviation.

3. Results

The mean concentrations of the trace elements and heavy metals in five fish species from Rajshahi are presented in Table 2. The fishes were collected from different culture and capture systems. The concentrations of trace and heavy metals were determined based on the sample dry weight (mg/kg d w). The mean concentrations of trace and heavy elements in different fishes were in the order of Zn> Fe> As> Cu> Sr> Mn> Cr> Se> Ni> Co> Pb> Mo> Cd> Sb> Be. Many differences were observed among the list of the analyzed elements. Beryllium (Be) was the least available trace element in the five fish tissues examined. Chromium (Cr) was detected in a significantly higher concentration in *T. ilisha* and *L. rohita* than in others. The highest Manganese (Mn) concentration (1.486) was found in *T. ilisha* and low concentrations were detected in the muscles of *P. pangasius* and *C. catla*.

Iron (Fe) concentration was found to be high in all the fishes, with the highest concentration (66.447) detected in *T. ilisha* and the lowest in the muscles of *C. catla*, however, there were significant differences among the species. Cobalt (Co) showed a comparatively high concentration in *T. ilisha* muscles, but a low concentration was detected in monoculture fishes. Nickel (Ni) was detected in all tissues, with higher concentrations in monoculture fishes and significantly lower concentrations in *T. ilisha*. Copper (Cu) showed comparatively higher concentrations in the muscles of *T. ilisha*, but there were no significant differences among the tissues. A comparatively high amount of Zinc (Zn) was found in all fishes, with the highest concentrations found in the muscles of *T. ilisha*, but there were significant differences among the tissues.

Arsenic (As) showed a very high concentration in *T. ilisha* muscles differing from the rest of the muscles, and the rest of the muscles did not show any significant difference. Selenium (Se) showed a clearly higher concentration in *T. ilisha* than in the other tissues, where significant differences were found. Higher concentrations of strontium (Sr) were found in *T. ilisha* and lower concentrations in *P. pangasius*, and they differed significantly from the other tissues. Molybdenum (Mo) showed a significantly higher concentration in *P. pangasius*, but the rest of the tissues did not show any significant difference. Cadmium (Cd) and Antimony (Sb) showed a lower concentration in all muscles, but significant differences were found when compared with the others. Lead (Pb) concentrations were found to be high in all fishes, however, no significant differences were observed in the tissues, except in *A. testudineus*

The mean heavy and trace element concentrations in the muscles were used to evaluate the human health risk assessment for fish consumption in humans. A conversion factor of 4.8 was used to transform wet weight to dry weight [5]; the result is shown in Table 3. Thus, the estimated daily intake (EDI) of trace elements and heavy metals were compared with a reference dose (Rfd), as established by the USEPA [16], to assess whether the trace levels found in the fish were safe for human consumption. The results showed that the values of EDI were lower than the Rfd values, except for Zinc in *T. ilisha* (Table 4). The values of the Hazard Quotient (HQ) were also less than 1 (Table 4).

Table 2. Concentration (mg/kg d w) lements in different fish tissues. Data are mean±SD (n=7). The values with non-common letter superscript are significantly different (Exell t-test, p<0.05) within the same row.

Element	Mono Fish Culture		Mixed Fish Culture		Capture Fishery	Average Concentration in Fish tissues
	P. pangasius	*A. testudineus*	*L. rohita*	*C. catla*	*H. ilisha*	
Be	0.002±0.001^{a}	0.001±0.000^{b}	0.001±0.000^{b}	0.002±0.001^{a}	0.002±0.001^{a}	0.002±0.000
Cr	0.772±0.23^{a}	0.705±0.18^{a}	0.881±0.33^{b}	0.694±0.25^{a}	0.925±0.32^{b}	0.795±0.104
Mn	0.503±0.145^{a}	1.027±0.215^{b}	0.728±0.074^{c}	0.515±0.032^{a}	1.486±0.23^{d}	0.852±0.413
Fe	40.288±4.12^{a}	66.447±3.22^{b}	41.403±3.84^{a}	31.994±2.41^{c}	57.057±10.46^{d}	47.438±13.96
Co	0.290±0.541^{a}	0.205±0.0364^{b}	0.439±0.048c	0.300±0.024^{a}	0.737±0.285^{d}	0.394±0.209
Ni	0.665±0.152^{a}	0.471±0.114^{b}	0.414±0.162^{b}	0.390±0.074^{bc}	0.347±0.065^{c}	0.457±0.124
Cu	3.480±0.56^{a}	3.825±0.66^{a}	4.480±0.71^{a}	3.862±0.621^{a}	4.949±1.421^{a}	4.12±0.587
Zn	38.016±2.98^{a}	67.759±6.59bc	71.224±5.32^{b}	59.435±8.55^{a}	82.468±16.34^{d}	63.780±16.608
As	3.613±1.59^{a}	3.294±1.74^{a}	3.061±1.93^{a}	3.236±1.38^{a}	10.507±4.57^{b}	4.742±3.229
Se	0.314±0.064^{a}	0.304±0.052^{ac}	0.244±0.039^{b}	0.270±0.017^{bc}	1.173±0.068^{d}	0.461±0.399
Sr	1.142±0.052^{a}	1.892±0.063bc	1.233±0.046^{a}	1.539±0.081^{c}	2.618±0.095^{d}	1.685±0.599
Mo	0.033±0.012^{a}	0.017±0.005^{b}	0.015±0.003^{bc}	0.014±0.004^{c}	0.016±0.006b^{c}	0.019±0.008
Cd	0.008±0.002^{a}	0.015±0.001^{b}	0.024±0.00^{c}	0.013±0.000^{b}	0.034±0.001^{d}	0.019±0.10
Sb	0.033±0.002^{a}	0.008±0.001^{b}	0.018±0.002^{c}	0.008±0.002^{b}	0.010±0.001^{b}	0.015±0.11
Pb	0.617±0.054^{a}	0.413±0.021^{b}	0.62±0.008^{a}	0.570±0.061^{a}	0.55±0.07^{a}	0.554±0.084

