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Toxicity Effects of Metals Bioaccumulation in Water and Fishes of the Balu River, Bangladesh

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ABSTRACT

The present study was conducted to determine the current state of water quality parameters and concentration of metals in the water and fish of Balu River. Samples were collected from three sampling sites in both wet and dry seasons. Temperature, color and odor, pH, electrical conductivity (EC), total dissolved solids (TDS), total solids (TS), optical density, salinity, and dissolved oxygen (DO) of water were tracked. Five fish species (*Puntius ticto*, *Channa punctatus*, *Glossogobius giuris*, *Amblypharyngodon mola*, and *Mystus vittatus*) were tested for metal concentrations (Cu, Pb, Cd, Zn and Cr). During the dry season, the river's water was slightly black, light grey, and black in color, and it smelled strongly pungent. Waterborne life was fatally affected by the DO values. The TDS, EC, and TS of water were higher in the dry than in the wet season. The trends of metal concentrations were Zn > Cu > Pb > Cr > Cd in water and Zn > Cu, Pb > Cr > Cd in fish species. During the dry season, the Cu in *G. giuris*; Pb, Cr, and Zn in *A. mola*, and the Cd in *C. punctatus* were the highest among the fishes investigated. However, during the dry season, *A. mola* had the highest total metal bioaccumulation while *P. ticto* had the lowest. When opposed to the wet season, the water had a significantly worse quality during the dry season, which may have been brought on by nearby industrial pollutants.

INTRODUCTION

One of the key elements of environment that play a significant part in every aspect of human life is water. It is regrettable that human activity is constantly contaminating water around the world. Many of the rivers have become contaminated by industrial effluents, sewage disposal, municipal garbage, agricultural waste, etc. However, given its importance in meeting human needs as well as those of the natural world, the source of water resources is of primary interest. In fact, Bangladesh is one of those polluted countries, which currently holds 1,176 industries that release about 0.4 million m^3 of untreated waste to the rivers per day (**JIKA, 1999**). The world's industrial boom has made pollution a severe threat to human life. Particularly in developing nations, the rivers are the primary options to hold and bear the responsibility of pollution. River ecology is impacted by chemical water contamination originated from metals (**Sikder *et al*., 2012**). Metal contamination is a major issue today in many developing nations including Bangladesh (**Islam *et al.*, 2015; Nahar *et al.*, 2017**). The unplanned urbanization and industrialization of Bangladesh have detrimental effects on the quality of water and other aquatic fauna. Urban garbage, untreated industrial effluents, and agrochemicals dumped into open water bodies (rivers) have reached alarming levels in Bangladesh, causing metal levels in water to rise and the water quality to deteriorate **(Khadse *et al*., 2008; Venugopal *et al*., 2009**). In general, most of the metals enter the river from different sources, which can be both natural from erosion and weathering or anthropogenic (**Gupta *et al*., 2013**).

Due to their toxicity and buildup in aquatic ecosystems, metal contamination in the environment is of utmost importance. While, some metals such as iron and manganese are necessary for an organism's metabolic processes, others, such as chromium, copper, mercury, nickel and lead are hazardous to aquatic life. Metals have drawn a lot of interest in aquatic ecosystems because of their toxicity and accumulation in fish and another biota. Fish with metal accumulation experience biomagnification in the food chain. These are continually released into aquatic systems causing serious threat because of their toxicity, bioaccumulation and long persistence (**Eisler, 1988**). Metal exposure has been associated to numerous human disorders, including kidney damage, cancer, abortion, effects on IQ and behavior, and in some cases, death due to exposure to extremely high concentrations of metals. Fish absorb a significant amount of metals from polluted water through water borne exposure (**Salem *et al*., 2014, Renieri *et al*., 2017**). Due to their superior nutritional value, fish are a significant component of the human diet (**Sioen *et al*., 2007**). About 17% of the animal protein ingested by the world's population and 7% of all protein consumed come from fish **(FAO, 2020**). Metal concentrations in fish tissues can be many times higher than those found in the water. Fish assimilate metals by ingestion of particulate material suspended in water, ingestion of food, ion-exchange across lipophilic membranes (e.g. the gills) and adsorption on tissue and membrane surfaces. Metal buildup in the body's tissues can lead to chronic sickness aside with having negative effects on the general population. The accumulation of metals in the tissues can result in chronic illness causing potential damage to the population (**Amirah *et al*., 2013**). Serious dangers include kidney failure, liver damage, cardiovascular conditions, and even death may fall under this category (**Rahman *et al*., 2012**). As a result, numerous international monitoring programs have been set up to evaluate the fish's suitability as human food and to keep track of the condition of the aquatic ecosystem (**Meche *et al*., 2010**). Additionally, a variety of variables, including sex, age, size, reproductive cycle, swimming habits, feeding behavior, and living environment (i.e., geographical location) can affect metal uptake (**Mustafa & Guluzar, 2003; Zhao *et al*., 2012)**. Bangladesh has extensive water bodies, both freshwater (culture 52.38%, capture 27.72%) and marine water (14.90%) production (**DOF, 2020**), which have a high potential for the fisheries sector, which plays an important role in the agro-based economics of Bangladesh by providing nutrition, increasing employment opportunities and earning foreign exchange. The freshwater inland capture production is the third highest in the world after China and India (**FAO, 2020**). Fisheries sector contributes 3.52% to the national GDP and more than one-fourth (26.37%) of the total agricultural GDP (**DOF, 2020**).

The Shitalakshya River has a tributary called the Balu River, which flows through Narayanganj City. The Shitalakhya River joins the Balu River near Demra ghat in the downstream portion of the river, which primarily flows through the vast wetlands of Belai Beel and to the east of Dhaka. It has a narrow connection with Shitalakhya River through Suti River as well as with Turag River by the way of Tongi canal that passes through the northwest of Dhaka. It carries floodwater from Shitalakhya and Turag during flood season. The Balu River water is heavily loaded with sewage and industrial waste, which contains various chemicals and harmful metals. It has become more and more contaminated as a result of the city's numerous industrial units and sewer pipes discharging massive amounts of hazardous waste into it day and night (**Islam *et al.*, 2006**). As the sewage from the capital is discharged into the river, the Balu River's contaminated waters have an impact on the local population. In the Dhaka watershed, there has been significant industrialization during the past ten years, particularly in the dyeing, washing and textiles sectors, according to a World Bank assessment. It is estimated that there are over 7,000 industries in Dhaka metropolis located mostly in three clusters area, Hajaribagh, Tejgaon and Dhaka-Narayanganj-Demra (DND).

In order to compare the amounts of metals in two seasons, water quality parameters, the presence of metals (Cu, Pb, Cd, Zn, and Cr) in water, and fish species from the Balu River were determined. A few species of fish are available in the River, such as *Puntius ticto, Channa punctatus, Glossogobius giuris, Amblypharyngodon mola* and *Mystus vittatus*, etc. They are commercially important small indigenous species (SIS). Having high protein composition along with micronutrients, vitamins, minerals and taste, those fishes have become very popular in Bangladesh (**Ross *et al.*, 2003**).

MATERIALS AND METHODS

Study area

Balu River, an important river in Bangladesh, passes through the wetlands of Belai Beel, Gazipur and Dhaka, joining the Shitalakshya River at Demra and also by way of the Tongi Khal with the Turag River. Although it inundates with floodwater from the Shitalakshya and the Turag rivers during flood season, it is an important channel for local drainage and other watercrafts like small boats. In Gazipur, it flows through densely populated town and agricultural fields that was used as a source of water in the past. Its downstream portion is 44 kilometers long. Samples were collected from three locations, viz. Ichapura (latitude 23°49'40.8"N and longitude 90°29'12.4"E), Nagarpara (latitude 23°46'22.1"N and longitude 90°28'58.4"E) and Chanpara (latitude 23°43'47.1"N and longitude 90°30'00.8"E) (Fig. 1). Water and fish samples were collected with three replications during the wet season (June 2017) and the dry season (December 2017).

