

Assessment of Heavy Metals in *Johnius Dussumieri* (Cuvier, 1830) at Downstream Pasur, the Sundarbans Bangladesh

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Abstract Heavy metals are raising worldwide concerns owing to their potential effects on the environment. The present research analyzed heavy metals in the estuarine fish (*Johnius dussumieri*) sampled from the downstream Pasur estuarine Kokilmoni area of the Sundarbans, Bangladesh, during summer (March) and winter (November), 2022. Lead (Pb), Chromium (Cr) and Cadmium (Cd) were measured following a validated and accredited test method using graphite furnace atomic absorption spectrometry (GFAAS; Varian AA Duo 240 FS and Varian 280Z). Mercury (Hg) and Arsenic (As) in fish extracts were determined by cold vapor and hydride generation atomic absorption spectrometry (CVAAS) respectively. Mean concentrations (mg/kg) of Pb, Cd, As, Hg and Cr, were 2.93 ± 0.91 , 0.057 ± 0.020 , 0.144 ± 0.054 , 0.164 ± 0.150 and 1.633 ± 1.450 . ANOVA show that mean concentrations of Pb was significantly ($p < 0.001$) higher than the FAO and WHO recommended value whereas Cd, As and Hg were significantly ($p < 0.001$) lower. The Levene's test show winter fish population has significantly higher Pb and Cr concentrations than summer populations ($p < 0.05$). Pearson Correlation analysis ($p < 0.05$) quantified that Cr is strongly correlated with Pb ($r = 0.974$) and Cd ($r = 0.612$) at the 0.01 level (two tailed), Cd is strongly correlated with Pb ($r = 0.666$). Principal component analysis was executed for the five heavy metals and revealed that two principal components covering 71.86% of the cumulative variance. The PC1 variable loading appeared to strongly be influenced by Pb, Cr, and Cd (0.970, 0.951, and 0.797 respectively). The variables loading on PC2 has influenced by As and Hg. Cumulative variance of component 1 accounts 51.423%, the component 2 accounts 20.445%. Therefore it can be concluded that Pb is supposed to be higher due to anthropogenic influences such as lead-acid battery industries, agricultural pesticides, industrial and different domestic uses of metals in the upstream Pasur. Heavy metals need to be monitored regularly for the sustainable management of the pristine Sundarbans ecosystem. Further time and space bound data is needed to understand the detailed metal trajectory in the coastal fishes of the Sundarbans.

Keywords: heavy metal, *Johnius dussumieri*, assessment, the Sundarbans, Bangladesh

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1. Introduction

The natural elements occurring in the earth surface are known as heavy metal. Heavy metals are considered as the top most pollutants in the aquatic ecosystems because of their harmfulness and manifold extended effects on the biological systems [1]. Rapid economic growth, human settlements, intensification of tourism, agricultural and aquaculture activities are damaging the ecosystems by releasing toxic elements and leading the coastal as well as aquatic environmental pollution [1]. The anthropogenic developments are leading the soil, water and environmental contamination as well as pollution because of mining and operations, local use of metal-composites and industrialization, manufacturing of pesticides using

metals in agricultural sector. Coastal fish *Johnius dussumieri* is a commercially important species in Bangladesh, locally known as Kalapoa, and found in estuarine and river mouth water from 0-40m depths. It is known as Silver Jawfish or sharptooth hammer fish also [2], belonging Sciaenidae family, the fish is distributed throughout the Malaysia, Philippines, Myanmar, Sri Lanka, Indonesia, Pakistan, Bangladesh, India and Thailand [2]. It is usually carnivorous in nature and fed on the coastal small fishes and invertebrates. The Kalapoa fish is available around the year at a reasonable cost, thus the coastal community as well as countrywide people consumes the fish in fresh owing to its good flesh and taste and in dried form also. Smaller sizes of the fish as trace fish or bi-catch during trawling are used in fishmeal production. The maximum recorded length for this species is 40 cm. In Bangladesh coast this fish species spawns

