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Exploring Bioaccumulation and Eco-toxicological potentiality of Heavy Metals in Natural Water Ways of the Padma River, Rajshahi

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Abstract: The study was envisioned to identify bioaccumulation and eco-toxicological potentiality of heavy metals in aquatic creatures of the Padma River water using several parameters for sustainable development. A complete set of 15 samples of water were collected from five sites Padma garden (S_1), Boro khuthi ghat (S_2), Lalonshah ghat (S_3), Police commissioner residential area ghat (S_4) and T-badh (S_5). Atomic Absorption Spectrometer was utilized to analyze the heavy metal Zn, Pb, Cu, Cd, Fe, Cr, and Ni concentrations in water and phytoplankton. The average concentration of physicochemical parameters in the sampling sites were pH (6.96), temperature (28.87 ℃), electrical conductivity (257.44 μs cm^{-1}), total dissolve solid (210.69 mgL^{-1}), alkalinity (207.33 mgL^{-1}), dissolve oxygen (6.81 mgL^{-1}), biological oxygen demand (1.77 mgL^{-1}) and total hardness (6.42 mgL^{-1}). Additionally, the heavy metal pollution index (HPI) excessed critical index value (100) for intake and aquatic life standard. The metal index (MI) value show that water was slight to moderate polluted by studied heavy metals. While the river water poses low ecological risk. The bioaccumulation risk was higher among all sites, particularly for Cu (1.89) and Cd (1.86). The ANOVA findings revealed that the dispersal pattern of heavy metal deliberation at different sites varied considerably ($P<0.05$) for Zn, Cu, and Ni. Consequently, effective management of heavy metals is mandatory for the sustainable water quality and well-being of the adjacent inhabitants of the sampling sites.

1. Introduction

Bangladesh is a phenomenal delta formed by the alluvial deposits of three mighty Himalayan Rivers; Padma is one of them. River valleys have historically been prominent centers of civilization because they provide transport routes and alluvial soils for productive agricultural lands (Sang *et al*. (2019). Padma River is well-known for its diversified use as a fishery, as well as for domestic (taking showers and rinsing clothes) and recreational purposes (Haque *et al*. (2019). During the 2007-2008 period, an approximate amount of 9392 metric tons of fish were captured in the Padma River, representing 6.87% of the total amount of fish harvested in Bangladesh's rivers (Ilie *et al*., 2017, Zhu *et al*., 2020). Anthropogenic acts and industries have resulted in a substantial increase in the incursion of heavy metals into the aquatic environment through nonspecific utilization and discharge of metal sewages, which have been recognized as chemical hazards (Hajeb *et al*., 2009, Li *et al*., 2015). Again contaminants are produced by printing facilities, chicken farms, material manufacturers, and manufacturing facilities located close or in the river's catchment region. Additionally, Farakka Barrage is located on the Ganges River in West Bengal, India, and the river's acceleration is shrinking on a regular basis (Liu *et al*. (2019).

Because of the prevalence, toxicity, persistence, accumulation features, and biomagnification prospects of heavy metals in aquatic ecosystems, this has drawn worldwide concern (Hossain *et al*. (2018). Heavy metals accumulate not only in reservoir waters and sediments but also within phytoplankton inhabiting heavy metal-contaminated water and are then reinstated into river water after aquatic creatures die and disintegrate through complex physical and chemical processes (Croteau *et al*., 2005, Yuan *et al*., 2020, Zhang *et al*., 2017). Some heavy metals can induce stomach and intestinal pain, as well as liver damage, reduced immune system function, and lower levels of high-density lipoproteins when taken in excess (Mohiuddin *et al*., 2011, Tessema *et al*., 2020). The average concentrations of studied heavy metals below the threshold, but Hg and Cr tended to be efficiently biomagnified in the food chain (Liu *et al*. (2019). Thus, heavy metals have been broadly studied for their toxic effects (Yu *et al*., 2012, Belbachir *et al*., 2013, Kim *et al*., 2016, Wang *et al*., 2018, Chris *et al*., 2021, Ahmed *et al*., 2021, Rahman *et al*., 2021) bioaccumulation in organisms (Yu *et al*., 2012, HKarim *et al*., 2016, Heshmati *et al*., 2017, Yilmaz *et al*., 2021, Ismail and Zokm, 2023, Akindele *et al*., 2020) in recent years.

Padma river water quality is degrading gradually, imposing a burden on living species (Pragnya *et al*., 2019). Padma River were unfit for drinking water and aquatic life standards using heavy metal pollution and metal indices (HPI and MI) (Haque *et al*., 2019). Heavy metal poisoning of watercourses is not only a concern for the environment, but it also poses a risk to human health. Therefore, it is crucial to assess how this metals distributed in the Padma River water to understand the current condition. Furthermore, the current study establishes a framework for policy implementation on the rehabilitation of the Padma River and its surrounding ecology from pollution. The current study was conducted to measure bioaccumulation risk, and assess heavy metal distributions using several indices such as Single-factor pollution index (I_i), Metal Index (MI), Heavy Metal Pollution Index (HPI), Ecological Risk Index (ER), Contamination degree (CD).