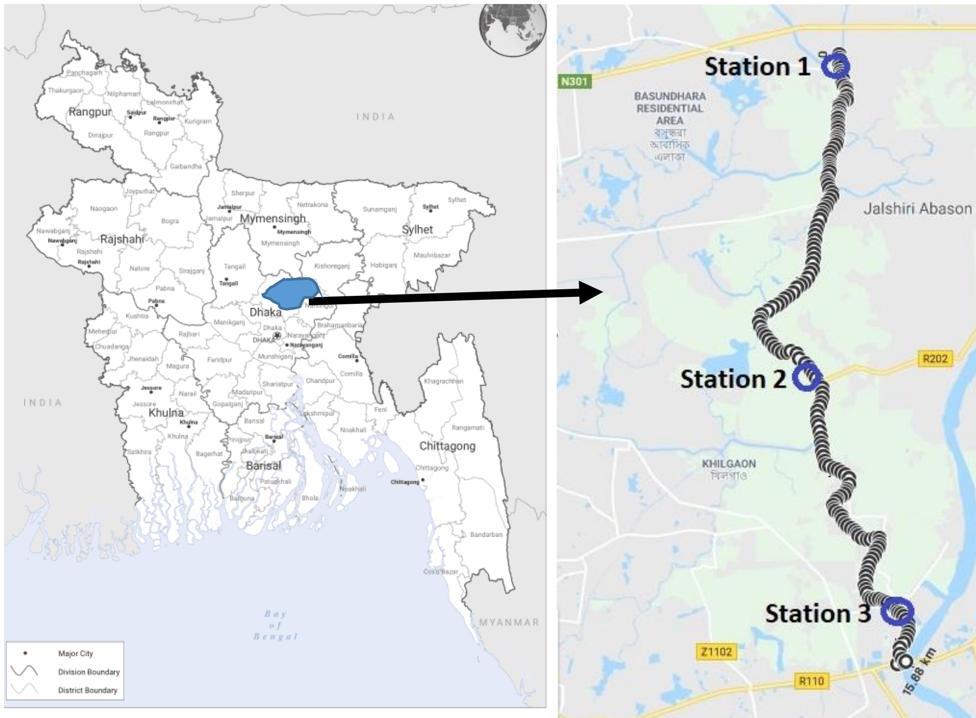


Fig. 1. Map of the Balu River showing sampling stations

Field sampling

In both the wet and dry seasons, water and fish samples were gathered from the sampling stations between 8:00 and 10:00 in the morning. Acid-washed plastic bottles were used to collect water samples (500 ml). The bottles were cleaned in diluted nitric acid and thoroughly rinsed with deionized water. In the field, a water sample was collected from a depth of around 20cm in bottles that were rinsed three times with river water. For additional analysis, nitric acid (1 ml conc.) was added to each sample. Nitric acid (1ml conc.) was added in each sample for further analysis. The samples were immediately sealed after collection in order to prevent air exposure.

In this study, five fish species (Fig. 2), including punti (*Puntius ticto*), taki (*Channa punctatus*), bele (*Glossogobius giuris*), mola (*Amblypharyngodon mola*) and tengra (*Mystus vittatus*) were collected from the river with the help of local fishermen using local fishing tools such as gill net hooks, lines and local traps. Samples were washed with clean water at the point of collection, separated by species, placed in an icebox, transported to laboratory, and frozen in the refrigerator until further analysis.

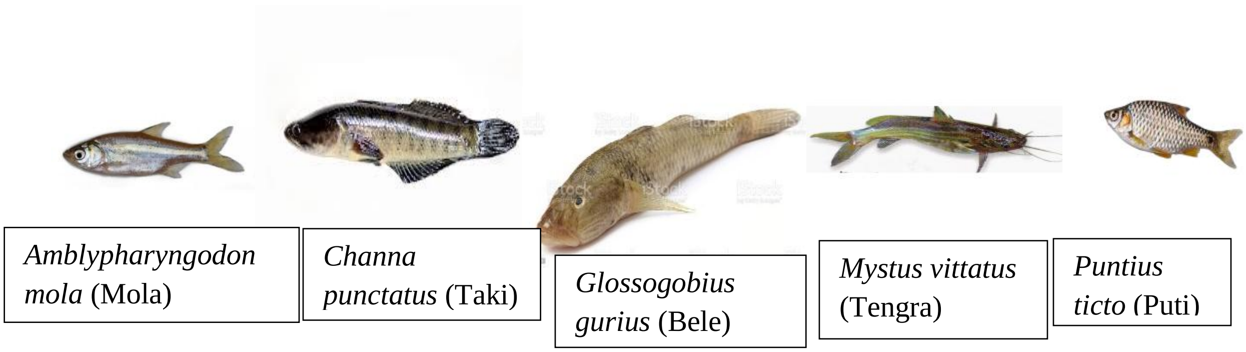


Fig. 2. Five fish species from Balu River, Bangladesh. (Italy letter: Scientific name; In parenthesis: Local name)

Water quality parameters

Color of the water was observed by naked eyes. A digital DO meter (Model: HI 8424, HANNA) was used to measure the temperature and dissolved oxygen (DO) at the river site. The pH meter was used to measure the pH levels (Model: pH 211, HANNA). Total solid (TS) was determined by difference between initial and final weight of a crucible.

It was placed into an oven and dried at 105°C temperature for 24hr. After cooling in desiccator, the crucible's initial weight was determined. Then, 50ml of water was taken in that crucible and further placed into an oven at 105°C temperature for 24hr. The final weight was taken after cooling it. Values of total dissolved solid (TDS) electrical conductivity (EC) and salinity were determined by using a conductivity meter (Model: DDSJ-308A).

Sample preparation for metal analysis

Water sample was digested with nitric acid (5mL), continued heating while adding nitric acid until digestion was completed. It was filtered through Whatman no. 42 filter paper and made up to 50ml with distilled water. Metals of the water samples were determined using flame atomic absorption spectrophotometer (Perkin Elmer Model 2380).

Fish samples (muscle) were washed, weighed and oven dried at 105°C. The weights were taken after drying the fishes until constant weights. The samples were homogenized into fine powder using a carbide mortar and pastel. The dry weight samples (0.5 g) were digested with tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4=10:4:1) at the rate of 5ml per 0.5 g sample and placed on a hot plate at 100°C **(Pyle *et al*., 2005)**. Digestion continued until liquor was clear (**AOAC, 1990**). Digested liquor was cooled and filtered with Whatman no. 42 filter paper. The samples were diluted to 50ml with distilled water and analyzed using flame atomic absorption spectrophotometer (Perkin Elmer Model 2380) as per standard conditions. Zinc, copper, cadmium, lead and chromium were estimated using an air- acetylene flame. The blanks and calibration standard solution were also analyzed in the same way as for the samples.

Bio-concentration factor

Bio-concentration factor (BCF) was used to evaluate bioaccumulation of metal elements in fish, and it can also be used for field investigation data (**USEPA, 1991**). The BCF is used to determine the ability of the aquatic organism to accumulate chemicals from the river water. If BCF>1, then fish individual has a potential to accumulate the metal but is generally not considered to be significant unless the BCF exceeds 100 or more (**USEPA, 1991**).