from June to August of a year [2]. The fish species reaches sexually maturity around 1-2 years of age. The physiology and species diversity of the fish depends on coastal estuarine habitat and impacted by habitat degradation [2,3]. *Johnius dussumieri* has most recently been assessed by IUCN and found as threatened species [4]. However, aquatic habitat is in stressed owing to the unplanned industries and hotels because of their inadequate waste treatment which contains a noticeable amount of heavy metal, ends up in the adjacent coastal water bodies [3,4]. Through the food chain the heavy metals then taken by the aquatic dwellers and bioaccumulation and bio magnification occurs [5]. Therefore, aquatic organisms may contain sufficient amount of inorganic or toxic metal pollutants and may cause the habitat pollution and health hazards to human. Toxic metal pollution affects different characteristics of the water such as physicochemical and biological components as well as changes sediment quality. Therefore, the quality and quantity of the aquatic dwellers are negatively affected by metal pollution [5].

In the aquatic ecosystems, fish can be considered as one of the vibrant bio indicator for the assessment of heavy metal contamination [6]. Fish are usually the top predator in the aquatic food chain and most vulnerable to the lethal effects of heavy metal manifestation. Fish are directly linked with human by the food chain because human regularly consumes the most overflowing vertebrates in this planet. Thus, the degree of pollution in an aquatic ecosystem can be assessed through the assessment of fish sample [7]. One of the best way to estimate this with selecting the edible as well as commercial fish for investigating the toxicity level of the ecosystem health [7, 8]. The ability of fish to accumulate different metals in their body differ among species [9]. In this regard, the reckless urban development, tourism, agriculture farming and intensive fisheries activities are deteriorating the world's largest mangrove ecosystem, the Sundarbans potential fisheries resources [7,8,9,10]. Recently, Sundarbans coastal system in Bangladesh is facing multiple threats for the boom and bust development designs of poultry and agriculture farms, battery and shipbreaking industries at the upstream Pasur, which are releasing toxic metals into the waterways of the ecosystem such as mercury, lead and cadmium [11]. A noticeable quantities of contaminants are discharging locally in the shipyard of south western Khulna, Bangladesh, which may be a concern of ecosystem safety and environmental efficiency. This may need to resolve the problems of trace metals like lead, cadmium, chromium, mercury as well as persistent organic compounds into the Pasur downstream [11]. Thus, Mongla port activities in the upstream Rupsha and Bhairab rivers, untreated wastes, several steel welding industries, pesticide residues from agricultural farming practices adding the toxic wastes to the downstream coastal water [12,13]. Considering the above situations, the present research was carried out to assess some potential heavy metals such as Mercury (Hg), Cadmium (Cd), Chromium (Cr) Arsenic (As) and Lead (Pb), in the coastal fish (*Johnius dussumieri*) to understand the present status of the integrated Sundarbans ecosystem.

2. Materials and Method

2.1. Study Area

Owing to the geographic positioning the largest mangrove Sundarbans (88°00'–89°55'E and 21°30'–22°30' N) ecosystem is situated in the south west Bangladesh, its area range about 10,000 km² and about 30% of the area is covering many interconnected rivers, canals and creeks as shown in Figure 1. The tidal Sundarbans freshwater discharge is controlled by tropical south-west monsoon. Mongla port is very closed to the Sundarbans and considered as the 2nd largest port, located at the Pasur confluence. The port has trade link with worlds many other ports and always busy with transporting goods with cargo, ships and other water vehicles. Thus different types of developmental activity like ship breaking, industrial effluents, agriculture, aquaculture activities are ongoing around the Sundarbans [14].