4. Discussion

In the environmental studies heavy metals and trace elements, such as, Cr, Mn, Fe, Co, Ni, Zn, As, Cd, Pb, and Cu are the most studied and significant elements with regard to the environmental impact and human health. Indeed, many common heavy metals (Cr, Mn, Fe, Co, Ni, Zn, As, Cd, Pb, and Cu) are known to form materials that are potentially toxic to the environment [17-20], and the anthropic effects of heavy metals such as Be, Se, Sr, Mo, and Sb on fish and other aquatic life are less studied [21]. The values obtained in this study have been found to be within the range of values found in other studies, after comparing their concentrations in the muscles of freshwater fish (Table 5).

The results among all these studies have been widely dependent on where and which species were caught. The metal content in various fish species from the Hooghly River, India [22]; Pearl River, China [23]; Okumeshi River, Nigeria [24]; Bagashi River, Bangladesh [5], Wadi Hanifah [25], and the Danube River, Serbia [26], was higher than that in this result, except for Fe, Co, Zn, and As. Nevertheless, the distribution patterns could be compared within the species analyzed in this study. As far as we could observe, most of the elements (Cr, Mn, Co, Cu, Zn, As, Se, Sr, and Cd) were preferentially accumulated in *T. ilisha*. This might be because of the regulatory ability, behavior, and feeding habits, which were factors that could influence the accumulation in different species [27].

The fishes were collected from three distinctive groups, based on the culture system, and could point out their different feeding habits (Table 1). Almost similar artificial feed was supplied to the mono-, and mixed-fish culture in Rajshahi, Bangladesh. There was no significant difference observed in the trace element accumulation, however, *T. ilisha* showed a significant difference in most of the trace element accumulations. This could indicate its different habitat preferences, *T. ilisha* foraged in open water. This could also act as a detoxification pathway in *T. ilisha*, for accumulating toxic elements [28]. This study found higher concentrations of Mn, Fe, and Ni in the carnivorous fish than in carps, which confirmed the findings of several authors [29-32]. We also detected elevated concentrations of Zinc in *T. ilisha* muscles, which confirmed the findings of other authors [26, 30, 31, 33]. This could be a result of the pollution in the Padma River [34], of inversely correlated Zinc concentrations in the trophic level [35], as well as the physiology of *T. ilisha* [[36-37]. It was recently shown that trace elements in the food contribute predominantly to metal accumulation in marine fish, due to a very low uptake from the dissolved phase [38-39]. Dietary accumulation of trace elements was determined by dietary assimilation efficiency, ingestion rate, as well as trace element concentrations in the ingested food. Dietary assimilation was inversely related to the ingestion rate in fish [14]. As the fish farmers generally fed huge volumes of food, it was possible that the dietary assimilation was low, leading to a relatively low accumulation of these trace elements in the fish. However, this needs to be further examined in future studies.

The mean muscle concentrations were used to conduct health risk assessments for human fish consumption. Heavy metals had the tendency to accumulate in various organs of the organisms, especially fish, which in turn could enter the human metabolism through consumption, causing serious health hazards [5, 40]. Therefore, the daily intake of some selected trace metals were estimated and compared with the recommended values, to assess whether the metal levels found in the fish samples collected from Rajshahi were safe for human consumption (Table 4). This study was conducted only for fish muscles, as this tissue was the most important part consumed by the human population. The EDI values for the examined fish samples were lower than the reference dose (Rfd) [16]; The Joint FAO/WHO Expert Committee on Food Additives [41], indicating that there was no health risk for humans through the consumption of these , except for the Arsenic (As) concentration in *T. ilisha*. In addition, the values of the hazard quotient (HQ) were lower than 1 (one), indicating no risk on consumption of these fishes [14].