It was calculated as:

$$BCF = C_{fish} / C_{water}$$

Where, C_{fish} is the concentration of the metal element (wet weight) in the muscle of fish, and C_{water} is the concentration of the metal element in surrounding water.

Statistical analysis

The results of the study were presented in charts and tabular forms, which were used to present and interpret the collected data. In the present study, the two-way analysis of variance (ANOVA) was performed to measure water quality parameters and trace metals of different locations and seasons. The mean differences were conducted by Duncan's multiple range test (DMRT). All the statistical analyses were done using Microsoft Office Excel 2010 as well as the statistical package of IBM SPSS software, version 28. The Pearson correlation of different metals was calculated using the different values for water and five fish samples at a significant level of $P< 0.05$.

RESULTS AND DISCUSSION

1. Physicochemical characteristics of river water

The values of physicochemical parameters measured in the Balu river water at different sites are given in Tables (1, 2). The study showed that water properties of Balu River were significantly varied between wet season and dry season. The mean water temperature of three sampling stations was recorded as 27.6ºC and 26.1ºC in wet and dry seasons, respectively. There was no significant difference in temperature among the three stations, with a below standard limit of WHO. Three colors of water were observed at the sampling sites (Slightly black, black and slightly grey). It appeared that neither domestic use nor aquatic life could thrive in the water. The lowest mean value of dissolved oxygen was found at sampling station 1, and there was a substantial difference between the two seasons (dry season: 1.24-1.60 mg/l, rainy season: 2.10-3.20 mg/l). The optimum level of the DO for aquatic organisms is 4-6 mg/l and the most of organisms cannot survive in low DO in aquatic environment (**Dara, 2006**). The observed DO values were lethal for aquatic life and created severe negative impact on aquatic life in the river. The DO condition of this river coincides with that recorded in the study of **Hasan *et al.* (2014)**. In addition, the DO condition of the sampling area was unsuitable for drinking, domestic, irrigation and industrial purposes. The mean pH was 7.45 and 6.51 in wet and dry seasons, respectively.

Table 1. Physical parameters of water at different locations of the Balu River in wet and dry seasons

	Wet season				Dry season				Standard value
Parameter	**Station 1**	**Station 2**	**Station 3**	**Mean±SD**	**Station 1**	**Station 2**	**Station 3**	**Mean± SD**	**DoE (2001)**
Color	Slightly Black	Transparent	Transparent	-	Black	Light grey	Slightly Black	-	-
Odor	Slightly pungent	Odorless	Odorless	-	Highly pungent	Pungent	Pungent	-	Odorless
Temperature (ºC)	27.5	27.2	28	27.57 ± 0.40^{a}	26.2	26	26	26.07 ± 0.12^{a}	20-30
TS (mg/l)	163.2	115.2	94.9	124.43 ± 35.07^{b}	400.3	349.1	305.6	351.67 ± 47.40^{a}	-
TDS (mg/l)	71	59.2	54.4	61.53 ± 8.54^{b}	267	243	237	249.0 ± 15.87^{a}	1000

Means in a raw with different superscripts are significantly different (*P*<0.05).

Table 2. Chemical parameters of water from different locations of the Balu River in wet and dry seasons

Parameters	**Wet season**				**Dry season**				**Standard value**
	Station 1	Station 2	Station 3	**Mean±SD**	Station 1	Station 2	Station 3	**Mean±SD**	**DoE (2001)**
DO (Dissolved Oxygen)	2.1	3	3.2	2.77 ± 0.48^{a}	1.24	1.33	1.60	1.39 ± 0.15^{b}	>5 mg/L
pH	7.31	7.36	7.45	$7.37\pm.07^{a}$	6.45	6.41	6.51	6.51 ± 0.05^{b}	6.5-8.5
EC (Electrical Conductivity)	126.7	116.52	107.4	116.87 ± 9.6^{b}	487	454.1	443.7	461.6 ± 22.6^{a}	800
Salinity	0.01	0.01	0.01	0.01^{a}	0.2	0.2	0.2	0.02^{a}	<.05ppm

Means in a raw with different superscripts are significantly different (*P*<0.05).

The TDS value of water was near four to five times lower during the wet season (54.4 - 71 mg/l) compared to the dry season (237 - 267 mg/l). Water with a high TDS value is unfavorable because it rises the water's pH, affects the osmoregulation of aquatic species and decreases the solubility of gases (like oxygen). The mean TS value increased from 305.6 in the rainy season to 400.3 in the dry season, but it remained within the acceptable range in the rainy and dry seasons, respectively, the EC values of water were reported as 107.4-126.7 S/cm and 443.7-487 S/cm. The high EC value during dry season might impose unpleasant impact on the existence, sustainability, growth and development of aquatic life.

2. Metals in water samples

The metal concentrations in water samples are presented in Table (3). The average concentrations of studied metals in the river water were decreasing in the sequence of Zn > Cu > Cr > Pb > Cd. Zinc concentration ranged from 0.077-0.0581(mg/l) in wet season and from 0.735-0.963 (mg/l) in dry season. The highest concentration (0.971 mg/l) of Zn was observed at station 1 (Ichapura) in the dry season, which was below the recommended limit of toxicity reference values (TRVs) and WHO. The average value of Zn was higher than that of Cu, Cr, Cd and Pb. Among the three sampling sites, station 1 showed the highest concentration of all metals in both seasons. The presence of high concentrations of Cr and Cd in water may cause health hazards to all populations of an ecosystem.

Table 3. Metal concentrations in water sample (mg/l) during dry and wet seasons

Metal	Wet season				Dry season				Standard value WHO (1993)	TRVs
	Station 1	Station 2	Station 3	Mean± SD	Station 1	Station 2	Station 3	Mean± SD		
Copper (Cu)	0.027	0.018	0.011	0.0186 [b] ±0.008	0.101*	0.035	0.032	0.056 [a] ±0.039	0.1	0.009
Lead (Pb)	0.013	0.009	0.005	0.0051 [b] ±0.004	0.11*	0.03*	0.021*	0.054 [a] ±0.05	0.02	0.0025
Cadmium (Cd)	0.0005	0.0003	0.0003	0.0003 [b] ±0.0001	0.0021	0.0014	0.0011	0.002 [a] ±0.0005	0.003	0.0022
Zinc (Zn)	0.077	0.0593	0.0581	0.065 [b] ±0.01	0.963	0.832	0.735	0.84 [a] ±0.11	3.0	0.118
Chromium (Cr)	0.002	0.001	0.001	0.0013 [b] ±0.0006	0.051*	0.041	0.038	0.0433 [a] ±0.007	0.05	0.011

*Indicates values exceeding permissible limits of WHO (World Health Organization) in water. TRVs: Toxicity reference values for fresh water proposed by **USEPA (1999)**. Means in a raw with different superscripts were significantly different (*P*<0.05).

3. Metals in fish samples

Due to their toxicity and accumulative behavior, metal discharge into rivers or any other aquatic environment can alter both aquatic species' diversity and ecosystems (**Heath, 1987**). Fish can accumulate metals in their tissue, either from their diet or through their gills (**Dallinger *et al.*, 1987**), and the metal accumulation in fish organs provided evidence of exposure to contaminated aquatic environment (**Qadir & Malik, 2011**). Fish are often at the top of the aquatic food chain and may contain concentrated, large amounts of metals, such as Pb, Zn, Fe, Cu, Cd, Cr and Hg. Many fishes show respiratory distress with metal toxicity (**Samad *et al*., 2015**). The concentration of metals in fish muscles (Fig. 3), which are edible components for Bangladeshi consumers, was assessed in the current study. The ranking order of mean concentrations of trace metals in fish was as follows: Zn (1.38)> Cu (0.47) and Pb (0.47) > Cr (0.46) > Cd (0.10) (mg/kg w/w), respectively. The concentrations of trace elements were considerably varied in fish species *A. mola* (1.03) > *G. giuris* (0.78) > *C. punctatus* (0.76) >*M. vittatus* (0.65) > *P. ticto* (0.64) and seasons.