2. Methodology

2.1 Overview of the study area and river water sampling

The Padma is a major river in Bangladesh, it is the main distributary of the Ganges. It is approximately 4 and 8 km wide and 120 km long. Rajshahi city is located on the bank of the Padma River.

This research was carried out at the Padma Garden, which is close to the river's edge in the Rajshahi Division, at 1 km intervals for five sample points (S_1-S_5).

The sites of sampling sections are given in **Figure 1**. In detail, S_1 (Padma garden, GPS location: 24.36 N ~ 88.59 E) was in an region of domestic sewage; S_2 (Boro khuthi ghat: 24.35 N

~ 88.58 E) was influenced by direct discharges of effluent from household septic tanks ; S_3 (Lalonshah ghat: 24.35 N ~ 88.57 E) was in an area of metal industry and glass industry; S_4 (Police commissioner residential area ghat: 24.36 N ~ 88.55 E) was in the region of household domestic sewage; and S_5 (T-badh: 24.37 N ~ 88.54 E) was in the area of direct discharges of effluent from some household garbage.

2.2 Water sample collection and physicochemical parameters analysis

In the dry season (October 2022 to March 2023), fifteen (15) water samples were taken from the five sampling sites (S_1 to S_5) with three replications taken using 500 ml plastic bottles from the Padma River. The obtained water samples were acidified with concerted HNO_3 at pH < 2 to prevent precipitation of heavy metals and preserved in a refrigerator to avoid further contamination (APHA (2012).

Water, pH, temperature, dissolved oxygen, electrical conductivity, and total dissolve solid were evaluated by using portable multimeter, respectively. Biochemical oxygen demand (BOD) were measured using 5-Days BOD test (Rikta (2016). Total hardness and Alkalinity were measured by Titration method (Hem (1984).

2.3 Heavy metal detection of collected water samples

The Atomic Absorption Spectrophotometer is utilized for measuring heavy metal concentrations in water and phytoplankton including Zn, Pb, Cu, Cr, Fe, Cd, and Ni. The 100 mL water sample has been mixed with 10 mL of pure nitric acid (HNO_3) in a beaker. The sample was gradually heated and allowed to dry out on a hot plate to acquire a minimum quantity of approximately 20 ml. It was heated further with adding HNO_3 until digestion was finished after chilling and receiving a further volume of concentrated HNO_3 (5 ml). After cooling, an additional amount of concentrated HNO_3 (5 ml) was added, and heating was maintained at a steady pace by adding supplemented HNO_3 until digestion was finished. The sample was vaporized and chilled before being treated with 5 ml of a 1:1 v/v HCl solution. After boiling the solution, 5 ml of 5 M NaOH was incorporated and strained. The filtrate sample was shifted to a 100 ml container, and the contents were remedied with distilled water to prepare it for elemental analysis. With each batch, a blank sample (100 mL of distilled water and two drops of HCl) was tested (Neila *et al.* (2021).

Water samples were sieved directly on board in the laboratory using a sieve column to detect phytoplankton groupings. The phytoplankton groups Chlorophyceae, Cyanophyceae, Euglenophyceae, Xanthophyceae, and Dinophyceae were determined and then put through a Whatman 0.45 m glass fiber filter, dried at 100°C for 24 hours, and examined. After igniting in the ash furnace, the samples were warmed to 80°C and digested on a hot plate with 1.5 N HCl. Distillate deionizer water was used to dilute the digest to an extent of 100 ml (Chouvelon *et al.*, 2019).

Figure 1. Sampling locations in the study area (a) ArcGIS 10.8; (b) Google Earth

2.4 Bioaccumulation Factor (BAF)

BAF is used to identify bioaccumulative chemicals and offers target metal concentration range estimation for a water reservoir to maintain the contaminant value below the daily intake that is considered acceptable or tolerable (Schāfer *et al*. (2015).

$$\text{BAF} = \frac{\text{Cph}}{\text{Cw}} \qquad \textbf{Eqn. 1}$$

where, C_{Ph} is heavy metal concentrations in phytoplankton (µgL^{-1}), C_W is heavy metal concentrations in water (µgL^{-1}).

2.5 Eco-toxicological indices

2.5.1 Single-factor pollution index (I_i)

A Single-factor pollution index assesses the main heavy metal pollutants with respect to their level of harm. It can be expressed as the proportion of the dignified value for an identified heavy metal to the associated permissible value.

$I_i = C_i \div S_i$ **Eqn. 2**

In the formulation, I_i denotes pollution index of the single water quality parameter i; C_i is assessment value of pollutant load (μgL^{-1}); S_i is the permissible concentration of heavy metals (μgL^{-1}) (BIS, 2012, DoE, 1997, USEPA, 2012, WHO, 2008). I_i divided by 5 degrees of water quality assessment level, like no pollution (≤1), slightly polluted (1-2), lightly polluted (2-3), moderately polluted (3-5), and seriously polluted (> 5) (Su *et al*., 2022).