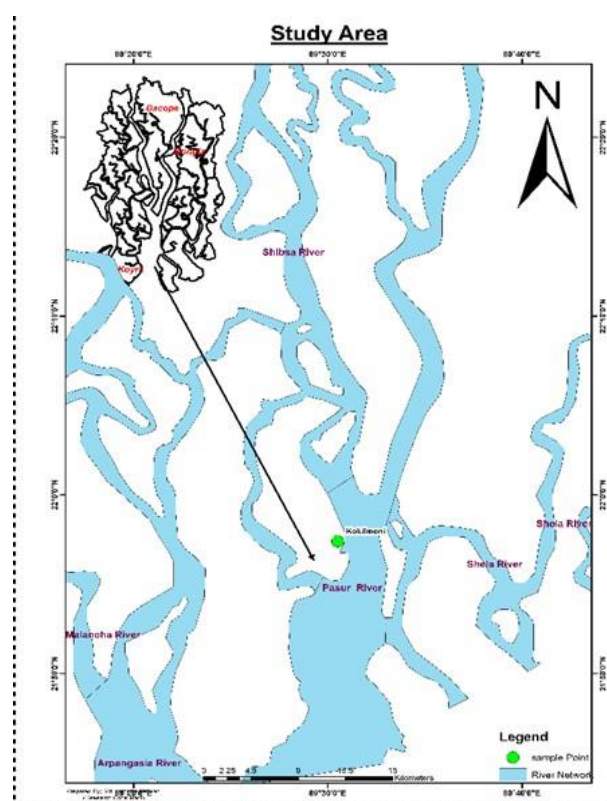


Figure 1. The location of the study area at the downstream Pasur (Kokilmoni area) of the Sundarbans

2.1.1. Sampling Station and Sampling Period

Twenty fish (*Johnius dussumieri*) samples were collected during the Summer (March; ten fishes) and Winter (November; ten fishes) seasons from the fishermen during fishing time from the downstream Pasur (Kokilmoni area) of the Sundarbans (Figure 1). Each fish sample was weighed about 300-350g (about 25cm).

2.1.2. Sampling Procedure

A Global Positioning System (GPS) was utilized to

determine the geographical location of the sampling station, in the study. Fish samples were collected from the fishermen after fishing placed into polythene bags and stored in an ice box of crushed ice with a 1:1 fish-to-ice ratio during the transportation time of around 8 hours from the sampling spot to the Atomic Energy Center Dhaka for further laboratory analysis to assess the heavy metal content in the fish sample.

2.1.3. Sample Preparation

In this research, fish were sampled from the Kokilmoni area located in downstream Pasur of the Sundarbans during above mentioned seasons. Fish samples were collected and stored in a labeled air-tight zip lock bag. After bringing them into the laboratory, the samples were immediately washed with tap water followed by washed with distilled water. At the beginning of the preparation, the fish were air dried at about 25°C room temperature. A tray washed with distilled water was used to put the sample on it. A nitric acid-washed knife was used to cut the flesh from each fish sample. The required amount of beaker, watch glass, and glass rod was taken for the sample digestion which was washed by nitric acid around 24 hours. Afterwards, deionized water was utilized to wash the sample and was oven-dried at about 70°C. Flesh from different parts of the body was collected by the knife and put into a pre-washed blender. After blending from each sample 2g sample was taken to the beaker for digestion and the rest of the blended sample was stored in a clean zip lock bag and stored in the refrigerator for further requirement.

2.1.4. Sample Digestion for Metal Analysis

By using an electric balance, two (02) grams of blended fish samples were weighed from each and put into a peracid-washed 100-mL beaker. According to the method of Ullah et al. 2017 [15], a total of 12 mL of suprapure nitric acid was added into the beaker with 2g of sample. Afterwards, the beaker was arrayed into a hot plate at 100 °C temperature and a watch glass was used to cover it. The samples were heated until no brown fumes were released. Heat was applied to evaporate the solution but without boiling. After completing the digestion, the maximal fish samples were dissolved in the acid to diminish the total volume of contents. It took around 8 hours when it reached a stage of near to dry. After that, the mixture was allowed to cool at room temperature. Then, a 125-mm membrane filter paper (Whatman® Schleicher & Schuell, Germany) was used to filter the digested acid solution. After digestion and cooling, each solution was diluted to a final volume of 20ml [16]. A sample blank, duplicate, and spike samples were prepared in the same manner as a sample. For the preservation of the digested sample. Then, up to the estimation of heavy metals, the digested samples were preserved at 4 °C in a 25-mL vial that is considered as a stock solution.