Copper (Cu) is a biogenic element that supports all organisms' metabolic functions and is crucial for the creation of hemoglobin (**Sivaperumal *et al*., 2007**). Cu can, however, have a negative impact on health when consumed in excess (**Gorell *et al*., 1997**). Cu was identified in all fish samples in the current investigation, with concentrations ranging from 0.3 to 0.67mg/ kg, with *G. giuris* having the highest concentration during the dry season and *P. ticto* having the lowest during the rainy season (Fig. 4). In a heavily polluted river in Bangladesh, Buriganga River, **Ahmed *et al.* (2010)** found the highest Cu concentration in *C. punctatus* (5.27 mg/kg) and the lowest in *Gudusia chapra* (4.25 mg/kg) in pre-monsoon fish samples. The present findings in Balu River fish are lower than the standard values and in agreement with the values recorded in other previous studies in Bangladesh (Table 4).

Lead (Pb) is a hazardous metal that has been linked to cancer in both humans and aquatic life (**Qadir & Malik, 2011**). It has a critical effect on the developing nervous system of children who are particularly sensitive to the Pb (**Castro-Gonzalez *et al*., 2008**). From this study, the highest Pb concentration was in *A. mola* (0.75 mg/kg) during dry season and the lowest value in *G. giuris* during wet season (Fig. 4). In this study, the Pb value of all fish samples was from 1.6 to 2.5 times higher than the standard limit (0.3 mg/kg) in the dry season. This indicates high concentration of lead in water and lead accumulation in fish through bioaccumulation. However, the high Pb concentrations (1.4 mg/kg) were detected in the fishes of Buriganga River, Bangladesh in the study of **Islam *et al*. (2015)** (Table 4), with a value of 12.32mg/ kg in pre-monsoon samples of *Mystus vittatus*. Furthermore, fishes of the Upper Lake, Bhopal, India (**Malik *et al*., 2010**) and River Chenab, Pakistan **(Qadir and Malik, 2011)** recorded the same Pb value previously reported.

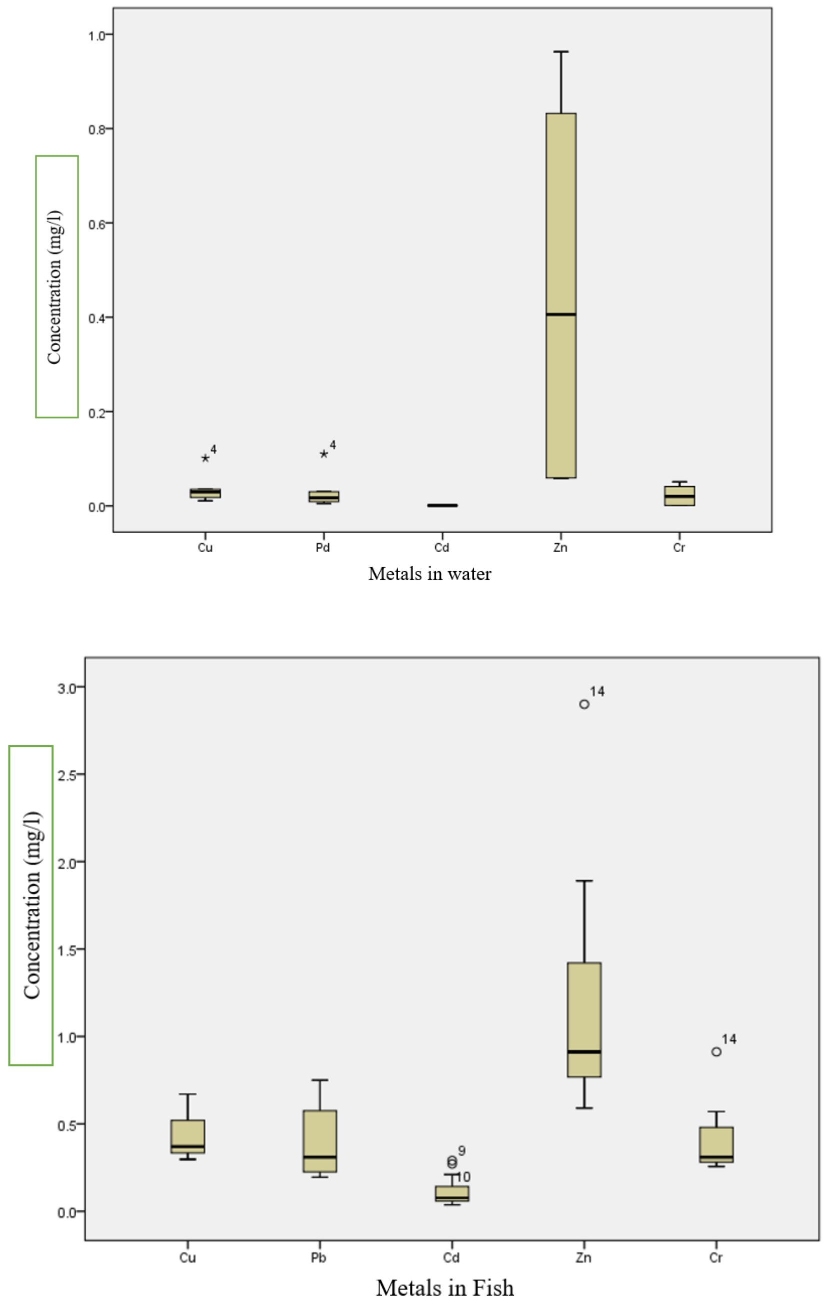
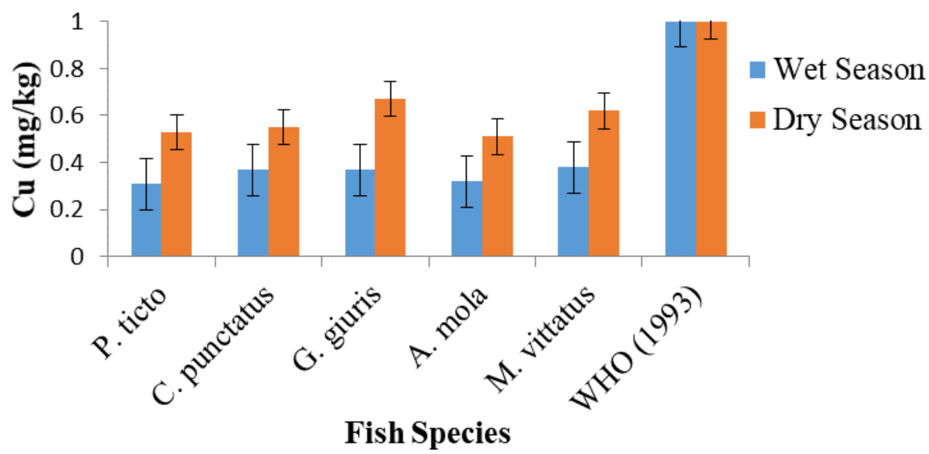
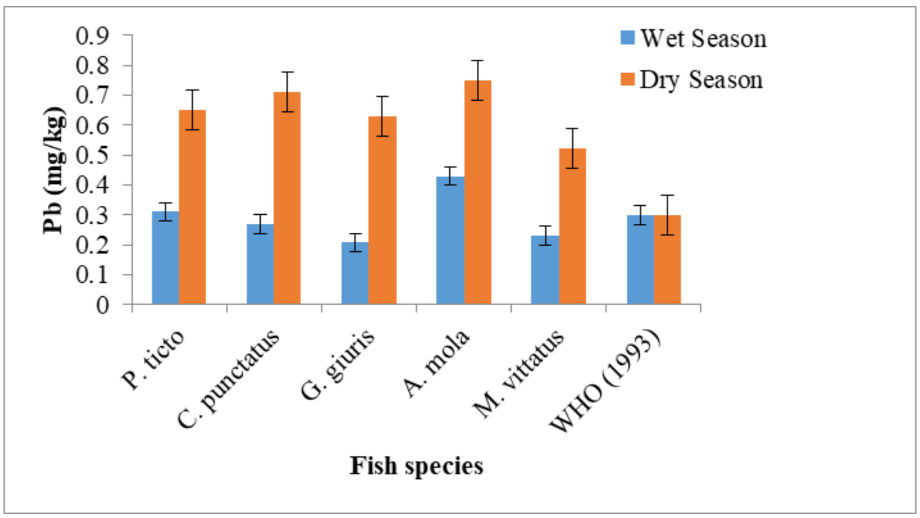
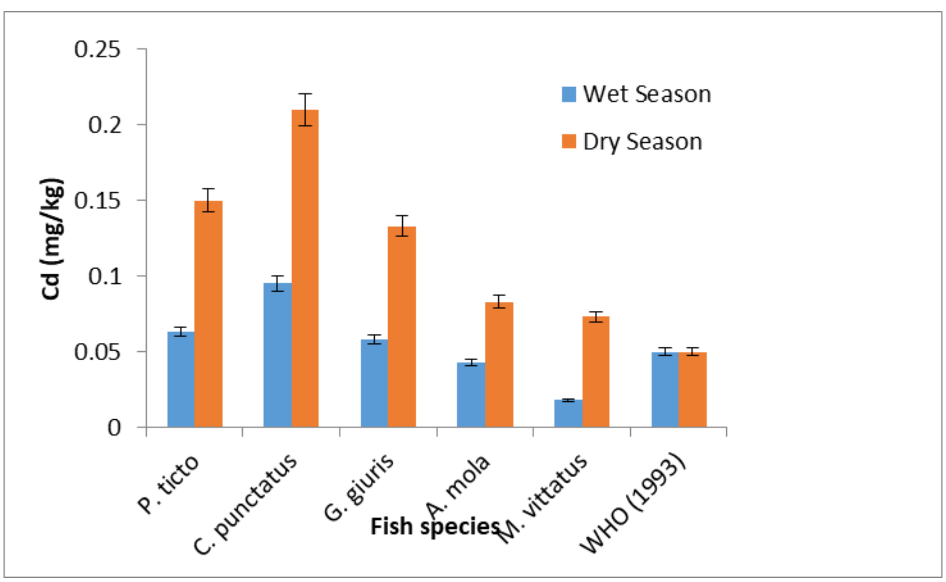
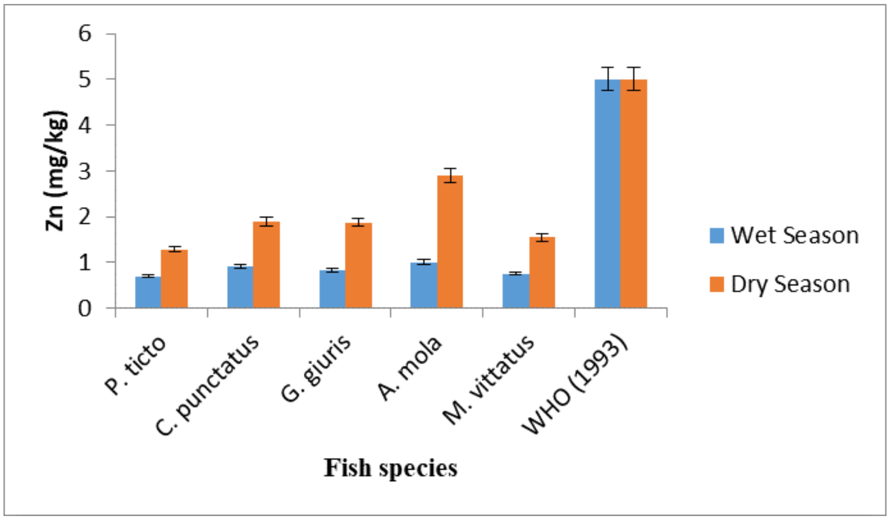