2.5.2 Metal Index (MI)

Metal Index is utilized to assess the general quality of drinking waters, considers the potential cumulative adverse effect of heavy metals on public health. MI is given by the appearance that given in **Eqn. 3** (Tamasi and Cini, 2004).

$$MI = \sum_{i=1}^{n} \frac{Ci}{(MAC)i} \quad \textbf{Eqn. 3}$$

Where, C_i is the value of an identified heavy metal and MAC is the highest recommended value.

The ratings of the index values are: very pure (< 0.3), pure (0.3 – 1.0), slightly affected (1.0 – 2.0), moderately affected (2.0 – 4.0), strongly affected (4.0 – 6.0), and seriously affected (>6) proposed by (Caerio *et al*., 2005, Lyulko *et al*., 2001).

2.5.3 Heavy Metal Pollution Index (HPI)

HPI is a rating procedure that demonstrates the complete influence of a particular heavy metal on water quality. The rating, which ranges from 0 to 1, specifies the relative weight apportioned to each quality factor and is inversely correlated with the suggested standard (S_i) for each indicator (Prasad and Mondal, 2008, Prasad and Sangita, 2008, Reza and Singh, 2010).

The following procedures are involved in calculating HPI:

- Firstly, calculate the weight of i^{th} parameter.
- Secondly, assign a quality ranking to each heavy metal.
- Thirdly, the sum of all these sub-indices is used to derive the overall index.

The i^{th} parameter's weight is defined via,

$$W_i = \frac{k}{Si}$$

where W_i denotes the unit weight, S_i is the tolerable standard for the i^{th} parameter, and k denotes the proportional coefficient constant.

$$Q_i = \frac{100 \times Vi}{Si}$$

Individual quality rating is supplied by the statement below, where Q_i is the i^{th} parameter's sub index, V_i is the i^{th} parameter's monitored value, and S_i is the i^{th} parameter's acceptable or appropriate range. The HPI is then computed in the following manner:

$$HPI = \frac{Qi \times Wi}{1} \quad \textbf{Eqn. 4}$$

Where Q_i is the parameter's subindex and W_i is the parameter's unit significance.

The critical pollution index for drinking water is 100 proposed by (Prasad and Bose, 2001). The three classes of low (<15), medium (15–30), and high (>30) for HPI values have been used in the current investigation.

2.5.4 Ecological Risk Index (ER)

The ecological risk index (ER) were determined to measure pollution, estimate the pattern of contamination and ascertain the possible risk resulting from exposure to ecological sensitivity, heavy metal content, and toxicity in water. The following ecological risk index (ER) formula was utilized to assess possible ecological dangers related to heavy metals given in **Eqn 5** (Sharifi *et al*., 2016).

$$ER = \sum_{i=1}^{n} Ti \times \frac{Ci}{Si} \quad \textbf{Eqn. 5}$$

Where, T_i represents a target heavy metal's potential ecological risk coefficient. The concentrations of T_i for all studied heavy metals 5 for Cu, Ni, and Pb, 2 for Cr and 1 for Fe, and Zn were selected from the literature Ref by (Sharifi *et al*., 2016, Håkanson, 1980).

The ranking criterion for ecological risks (ER) index as low risk (ER < 40), moderate risk (40 ≤ ER < 80), considerable risk (80 ≤ ER < 160), high risk (160 ≤ ER < 320), and very high risk (320 < ER) (Håkanson, 1980).

2.5.5 Contamination degree (CD)

The degree of contamination (CD) indicates impact or contribution of a single element in water contamination (Islam *et al*., 2013), and calculated by following **Eqn. 6** (Sharifi *et al*. (2016).

$$CD = \sum_{i=1}^{n} \frac{Ci}{Si} \quad \textbf{Eqn. 6}$$

where, C_i and S_i represent for the target heavy metal's measured concentration (μgL^{-1}) and assessment standard value (μgL^{-1}) of the studied heavy metal in the Padma River water.

The evaluation criterion are as follows: low pollution (Class I, CD < 6), moderate pollution (Class II, 6 ≤ CD < 12), considerable pollution (Class III, 12 ≤ CD < 4), and very high pollution (Class IV, CD ≥ 24) proposed by (Sharifi *et al*. (2016).

2.6 Statistical analysis

The mathematical computations were carried out by the practice of Microsoft Excel 13. The SPSS 25 and OriginPro (2023) were performed for employing multivariate statistical analysis, including principal component analysis (PCA), cluster analysis (CA), and the correlation matrix

(CM). Principal component analysis and One-way ANOVA were used at significant levels of $P \leq 0.05$ and 95% confidence interval.