5. Conclusion

In general, the data in this study suggested that the heavy and trace element concentrations found in the fish muscles sampled from Rajshahi, with the exception of the Arsenic content in *T. ilisha*, were within the standard limits proposed by the USPEA. It should be noted that the concentrations of Zinc and Iron were found to be considerably higher among the 15 heavy and trace elements in all the examined fish species. The differences in heavy and trace element concentrations among the five different fish species were statistically significant. However, these results could be used to provide baseline information for the risk assessment associated with their consumption, as the estimated daily intake (EDI) for the examined fishes and the trace elements found were far below the daily dietary allowance recommended by the different authorities [16, 41]. Therefore, we concluded that these trace elements should not pose any health threat to the consumers, except for Arsenic, after consumption of the studied fish. Furthermore, constant monitoring of the fish culture of Rajshahi was recommended, in view of the artificial feed for the fish culture in the freshwater aquaculture of this region, which disturbed the natural cycle of the chemical elements.

Table 3. Concentration (mg/kg w w) of trace elements in different fish tissues. Estimated daily intakes of trace elements (EDI) through freshwater fisher consumption by people in Rajshahi city, Bangladesh.

Elements	Mean concentration (mg/kg ww)					Estimated daily intake (EDI) (mg/kg bw/ day)				
	P. pangasius	*A. testudineus*	*L. rohita*	*C. catla*	*H. ilisha*	*P. pangasius*	*A. testudineus*	*L. rohita*	*C. catla*	*H. ilisha*
Be	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cr	0.161	0.147	0.183	0.144	0.193	0.056	0.051	0.064	0.051	0.067
Mn	0.105	0.214	0.152	0.107	0.309	0.0367	0.075	0.053	0.037	0.108
Fe	8.393	13.843	8.625	6.665	11.887	2.938	4.845	3.019	2.333	4.160
Co	0.060	0.043	0.091	0.062	0.153	0.021	0.015	0.032	0.022	0.053
Ni	0.138	0.098	0.086	0.081	0.072	0.048	0.034	0.030	0.028	0.025
Cu	0.725	0.797	0.933	0.804	1.031	0.254	0.279	0.327	0.282	0.361
Zn	7.92	14.116	14.838	12.382	17.181	2.772	4.941	5.193	4.333	6.013
As	0.753	0.686	0.638	0.674	2.189	0.263	0.240	0.223	0.236	0.766
Se	0.065	0.063	0.051	0.056	0.244	0.023	0.022	0.018	0.02	0.085
Sr	0.238	0.394	0.257	0.321	0.545	0.083	0.138	0.09	0.11	0.191
Mo	0.007	0.003	0.003	0.003	0.003	0.002	0.001	0.001	0.001	0.001
Cd	0.002	0.003	0.005	0.003	0.007	0.001	0.001	0.002	0.001	0.002
Sb	0.007	0.002	0.004	0.002	0.002	0.002	0.001	0.001	0.001	0.001
Pb	0.0128	0.086	0.129	0.119	0.114	0.045	0.030	0.045	0.041	0.040

Table 4. EDI, estimated daily intake; Rfd, reference doses of trace elements and heavy metals as established by the United States Environment Protection Agency (2005); Hazard quotient = EDI/Rfd. If the ratio is less than 1, there is no obvious risk. [a]Average concentration of inorganic As was estimated as 10% of total As (United States Food and Drug Administration, 1993).

Elements	Estimated daily intake (EDI) (mg/kg bw/ day)					Rfd (mg/kg bw/ day)	Hazard quotient (HQ)				
	P. pangasius	*A. testudineus*	*L. rohita*	*C. catla*	*H. ilisha*		*P. pangasius*	*A. testudineus*	*L. rohita*	*C. catla*	*H. ilisha*
Be	0.000	0.000	0.000	0.000	0.000	-	0.000	-	-	0.000	0.000
Cr	0.056	0.051	0.064	0.051	0.067	3	0.019	0.017	0.021	0.017	0.022
Mn	0.0367	0.075	0.053	0.037	0.108	140	<0.001	<0.001	<0.001	<0.001	<0.001
Fe	2.938	4.845	3.019	2.333	4.160	700	0.004	0.001	0.004	0.003	0.006
Co	0.021	0.015	0.032	0.022	0.053	0.3	0.070	0.05	0.107	0.073	0.179
Ni	0.048	0.034	0.030	0.028	0.025	1.6-5	0.03-0.010	0.021-0.007	0.019-0.006	0.017-0.006	0.016-0.005
Cu	0.254	0.279	0.327	0.282	0.361	40	0.006	0.007	0.008	0.007	0.009
Zn	2.772	4.941	5.193	4.333	6.013	300	0.009	0.016	0.017	0.014	0.020
As	0.263	0.240	0.223	0.236	0.766	0.3	0.878	0.800	0.744	0.786	2.554
Se	0.023	0.022	0.018	0.02	0.085	5	0.004	0.004	0.003	0.004	0.017
Sr	0.083	0.138	0.09	0.11	0.191	600	<0.001	<0.001	<0.001	<0.001	<0.001
Mo	0.002	0.001	0.001	0.001	0.001	5	<0.001	<0.001	0.001	<0.001	<0.001
Cd	0.001	0.001	0.002	0.001	0.002	1	<0.001	0.001	0.001	<0.001	0.002
Sb	0.002	0.001	0.001	0.001	0.001	-	-	-	-	-	-
Pb	0.045	0.030	0.045	0.041	0.040	0.21*	0.045	0.030	0.004	0.041	0.004