2.1.5. Sample Analysis with Atomic Absorption Spectrometry (AAS)

The chemical reagents used for this research were of Suprapure quality or analytical grade (E. Merck, Germany) and for the preparation of all solutions, double-deionized

water (Milli-Q System, Millipore) was utilised [17]. In the calibration processes, element standard solutions were used and was prepared by diluting stock solutions of 1000 mg/l of each metal element. For the determination of metals such as Lead (Pb), Chromium (Cr), and Cadmium (Cd) a graphite furnace atomic absorption spectrometry (GFAAS; Varian AA Duo 240 FS and Varian 280Z) was generally utilized. In addition, to estimate Mercury (Hg) and Arsenic (As) in extracts, cold vapor and hydride generation atomic absorption spectrometry (CVAAS) were utilized respectively in the laboratory of Atomic Energy Center, Dhaka, Bangladesh. In this procedure, replicate standards or samples, method blanks, and spike samples were utilized to monitor the instrument's performance and data quality.

2.1.6. Statistical Analysis

Dry mass of fish samples were calculated as mg/kg weight. The data were used for analysis were mean and standard deviations. One-way ANOVA was performed to observe the significant difference of population mean concentration and Levene's test was performed to test the significant difference of variances of mean concentration of heavy metals among different season's fish. Pearson correlation matrix was used to assess the relationship between heavy metal concentrations in fish. Principal component analysis was performed to observe the link between different variables and to clarify the strategic component controlling the heavy metals in *Johnius dussumieri* of the Sundarbans ecosystem, Bangladesh. The data was analyzed with the statistical package, SPSS 20.0.

3. Results and Discussion

3.1. Concentration of Heavy Metal

One sample T test shows (Table 1) that mean concentration of lead (Pb) was 2.93 ± 0.91 , ranged from 2.87 to 1.99 mg/kg and the population mean (n=20) was significantly higher ($p < 0.001$) than the FAO/WHO recommended level 0.50 [18].

Table 1. ANOVA of One sample Test ($p < 0.05$) Show the Mean Differences of Each Heavy Metal Concentrations in the Coastal Fish *Johnius dussumieri* (n=20) Population (2 Tailed)

Variables	(FAO/WHO recommended value)	df	P	mean differ	lower	upper
Pb	0.5	19	0.001	2.43	1.99	2.87
Cd	1.0	19	0.001	-0.04	-0.052	-0.03
Cr	1.0	19	0.081	0.63	-0.09	1.35
As	1.0	19	0.001	-0.86	-0.88	-0.83
Hg	0.5	19	0.001	-0.34	-0.42	-0.25

Concentrations are in mg/kg

Similarly the mean concentrations of Cd, As and Hg were 0.057 ± 0.020 , 0.144 ± 0.054 and 0.164 ± 0.150 respectively. Mean concentrations of Cd, As and Hg were significantly ($p < 0.001$) lower than the FAO recommended value and ranged between 0.033 to 0.052; 0.829 to 0.883; and 0.252 to 0.419 respectively. ANOVA revealed insignificant value for the Cr concentration. In

the fish or in a living cell heavy metals such as Pb, Cd and Cr have unknown role and become toxic at a low concentration, these elements are usually not available in the living areas of the nature and thus, are introduced into the environment through anthropogenic activities [19-20].

In the study, significantly higher concentration of the Pb indicates the anthropogenic interference into the ecosystem through the agricultural intensification and unplanned mushrooming of industries usually releases the waste into the coastal water at upstream Pasur. The waste with heavy metal then mix up with downstream water owing to the tidal effect and finally affects the ecosystem health [21]. These elements then become a source of pollution in the adjacent environment such as land and water ecosystem, accumulated in the environmental substrates like soil, sediments, water and biota [22].

The Levene's test (Table 2) show the summer and winter seasons (n=10) heavy metal concentrations. Pb and Cr concentration were ranged from 1.59 to 1.99 and 2.95 to 3.04 respectively (Table 2) and winter fish population has significantly higher Pb and Cr concentrations than summer populations ($p < 0.05$).