Fig. 3. Boxplot showing the concentration of metals in water and fish species from the Balu River


Cu (mg/kg)
1
0.8
0.6
0.4
0.2
0
P. ticto
C. punctatus
G. giuris
A. mola
M. vittatus
WHO (1993)
Fish Species
Wet Season
Dry Season


Pb (mg/kg)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
P. ticto
C. punctatus
G. giuris
A. mola
M. vittatus
WHO (1993)
Fish species
Wet Season
Dry Season


Cd (mg/kg)
0.25
0.2
0.15
0.1
0.05
0
P. ticto
C. punctatus
G. giuris
A. mola
M. vittatus
WHO (1993)
Fish species
Wet Season
Dry Season



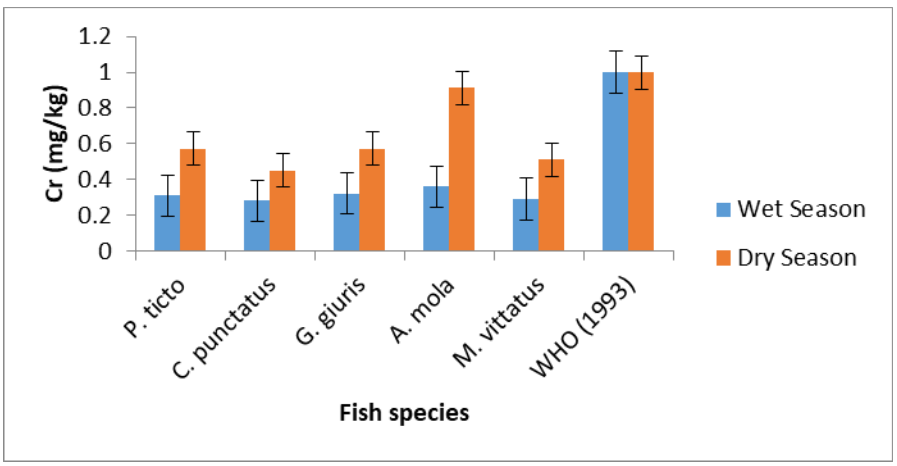


Fig. 4. Comparison among concentrations of metals with standard value in the examined fish species during both seasons (wet & dry)