3. Results and Discussion

3.1 Physicochemical parameters of the Padma River water

The physicochemical parameters pH, temperature, EC, TDS, alkalinity, DO, BOD, and TH values were recorded at the studied sites S_1, S_2, S_3, S_4, and S_5 (**Figure 2**). The outcome shows pH value at the sampling sites extended from 6.97 to 7.00. Turag River water pH values varied from 6.98 to 7.93 (Afrin *et al*., 2015). In this study, all the determinations of pH were within the acceptable limit (DoE, 1997, DPHE, 2019, USEPA, 2012, WHO, 2017). Measured average temperature values were 30.10℃, 28.50℃, 28.93℃, 28.30℃ and 28.50℃ in S_1 (Padma garden), S_2 (Boro kuthi ghat), S_3 (Lalon shah mukto moncho ghat), S_4 (Police commissioner residential area ghat) and S_5 (T-badh), respectively. Furthermore, the time of sampling, season, groundwater influx, air circulation, and water depth can all affect temperature (Peirce *et al*. (1998). Electrical conductivity varied from site to site. The EC values in the sampling points (S_1-S_5) ranged from 213.30 µs cm^{-1} to 271.63 µs cm^{-1}. Although conductivity is not considered a risk to human or environmental health, it can be used to detect other water quality issues (Dan *et al*., 2014). EC values in the Meghna River varied from 728.75 to 1980.00 µs cm^{-1} in water at various sampling locations (Hassan *et al*., 2015).

Total dissolve solid values ranged between 192.30 mgL^{-1} to 229.10 mgL^{-1} in **Figure 3**. The highest TDS value was found 229.10 mgL^{-1} at S_3 and the lowest value was found 192.30 mgL^{-1} at S_2. TDS readings of the Turag River water varied from 582 to 655 mgL^{-1} (Afrin *et al*., 2015). The Padma River's water contained an alkalinity range of 201.00-219.67 mgL^{-1} with the maximum level of alkalinity obtained at studied site S_4 and the minimum level at studied site S_5. Alkalinity value of all collected water samples from three sampling points ranged from 95.6 to 417.6 mgL^{-1} with an average concentration of 237.66 mgL^{-1} in the Turag River (Afrin *et al*., 2015). The result reveals that the DO levels in the study sites varied from 6.43 to 7.20 mgL^{-1}. DO mean values in water 6.81mgL^{-1} exceeding recommended level of 6 mgL^{-1} for drinking water in **Table 1** (DoE, 1997). As the water level gradually dropped from the monsoon to the post-monsoon, the aquatic plants received much more intense sunlight than they would have during the monsoon season, which enhanced photosynthesis and increased DO production. DO concentrations in the Halda River water ranged from 6.5 to 7.5 mgL^{-1} (Hasanuzzaman *et al*., 2020).

The average BOD values from the five sampling points (S_1-S_5) were within the range of 1.67-1.93 mgL^{-1}. BOD readings less than 6 mgL^{-1} indicate water is least contaminated with organic materials (Oluyemi *et al*. (2010). On the other hand, the BOD limit in drinking water set by the Bangladeshi standard is limited to 0.2 mgL^{-1} given in **Table 1** (DoE, 1997).