*JECFA, 2000

Table 5. Comparison of heavy metal concentrations (mg/kg d w) in fish muscle with the reported values in the literatures.

Sample Area	Be	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Cd	Sb	Pb	References
Rajshahi, Bangladesh	0.001-0.002	0.694-0.925	0.503-1.486	31.994-57.057	0.205-0.737	0.39-0.665	3.480-4.949	38.016-82.468	3.061-10.507	0.244-1.173	1.233-2.618	0.014-0.017	0.008-0.034	0.008-0.033	0.413-0.62	This work
Bagashi River, Bangladesh	-	0.47-2.07	9.43-51.17	-	-	0.69-4.36	8.33-43.18	42.83-418.05	1.97-6.24	-	-	-	0.09-0.89	-	1.76-10.27	[5]
Hooghly River, India		<0.001-3.89				2.20-3.69	16.22-47.97	12.13-44.74					0.62-1.20		12.40-19.96	[22]
Perl River, China	-	<5.36				2.92-5.77	1.17-6.72	2.62-20.2	0.17-1.45				0.000-33.2		0.05-1.94	[23]
Wadi Hanifah, Saudi Arabia	-	0.94-2.11	19.81-37.18	8.06-14.67			18.71-43-61	178.91-410.71	3.25-7.31				1.60-2.48		2.88-5.12	[25]
Danube River, Serbia	-	0.04-0.08	0.12-0.87	13.64-27.06	0.0001	-	0.75-1.42	15.14-59.01	0.17-0.93	0.001-0.11	0.57-6.44	0.0004-0.12	0.005-0.01	-	-	[26]
Chascomus lake, Argentina	-	0.24-20.8	0.84-2.4	25.5-142	0.022-0.22	0.39-15.2	0.95-4.01	20.8-237	0.013-0.78	0.70-2.282	2.0-22.9	0.052-0.42	<0.01-0.11	0.007-0.088	0.37-31.4	[20]
Okumeshi River, Nigeria	-	0.06	1.97			0.17							0.63		-0.01	[24]

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Authors' Biosketches

Prof. M. Golam Mortuza

Dr. Mortuza is working in Environmental Pollution Research Laboratory in the Department of Zoology at king Saud University, Riyadh, Saudi Arabia. He received his BSc and MSc from University of Rajshahi, Bangladesh. He received DSc degree from Hiroshima University, Japan. He worked at Pusan national University as Postdoctoral Researcher. Presently his Lab deals with environmental pollution, toxicology and risk assessment. His research tropic addresses environmental pollution, fish ecology, habitat use, biotic interaction, relationship, fish behaviour, systematic study of fish, fisheries biology and floodplain fisheries. His overarching goal is to carry out an integrated research program to develop a sustainable biodiversity. He is also actively engaged in collaborative research with research students and colleagues in such area of fisheries biology, conservation and management, Environmental pollution and risk assessment. Dr. Mortuza also a permanent Faculty member of the University of Rajshahi, Bangladesh. For more contact at: mmortuza@ksu.edu.sa.

Prof. Fahad A. AlMisned

Prof. Fahad AlMisned is working at King Saud University in the Department of Zoology. He is also working as Deputy Vice-Rector at King Saud University. Professor Misned received his M.Sc. with distinction in Biological Science from the Manchester University (UK). He has also received his PhD in Biological Science from the Manchester University (UK). His laboratory is focused at Environmental pollution and toxicology. His research interests include environmental pollution, metal toxicity, risk assessment, biodiversity, insect ecology, environmental pollution assessment and management. He has published over 100 peer-reviewed ISI journals and book chapters. Dr. Misned held many important academic and administrative posts and chairs at King Saud University. For more information, visit at: faculty.ksu.edu.sa/4562/Pages/CVEnglish.aspx