Natural water rarely contains cadmium (Cd) (**Reash *et al*., 2006**), while 0.50mg/ kg of cadmium in food is safe for intake by humans (**Sanstead, 1976**). It is hazardous to reproduction, hepatic, hematological and immunological systems, as well as kidney, lung, bones, placenta, brain and central nervous system (**Castro-Gonzalez *et al*., 2008)** as well as reproductive toxicity, hepatic, hematological and immunological effects (**ATSDR, 2008**). In the present study, Cd concentration was the lowest among the metals in the examined fish species. The sequence of bioaccumulation of Cd in the fish samples of this study was as follows: *C. punctatus* > *P. ticto* > *G. giuris* > *A. mola* > *M. vittatus* in wet season (Fig. 4). Here, the lowest Cd value was found in *M. vittatus* (0.073 mg/kg) and the highest in *C. punctatus* (0.21 mg/kg) in the dry season. These variations in concentration are mainly due to feeding habits of fish (e. g. *C. punctutus* is bottom feeder), the chemical form of freshwater environment and season of the year as well as detoxification process. The average value of Cd was 2.4 times higher in the wet season than dry season, which might be due to the differences in water capacity of the river where low water flow in dry and industrial activity, atmospheric emission, leaches from defused Ni-Cd batteries and Cd plated items [**Islam *et al*., 2014**]. In this study, the Cd value in all fish samples exceeded the standard level in dry season and slightly higher than Bangshi, Turag, Buriganga Rivers in Bangladesh (Table 4). The high Cd concentration was recorded in fishes of Lake Qarun, Egypt in the study of **Awasthi (2000**), fishes of the River Chenab, Pakistan by **Vildana *et al.* (2007)**, salmonid fishes of Una River basin, Bosnia and Herzegovina by **Vildana *et al.* (2007)**, fishes of the Buriganga River, Bangladesh by **Anonymous (2009**) and in fishes of *Labeo rohita* and *Ctenopharyngodon idella* of Upper Lake, Bhupal, India, as reported in the study of **Malik *et al.* (2010**),

Table 4. Comparison of metals in fish (mg/kg wet weight) with different international guidelines and other studies in the world

Study area	Cu	Pb	Cd	Zn	Cr	Reference
Fish						
Wet season	0.350 (0.31-0.38)	0.290 (0.21-.43)	0.055 (0.018-0.095)	0.844 (0.71-1.01)	0.312 (0.29-0.36)	This study
Dry season	0.576 (0.51-0.67)	0.652 (0.52-0.75)*	0.130 (0.073-0.21)*	1.902 (1.29-2.9)	0.603 (0.45-0.912)	This study
Bangshi river [a,b] (Bangladesh)	8.33– 43.18, 1.7-9.0	1.76– 10.27, 0.37-2.1	0.09– 0.87, 0.019-0.18	42.83– 418.05,	0.47– 2.07, 0.098-0.43	(**Rahman *et al*., 2012**)
Dhanmondi lake µg/g (Bangladesh)[a]	5.07	2.08	-	60.1	-	(**Begum *et al*., 2005**)
Gumti river (Bangladesh)[a]	1.48– 21.30	0.5–4.05	-	3.14– 186.9	-	[**Amin *et al*., 2011**]
Turag river (Bangladesh) [b,c]	2.9 (1.1-5.7), 0.30-0.74	0.84 (0.052-1.6), 0.01-0.13	0.018 (0.008-0.03), 0.001-0.02	-	2.2 (0.97-3.6), 0.17-0.48	(**Islam *et al*., 2015; Afrin *et al*., 2015**)
Buriganga River [b] (Bangladesh)	4.5 (2.3-7.2)	1.4 (0.78-2.7)	0.05 (0.022-0.13)		2.8 (1.0-4.8)	(**Islam *et al*., 2015**)
Shitalakha River [b] (Bangladesh)	3.8 (2.3-6.0)	0.83 (0.13-1.7)	0.036 (0.011-0.086)		2.1 (0.75-4.0)	(**Islam *et al*., 2015**)
Hooghly River (India) [a]	16.22– 47.97	12.40– 19.96	0.62– 1.20	12.13– 44.74	3.89	[**De *et al*., 2010**]
Okumeshi River [a] (Nigeria)	-	<0.01, 0.45	0.62, 1.32	-	0.06, 0.87	[**Raphael *et al*., 2011; Ali *et al*., 2016**]
Pearl River (China)[a]	1.17-6.72	0.05-1.94	Nd-33.2	2.62-20.2	Nd-5.36	[**Xie *et al*., 2010**]
WHO	1.0	0.3	0.05	5.0	1.0	[**WHO, 1993**]

*indicates values exceeding permissible limits of WHO (World Health Organization) in fish.
a Values present the ranges or mean expressed as mg/kg dry wt.
b Values present the ranges or mean expressed as mg/kg wet wt (mg/kg dry wt)
c not mansion weather dry or wet wt

In addition, zinc (Zn) is the essential mineral for both animal and human. It showed a protective effect against the Cd and the Pb toxicity (**Sanstead, 1976**). The order of accumulation of Zn in the fish samples was *A. mola* > *C. punctutus* > *G. giuris* > *M. vittatus* > *P. ticto* in both seasons (Fig. 4). Zn levels in the fishes of the Balu River fluctuated from 1.29 to 2.9mg/ kg in dry season which was 3.8 to 1.7 times lower than the standard values given by WHO (Table 4). Similar result was found in the fish samples from Campaign Creek stream, USA (**Reash *et al.*, 2006**) and northern Jordan valley, Jordan (**Al-Weher, 2008**). Relatively lower Zn accumulation was found in *Labeo rohita* from Upper Lake, Bhupal, India (**Malik *et al.*, 2010**).

In this study, *C. punctutus* had the lowest chromium value (0.28 mg/kg) during the wet season, while *A. mola* had the highest value (0.912 mg/kg) during the dry season (Fig. 4). In the present study, the Cr value in all fish samples was significantly higher during dry season, compared to the wet season; however, it was below the standard level as well as below values recorded other studies for several rivers in Bangladesh (Table 4). During the dry season, the Cr concentration in *A. mola* was much closed to the standard value. Marked Cr concentration was detected in the tissues of fishes dwelling in the Balu River in the dry season, which might be due to the impacts of untreated wastewater discharged from various industries such as dyeing and tanneries (**Islam *et al.*, 2014**).

The concentration of metals was slightly higher in the examined fish species during dry the season than wet season. Pb and Cd, in particular, exceeded the standard level which could be due to effects of point and non-point sources; such as leaded gasoline, petroleum, municipal runoffs, atmospheric deposition (**Mohiuddin *et al.*, 2012**), etc. In comparison with the concentration of metals in fish and some other studies from Bangladesh and other countries near the industrial area indicated that fishes were contaminated by trace metals (Table 4). The enrichment of metals in fish species could be due to the metal contaminated feed from effluents discharged into rivers from several industries and other sources in the urban area.

4. **Bio-concentration factor (BCF)**

The calculated BCF values of the five metals for different fish species are listed in Table (5). The BCF values of Cd and Cr were higher than 100 for all fish species, except Cd in *M. vittatus* in wet seasons. The BCF value of Cd was greater than other studied metals during dry season and was more than 100 in two fish species, *P. ticto* and *C. punctatus*; this can be due to the higher capacity to enrich metals from the water environment. Moreover, these two species are increasing metals from the water column through ingestion. The BCF values are not consistent among different fish species and seasons for fishes are more widely distributed and may migrate between river areas in response to several environmental conditions of the river **(Abal *et al.*, 2005**). In addition, the variation of BCF values for different fish species may be due to the five fish species having different behaviors and responses to different trace metals (**Tao *et al.*, 2012**).