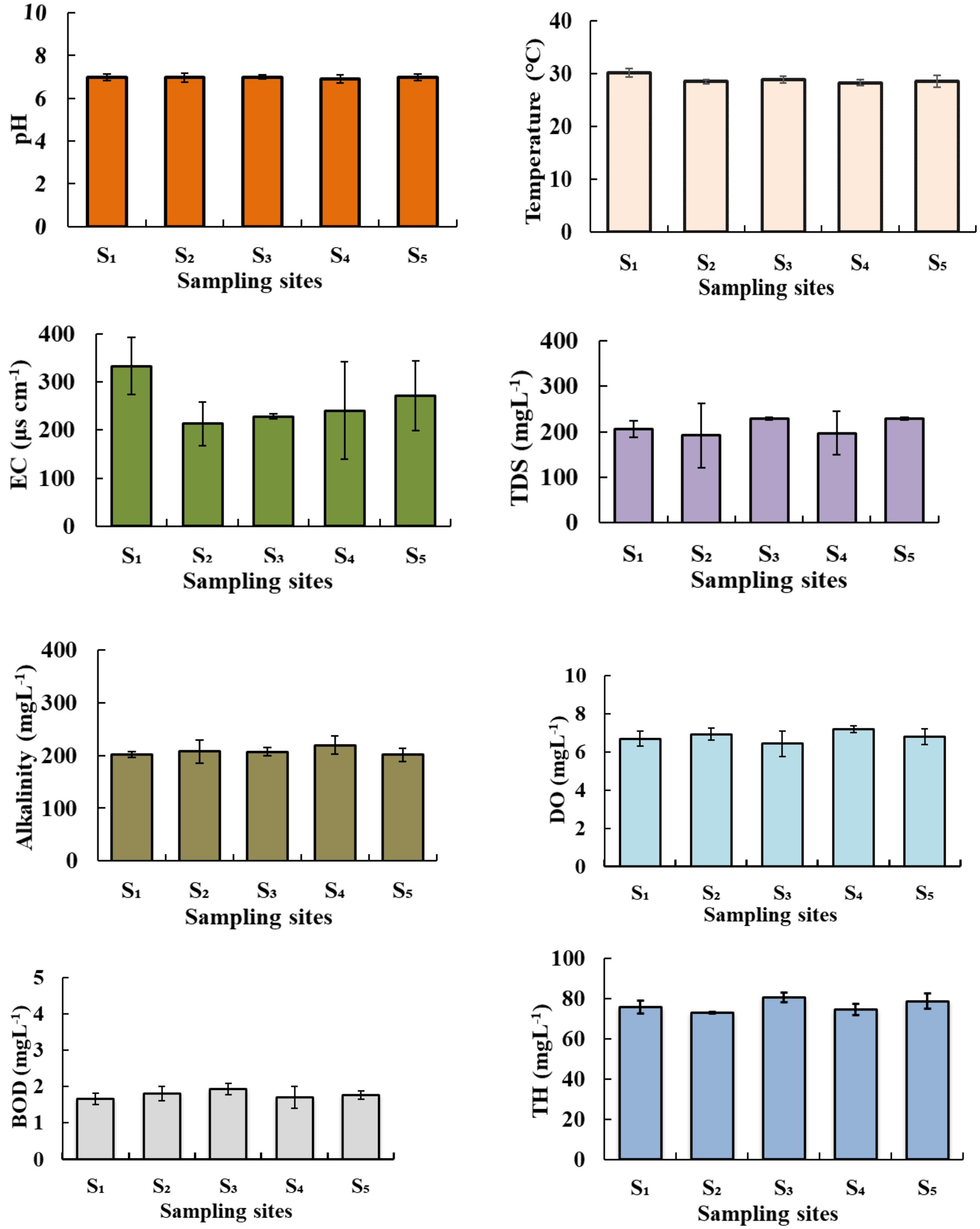

Figure 2. Physicochemical parameters in the Padma River water at different sampling sites (each site, n=3)