Table 2. Levene's Test for Heavy Metal Concentration of the Coastal Fish (*Johnius Dussumieri*) Population, During Summer And Winter Season ($P < 0.05$, Two Tailed). Showing equal variance assumed for Pb and Cr, equal variance not assumed for Cd.

Variables	Levene's Test			t-Test for Equality of Means				
	F	Sig.	Df	P	Mean.differ	StdE.differ	Lower	Upper
Pb	2.21	0.15	18	0.001	-1.79	0.09	-1.99	-1.59
Cr	1.95	0.18	18	0.001	-2.99	0.02	-3.04	-2.95
Cd	7.65	0.01	18	0.005	-0.03	0.01	-0.04	-0.01
			12.53	0.007	-0.03	0.01	-0.04	-0.01

The results of this research are somehow coincides with the results documented by reference [23] in coastal fishes of Gulf of Cumbay, and reference [24] for Pb and Cd concentrations in coastal fishes of Baluchistan coast, Pakistan. Table 2 also revealed that Cd concentration was ranged from 0.009 to 0.041 and winter population mean Cd concentration was significantly higher ($p = 0.001$) than summer population [23,24]. The Levene's test revealed that there is no seasonal effect of As and Hg on the fish populations ($p < 0.05$). Fish tend to be less active in the cold their metabolism dips when temperatures take drops [25]. This reduced metabolic rate can lead to a decrease in the rate of elimination of contaminants from their bodies. As a result, heavy metals that are present in the water may be taken up and stored in fish tissues more readily during the winter, leading to higher contamination levels. Food consumption of fish drops with a decrease in temperature as its digestive coordination becomes lethargic [26]. Decreased activity levels and lower food availability can lead to a lower dilution of contaminants in their bodies, resulting in higher concentrations of heavy metals. Thus higher winter value of Pb and Cd can be explained in the way that the species has the higher intake capability during this time [25,26], though the As and Hg intake was equal in two season but this may need more data to have a clear clarification. Lead, mercury arsenic cadmium, and chromium are considered among the important metals owing to their degree of toxicity to the ecosystem [26].

3.2. Correlation Analysis

The Pearson correlation coefficient matrix ($p < 0.05$) of heavy metals in the fish (*Johnius dussumieri*) of the downstream Pasur (Kokilmuni area) of the Sundarbans ecosystem are presented in the Table 3. The analysis quantified that Cr is strongly correlated with Pb and ($r = 0.974$) and Cd ($r = 0.612$) at the 0.01 level (two tailed), Cd is strongly correlated with Pb ($r = 0.666$).

The significant positive relations between fish heavy metals revealed the proximities of the comparable source of heavy metal inputs could be anthropogenic or manmade and natural earth system [27]. It is noteworthy that in this research the geochemical informations are not provided which will be the future focus for the Sundarbans ecosystem.

Table 3. Pearson Correlation Coefficient Matrix Among Metals in the Fishes (*Johnius dussumieri*) of Downstream Pasur (Kokilmuni area), the Sundarbans Ecosystem

	Pb	Cd	Cr	As	Hg
Pb	1				
Cd	0.666**	1			
Cr	0.974**	0.612**	1		
As	-0.004	0.000	-0.010	1	
Hg	-0.224	-0.080	-0.0200	0.040	1

3.3. Principal Component Analysis (PCA)

The PCA was executed for the five heavy metals and revealed that two principal components covering 71.86% of the cumulative variance (Table 4). The two principal components variable loading appeared to be strongly influenced by Pb, Cr, and Cd (0.970, 0.951, 0.797 respectively), with a negative loading of As and Hg showed on the PC1.