Pearson’s correlation matrix between each analyzed trace metal, listing the Pearson product moment correlation coefficients was calculated to find if some of the elements interrelated with each other (Table 6). The concentrations of the investigated metals in water were significantly high correlated with each other; Cu and Pb ($r = 0.989$), Cu and Cd ($r = 0.977$), Pb and Cd ($r=0.992$), Cu and Cr ($r = 0.993$), Pb and Cr ($r = 0.989$), Cd and Cr ($r = 0.992$) were correlated at $P< 0.01$ level, except for Zn (Table 6). High correlations between specific metals in water may indicate same levels of contamination or release from the same sources of pollution, mutual dependence and identical behavior during their transport in the river system (**Ali *et al.*, 2016).**

Significant positive correlations in fish muscle were found between all trace metals except Cd, where Pb-Cu, Zn –Cu, Zn –Pb, Cr-Cu, Cr- Pb were moderately and Cr-Zn was highly significant correlated at P< 0.01 level. These correlations might indicate that the distributions of these pairs of metals were regulated by common local inputs and similar dispersion processes in the study area.

Table 5. BCF values of different fish species collected from the Balu River in wet and dry seasons

	Fish species	Cu	Pb	Cd	Zn	Cr
Wet season	*P. ticto*	16.67 ^{d}	60.78 ^{b}	170.27 ^{b}	10.85 ^{d}	239.46 ^{b}
	C. punctatus	19.89^{b}	52.94 ^{c}	256.76 ^{a}	14.05 ^{b}	215.38 ^{c}
	G. giuris	19.89 ^{b}	40.59 ^{d}	156.76 ^{b}	12.77 ^{c}	246.15 ^{b}
	A.mola	17.20 ^{c}	84.31 ^{a}	116.22 ^{c}	15.54 ^{a}	276.92 ^{a}
	M. vittatus	20.43 ^{a}	45.29 ^{d}	48.65 ^{d}	11.69 ^{d}	223.08 ^{c}
Dry season	*P. ticto*	9.46 ^{b}	12.04 ^{b}	100 ^{b}	1.54 ^{c}	13.19 ^{b}
	C. punctatus	9.82 ^{b}	13.15 ^{a}	140 ^{a}	2.25 ^{b}	10.39 ^{c}
	G. giuris	11.96 ^{a}	11.67 ^{b}	88.67 ^{c}	2.24 ^{b}	13.16 ^{b}
	A.mola	9.11 ^{b}	13.89 ^{a}	55.33 ^{d}	3.45 ^{a}	21.06 ^{a}
	M. vittatus	11.07 ^{a}	9.65 ^{c}	48.67 ^{d}	1.85 ^{c}	11.78 ^{c}

Means in a column with different superscripts are significantly different (P<0.05).

Table 6. Correlation matrix of different metals in water and fish species collected from the Balu River

	Cu	Pb	Cd	Zn	Cr
Water					
Cu	1				
Pb	**0.989***	1			
Cd	**0.977***	**0.992***	1		
Zn	0.448	0.574	0.566	1	
Cr	**0.993***	**0.989***	**0.992***	0.466	1
Fish					
Cu	1				
Pb	**0.783***	1			
Cd	0.222	0.065	1		
Zn	**0.739***	**0.887***	0.010	1	
Cr	**0.698***	**0.869***	-0.019	**0.937***	1

*. Correlation is significant at the 0.05 level.

However, the influence of the studied seasons and the different fish species captured from the Balu River, Bangladesh is demonstrated in Table (7). The metal concentrations in the edible fish muscle of each fish species for two seasons were subjected to two-way variance analysis ANOVA (Table 7). The data obtained from ANOVA clearly demonstrated that there was significant variation (CI = 95%) of the metal concentrations in the two seasons in the Balu River. However, the variation was not significant in five species of fish available in Balu River, Bangladesh. The variation in the level of metals between different fish species depends on its feeding habit, age, size, and length of fish and their habitats (**Amundsen *et al*., 1997; Wantanabe *et al*., 2003**).

Table 7. Two-way ANOVA applied to detect the effect of inter-season and inter-fish species on the variability of metal concentration in edible fish fillet

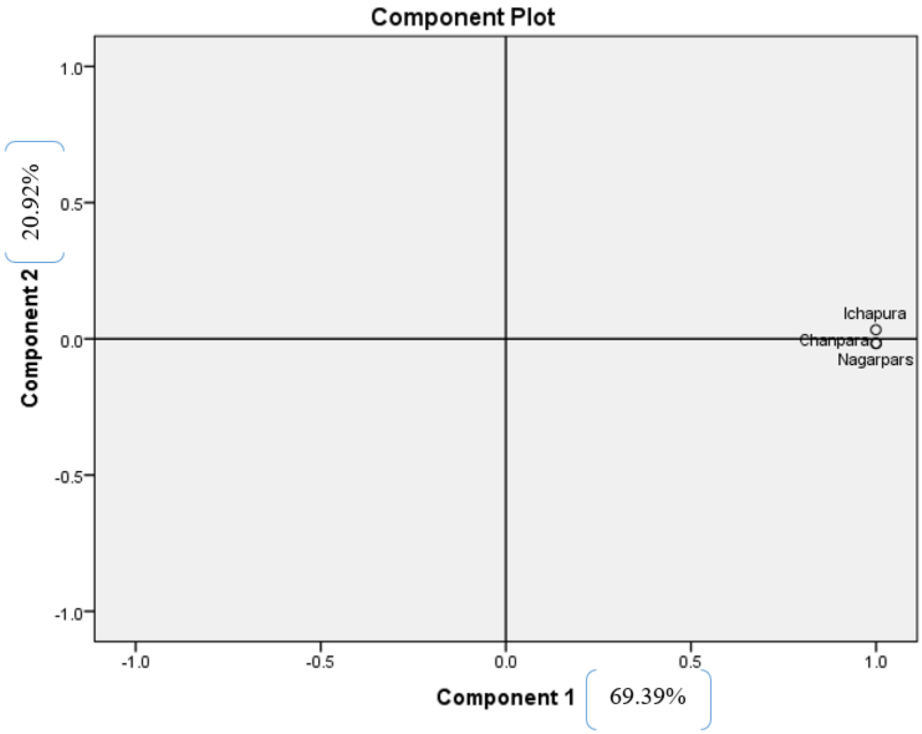
Effect	**Cu**			**Pb**			**Cd**			**Zn**			**Cr**		
	df	F	*P*(2-tails)	df	F	*P* (2-tails)	df	F	*P*	df	F	*P* (2-tails)	df	F	*P* (2-tails)
Season[a]	1	1027.6*	<.001	1	300.68*	<.001	1	26.62*	.004	1	698.05*	<.001	1	353.56*	<.001
Fish species[b]	4	30.99*	.001	4	8.57*	.018	4	67.02*	<.001	4	60.87*	<.001	4	29.26*	.001
Fish*season	4	9.98*	.013	4	1.7	.285	4	143.71*	<.001	4	27.55*	.001	4	19.93*	.003
Error	5						5						5		
Total	15						1 5						15		

F critical value for Fishes = 5.19 (α = 0.05).
F critical value for Seasons = 6.61 (α = 0.05).
a Seasons: Wet season and Dry season.
b Fish Species: *P. ticto*, *A. mola*, *C. punctatus*, *G. giuris*, *M. vittatus*
* Significant at 95% confidence level

5. Metals distribution patterns by principal components analysis

In Fig. (5), the score plot of the PCA shows a cluster of metals distribution patterns for water in components 1 and 2, with total 90.31% variation in data set. The component 1 made 69.39% and c component 2 made up 20.92% of total variation accounted in the data set. The loading plot describes the correlation between metal distribution and components 1 and 2. Although Zn and Cr have dissimilar distribution spreads, they have positively correlated with component 1. The positive factor loads for Zn and Cr indicates that these metals come from same sources viz. anthropogenic pollution such as industrial effluents along the catchment of study area.