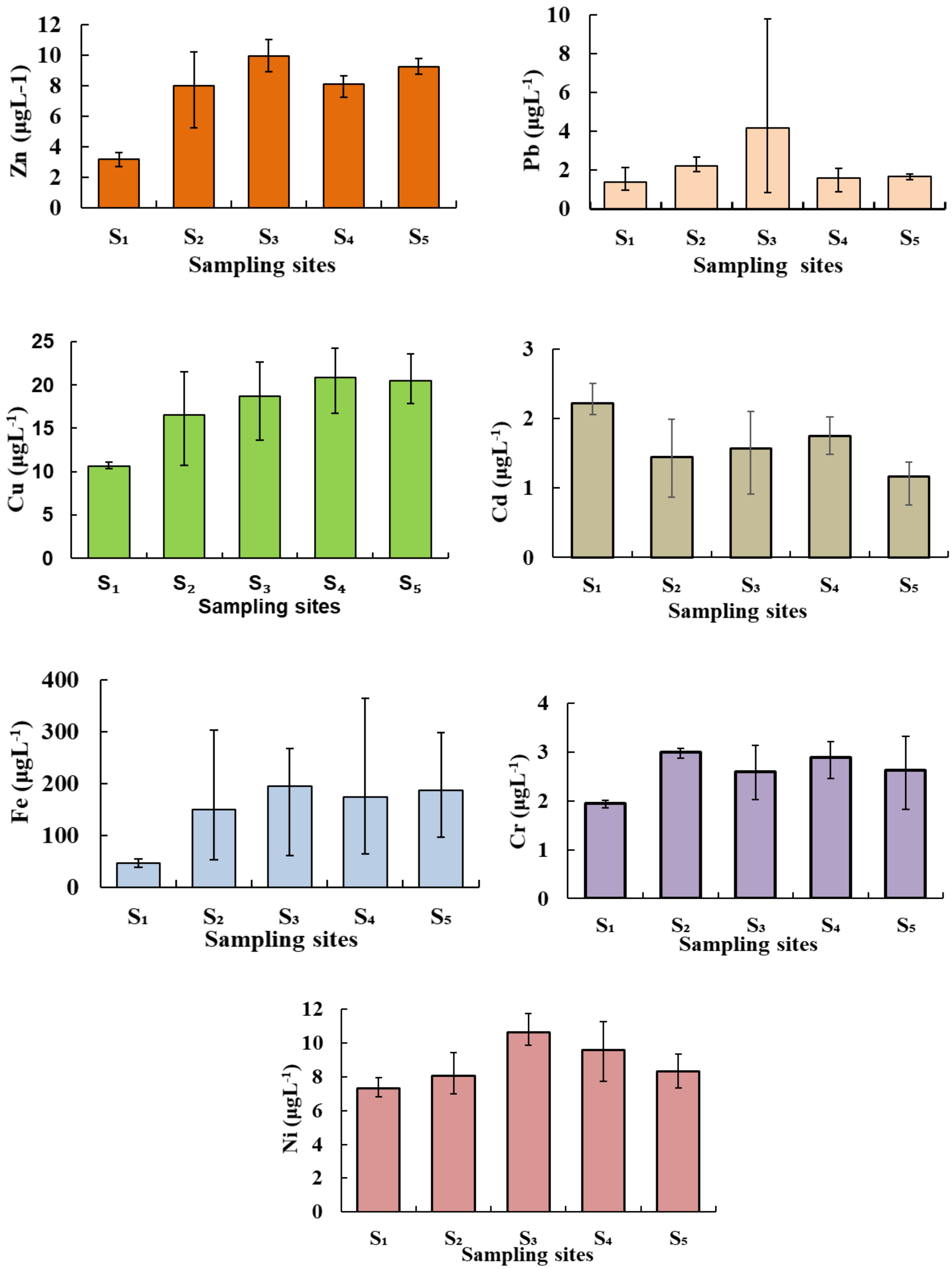

Figure 3. The dispersed patterns of the heavy metals at different sampling sites (each site, n=3)

The maximum BOD value of 31 mgL^{-1} in the Gomti River (Ahmad *et al*. (2021). The high BOD level indicates the presence of excess microorganisms in the water, presumably from household and industrial effluent, which absorbed dissolved oxygen and enhanced the biochemical oxygen demand in river water (Zeng *et al*. (2020). The mean TH values with respect to their five sampling sites were 75.73 mgL^{-1}, 72.77 mgL^{-1}, 80.50 mgL^{-1}, 74.47 mgL^{-1} and 78.63 mgL^{-1}, respectively. The mean TH values in the Buriganga River water samples were within the range of 180 - 345 mgL^{-1} (Majed *et al*., 2022). In winter, the maximum TH in the Turag River water was (328.00 ± 10.40) mgL^{-1} (Afrin *et al*., 2015).

3.2 Description of heavy metal concentrations in the Padma River water

The levels of heavy metal (Zn, Pb, Cu, Cd, Fe, Cr, Ni) in the Padma River water at five distinct sampling points is shown in **Table 1**. The value of Zn at the sampling points ranged from 3.20 to 9.95 µgL^{-1}. The mean value of Zn was 56 µgL^{-1} in Shitalakya River water (Jolly *et al*., 2018). Zn enters waterbodies through man-made channels, including steel manufacturing, waste material burning, coal-fired power plants, etc (Ewa *et al*., 2013). The average concentration of Pb for five sampling sites (S_1-S_5) were 1.37 µgL^{-1}, 2.22 µgL^{-1}, 4.16 µgL^{-1}, 1.62 µgL^{-1} and 1.69 µgL^{-1}, respectively. The Old Brahmaputra River average Pb concentration was found to be 110 µgL^{-1} (Bhuyan *et al*., 2019). The Shitalakhya River water Pb concentration was detected at 16 µgL^{-1} (Jolly *et al*., 2018). Pb can be found in several locations, which can be such as metal plating, fertilizer, wastewater discharge, and automobile exhaust (Haque *et al*., 2019).

Table 1. Comparison of heavy metal mean value (µgL^{-1}) in the Padma River water with the previously published values

River	Zn	Pb	Cu	Cd	Fe	Cr	Ni	References
Padma	7.71	2.21	17.45	1.62	150.46	2.61	8.78	This study
Old Brahmaputra	10	110	120	1	-	10	440	(Bhuyan *et al*. (2019)
Meghna	36.4	-	-	3	1022	34.6	-	(Hassan *et al*. (2015)
Buriganga	332	119	239	59	612	114	150	(Bhuiyan *et al*. (2015)
Dhaleshwari	-	-	150	6	-	440	7	(Ahmed *et al*. (2009)
Karnofully	280	140	50	10	2060	250	-	(Islam *et al*. (2013)
Shitalakhya	20	1	5	10	-	-	-	(Mokaddes *et al*. (2013)
Turag	20	2	4	10	-	-	-	(Mokaddes *et al*. (2013)
	5000	15	1300	5	300	100	100	(USEPA (2012)
	-	10	2000	3	-	50	70	(WHO (2008)
	5000	10	50	3	300	50	20	(BIS (2012)
	5000	50	1000	5	300–1000	50	100	(DoE (1997)

The minimum levels of Cu were found at S_1 (Padma garden), whereas S_4 (Lalon shah mukto moncho ghat) recorded the maximum readings. The average concentration of Cu in the Burigang River was detected at 239 μgL^{-1} (Bhuiyan *et al*., 2015). The mean concentration of Cu in the Dhaleshwari River at different sampling sites was 150 μgL^{-1} (Ahmed *et al*., 2009). The main causes of copper contamination are agrochemical factories, urban sewage systems, and agricultural fields (Koffi *et al*., 2014, Kundu *et al*., 2016) shown in **Table 1**. The average Cd concentration of the water samples at the five sampling points fluctuated from 1.17 to 2.22 μgL^{-1}. Due to Cd skeletal demineralization, the subsequent consequences amplified the risk of bone fragility and fractures (Liu *et al*., 2019). The Cd contents of Karnofully River was 10 μgL^{-1} (Islam *et al*., 2013). All the values of sampling sites were within the acceptable limit in **Table 1**.