Table 4. Component Matrix for Two Principal Component Factors (PCFs)

Variables	PCF1	PCF2	Communalities
Pb	0.970	0.033	0.942
Cd	0.797	0.124	0.650
Cr	0.951	0.034	0.906
As	-0.016	0.872	0.760
Hg	-0.300	0.494	0.334
Eigenvalue	2.571	1.022	-
% of variance	51.423	20.443	-
% of Cumulative variance	51.423	71.866	-

The variables loading on PC2 (Figure 2) has strongly influenced by As and Hg and Cd (0.872, 0.494 and 0.124 respectively). Thus the correlation matrix data set might be explained by the PCA analysis [28]. It is appeared that the PC1 was influenced by man-made developmental activities such as industrialization, waste disposal from farmlands whereas the PC2 might be influenced by the natural earth processes [29].

This result is in line with the results found by Nour 2019 at the southern coast of the Sinai Peninsula, who reported the anthropogenic influence linked with lead and relevant heavy metals on the Senai area, Gulf of Aqaba ecosystem. Similar result was reported by Singh et al. 2020 in sediments of West Coast of India. Figure 2 presented the dominant source of variance (Pb, Cd, Cr) and the cumulative variance of component 1 accounts

51.423%, indicating that this component alone accounts for over half of the total variance. Similarly, the component 2 represents the second source of variance the cumulative variance explained after Component 2 accounts 20.445% and % of cumulative variance accounts 71.866 (Figure 2). Poultry farming, pesticides in agriculture farm, textiles, tanneries, electroplating workshops, dyeing, printing-photographic add the above heavy metals in the coastal systems of the study area. Pb has many different occurrences such as lead-acid battery industries, agricultural pesticides, and different domestic uses in the upstream Pasur. Environmental occurrence the chromium is from industrial welding, chrome plating, dyes and pigments, leather tanning, and wood preservation. Occurrence of cadmium in the environment is from batteries and pigment production sectors. Hg is also occurring from batteries, dental amalgams and wood preservation activities [30]. Thus this research can be considered as benchmark for the future research. More inclusive data is needed to have a clear trajectory of the Sundarbans ecosystem health.

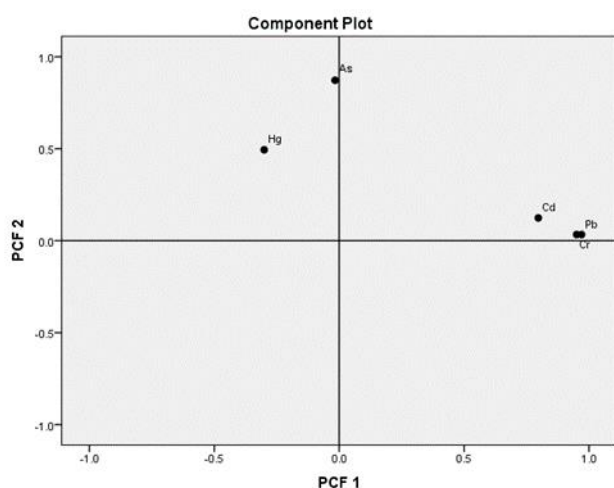


Figure 2. Component plot showing the dominant source of variance in the fish (*Johnius dussumieri*) at the downstream Pasur (Kokilmuni), the Sundarbans Bangladesh

4. Conclusion

Heavy metals assessed by analyzing Pb, Cd, Cr, As, and Hg concentration in fish species (*Johnius dussumieri*) collected from the Kokilmuni area of the Sundarbans, Bangladesh. The source and behavior of the metals in the mangrove ecosystem were explored by using multivariate statistical analysis (Pearson's correlation analysis and principal component analysis). Pb concentration indicates a clear anthropogenic influence in the Sundarbans which can be clarified by the unplanned battery industries and agricultural waste disposal to the water body. While lead is a worldwide concern of the mangrove ecosystems presently. Overall, the data of the present research shows almost the pristine condition of the Sundarbans area and need more expanded study. In addition, fish species and benthic organisms for heavy metal examination is worthy for inhabitants in the area to draw a consistent conclusion. The results indicate that the heavy metals need to be

monitored for avoiding further risk of pollution as heavy metal residues can be accumulated by aquatic biota, linked with human health hazards through the food chain.

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