Fig. (6) shows the PCA of metal distribution for fish sampled. The score plot of the PCA describes a cluster distribution in components 1 and 2 with a total variation of 90.7%. Component 1 accounted for 73.93% of the variation in the dataset, while component 2 accounted for 16.77% of total variation in dataset. The loading plot shows that Cu, Pd and Zn, Cr were cluster together except Cd and positively correlated with component 1.



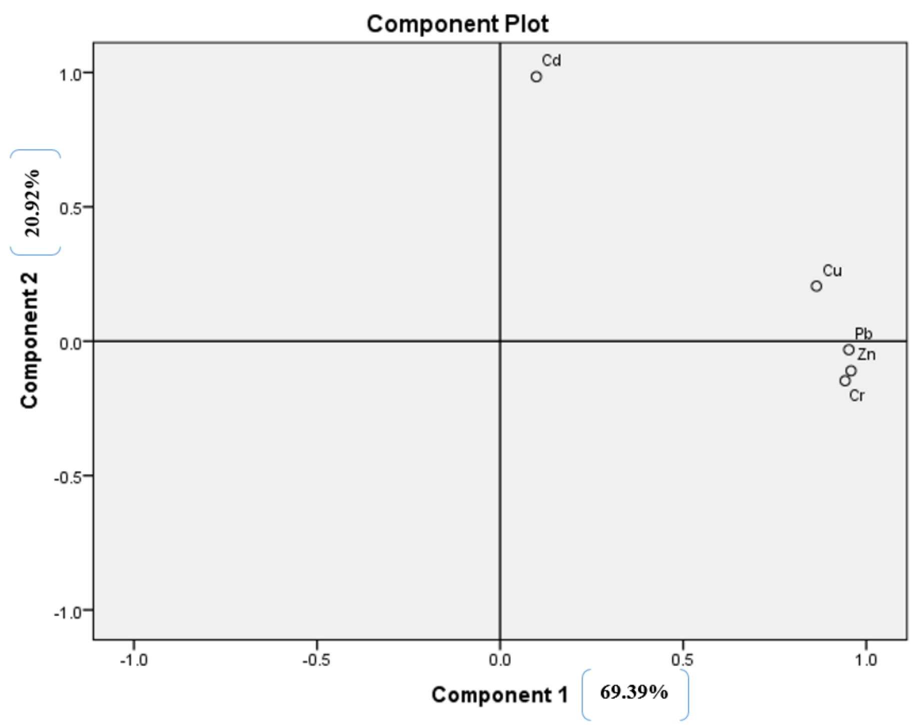


Fig. 5. Metal distribution in different locations of water surface at station 1 (Ichapura), Station 2 (Nagarpara), and Station 3 (Chanpara)

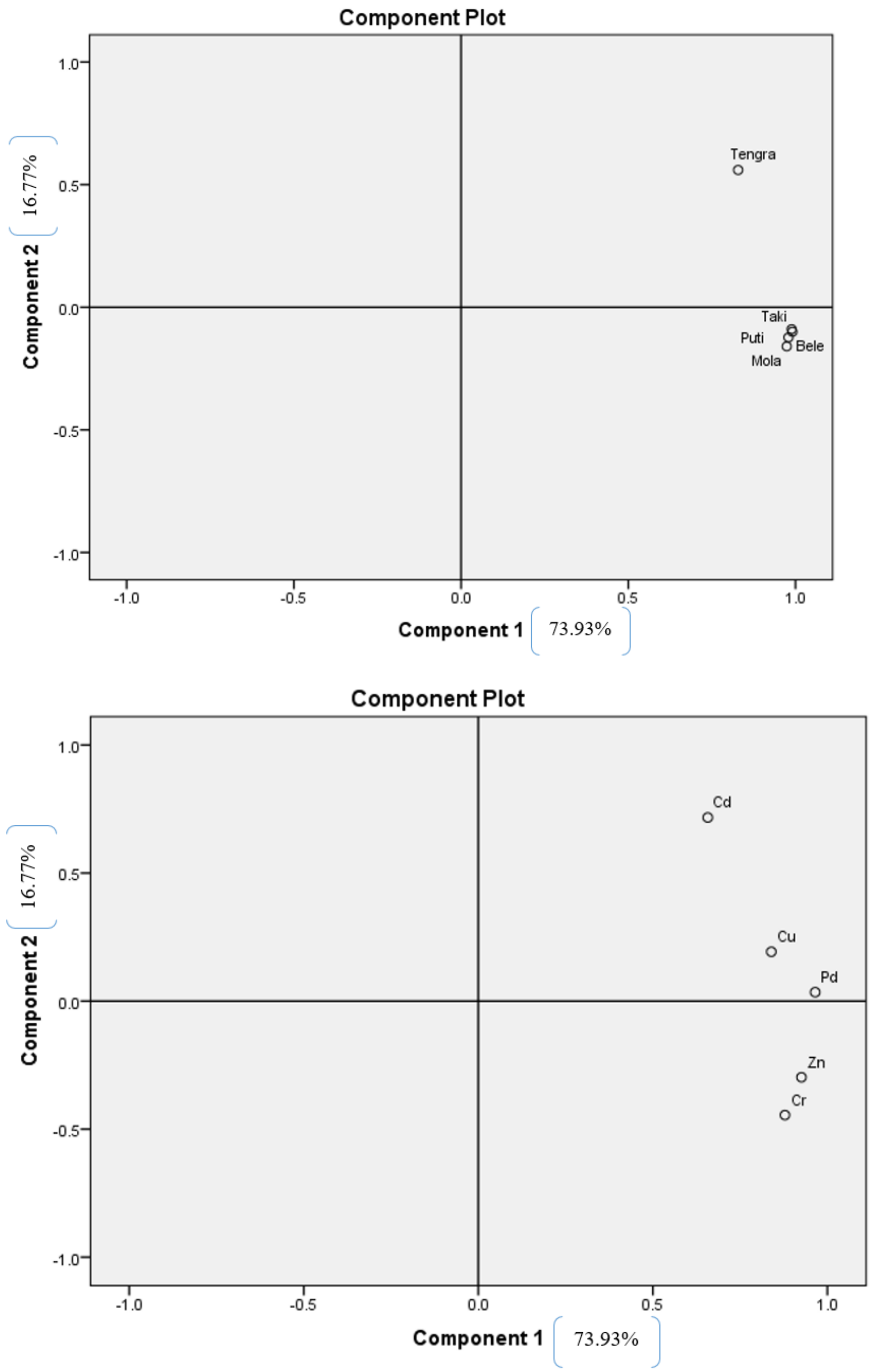


Fig. 6. Principal component analysis (PCA) showing metal relationship in five fish species

CONCLUSION

The findings of the present study show that, some of the water quality parameters of the Balu River were unfavorable for all aquatic lives due to discharging untreated industrial effluents into the river without considering aquatic lives existing in the Balu River water bodies. The DO concentration of the sampling site was very poor and found lethal condition for sustaining aquatic lives in the river. The EC, TS, and TDS concentrations were higher during the dry season than wet season. This might be due to low volume of water in the river and relatively high dissolved materials in water. The study revealed that among the three sampling sites, higher concentrations of all metals were found in the water of station 1 (Ichapura). The metal contamination varies according to the nature of the sites and the intensity of the industrial and agricultural runoff. High concentration of all metals was found in *A. mola*, *C. punctatus* and *G. giuris* among the five fish species. These variations in the metal concentration are mainly due to feeding habits, chemical form of freshwater environment, seasons of the year and detoxification process. The concentration of metals in water and fishes were significantly higher in dry season than the wet season. In addition, low discharge from the upstream during the dry season may have led to the increased concentration of metal in water and fish species.

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