The concentration of Fe ranged from 38.62 μgL^{-1} to 364.49 μgL^{-1} which exceeded the permitted limit of 300 μgL^{-1} specified by the WHO guideline (WHO, 2008). Excessive levels of iron in water may be linked to discharges of waste from metal alloy companies. Fe concentration in water varied from 311.0 to 494.0μgL^{-1} in the Halda River water (Dey *et al*., 2021).

The average deliberation of Cr was found to be 2.61 μgL^{-1} that was below the permissible limit of 100 μgL^{-1} DoE (**Table 1**). An extremely high level of Cr found in the Dhaleshwari River water was 440 μgL^{-1} (Ahmed *et al*., 2009), Cr content of the Karnofully River was 250 μgL^{-1} (Islam *et al*., 2013). Cr compounds are widely used in leather coloring, paints and coloring agents, metal plating, industrial welding processes, and timber preservation (Liu *et al*., 2019). Ni was found at all sampling sites, with levels ranging from 6.81 to 11.71 μgL^{-1}, with an average concentration of 8.78 μgL^{-1} that was below the WHO recommended value of 70 μgL^{-1}. The mean concentration of Ni in the Burigang River was 150 μgL^{-1} (Bhuiyan *et al*., 2015). Ni contents of Old Brahmaputra was 440 μgL^{-1} (Bhuyan *et al*., 2017).

3.3 Bioaccumulation Factor (BAF)

The values of BAF for Zn, Cu, Ni, Fe, Cd, Cr, and Pb varied from (1.70 to 1.89), (1.31 to 1.51), (1.13 to 1.21), (1.29 to 1.95), (1.31 to 1.60), (1.31 to 1.86), and (1.30 to 1.57), respectively. BAF mean values possessed higher for Cu, whereas Fe had comparatively low BAF mean values shown in **Figure 4**. The findings exhibited that maximum value for Zn (1.95) was recorded at S_4. The exception was for Fe, which was lowest (1.13) at S_1. In this study, all the studied heavy metals are BAF>1, demonstrating heavy metal accumulation from food to predators.

Water-to-plant transfer is a main source of harmful heavy metal exposure for humans via the food chain. BAF < 1 or BAF = 1 indicate that, nutrient-absorbing plants have heavy metals but do not accumulate them. BAF > 1 shows that, plant accumulates the heavy metals while up taking nutrients (George *et al*., 2017, Khatun *et al*., 2020). Heavy metal values in the Padma River water were relatively low; the low amounts detected could be explained by dilution effects, which mask the local concentration effects of low and chronic metal exposure by substantially decreasing concentration values in the water (Tessema *et al*., 2020). Higher Cu and Zn concentrations are found in phytoplankton samples, which may eventually be deposited into bony tissues and not transferable to food web (Liu *et al*. (2019). Cu is a functional element of the respiratory enzyme haemocyanin (Borrell *et al*., 2016), as well as Zn is an imperative element of various substances (Griboff *et al*., 2018). Fe is generally present in the natural ecosystem, and numerous species, including humans, have been observed to have the inherent ability to eliminate excess elements without much harm in healthy conditions (Kundu *et al*., 2016).

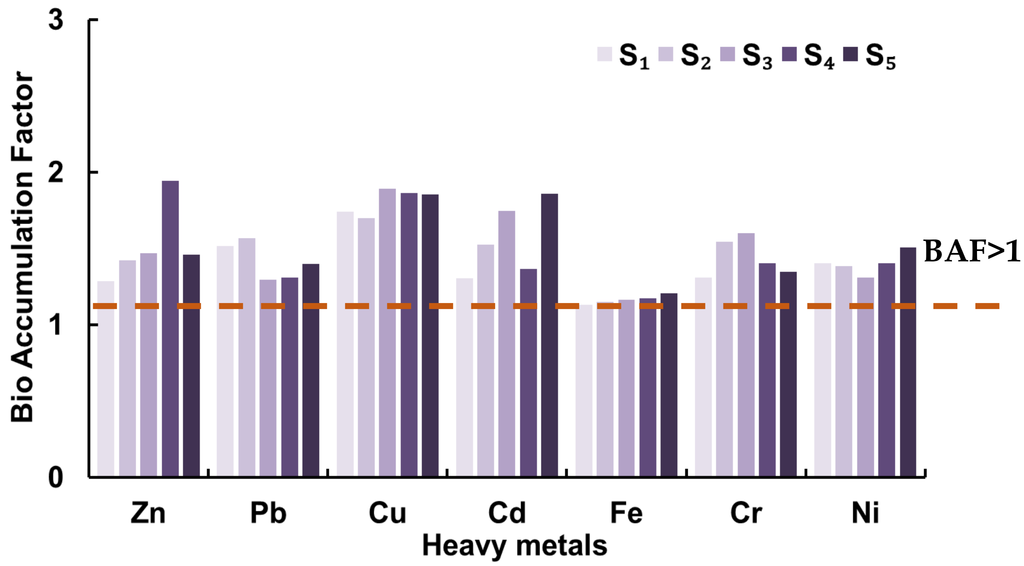

Figure 4. Variations of the bioaccumulation factor of phytoplankton in respect to sampling sites (BAF values > 1 suggest accumulation of heavy metal corresponding to the trophic level; BAF values ≤ 1 reveal active eradication of the element or disturbed trophic transfer)

3.4 Eco-toxicological indices

The single pollution indexes (Ii) of Pb are (2.44) at S_3. It concludes that sampling site is lightly polluted with Pb. Except that, the study sites of Padma River is Category I, which means there is no pollution given in **Table 2**. Lugu Lake in China was free of pollution using same index (Su *et al*., 2022).

Table 2. Single-factor pollution index value of investigated heavy metals

Sites	Zn	Pb	Cu	Cd	Fe	Cr	Ni
S_1	0.00	0.09	0.01	0.44	0.16	0.02	0.07
S_2	0.00	0.15	0.01	0.29	0.50	0.03	0.08
S_3	0.00	**2.24**	0.01	0.31	0.65	0.03	0.11
S_4	0.00	0.11	0.01	0.35	0.58	0.03	0.10
S_5	0.00	0.11	0.01	0.23	0.62	0.03	0.08

Metal Index (MI) values, at S_3 (3.34), the Padma River was moderately influenced with investigated heavy metals that may disrupt the existence of aquatic organisms due to direct discharge of heavy metals from the glass industry in **Table 3**. The mean concentration of investigated heavy metals is monitored by a decreasing order: Pb>Fe>Cd>Ni>Cr>Cu>Zn. The maximum value of Cr was also described in the Buriganga River during the dry months, Bangladesh due to direct discharge from commercial units, markets and small industries (Ahmad *et al*., 2010). The dilution effect of heavy metal concentrations has also been examined in previous studies (Giri and Singh, 2014).

Table 3. Properties of Metal Index found in the Padma River

Sampling sites	MPI values	Class	Properties
S_1	0.79	II	Pure
S_2	1.06	III	Slightly affected
S_3	3.34	IV	Moderately affected
S_4	1.17	III	Slightly affected
S_5	1.09	III	Slightly affected

Heavy metal pollution index value was found at 35, 61, 108, 49, and 69 at S_1, S_2, S_3, S_4, and S_5 sampling points, respectively given in Table 4. HPI value was in order $S_3 > S_5 > S_2 > S_4 > S_1$ during the study period. The HPI value at S_3 is higher than the minimal index value (100), which must exist for drinking water quality along with aquatic life standards. The river basin was accessible for agricultural purposes throughout the dry season, which contributes to the rising level of HPI. Similarly, agricultural practices contributed enormously to the heavy meal found in India's tropical mountainous river (George *et al*., 2017).

Table 4. Heavy Metal Pollution Index in the Padma River water

Sampling sites	HPI values	Class
S_1	35	High
S_2	61	High
S_3	108	High
S_4	49	High
S_5	69	High

The contamination degree (CD) of studied heavy metals was found to be 0.79, 1.06, 3.34, 1.17, and 1.09 at S_1, S_2, S_3, S_4 and S_5, respectively. The results show a low contamination degree according to the following classification that displayed in **Table 5** (Sharifi *et al*., 2016). The potential ecological risk (ER) index approach proposed by (Håkanson, 1980) for determining heavy metal pollutants' natural and environmental comportment. The ecological risk evaluation outcomes depend on the calculated compounds at the points of sampling had ecological risk index (ER) values less than 40, indicating that heavy metals represent a minimal ecological risk.

Table 5. Contamination Degree (CD) and Ecological Risk index (ER) in the Padma River

Sampling sites	CD	Quality class	ER	Ecological risk grade
S_1	0.79	Low pollution	14	Low risk
S_2	1.06	Low pollution	10	Low risk
S_3	3.34	Low pollution	22	Low risk
S_4	1.17	Low pollution	12	Low risk
S_5	1.09	Low pollution	9	Low risk

The possible ecological risk configuration for the studied heavy metals declined in the order of Cd > Pb > Fe > Ni > Zn > Cu > Cr. The principal sources of Cd are the metal industry (S_3), coal combustion, and waste disposal (Dey *et al*., 2021). The Ganges River water was judiciously contagion with Cr, Pb, Ni, As, Cu and Zn heavy metal (Haque *et al*., 2019). One of the biggest causes of contaminants entering the river water might be open city drains, residential garbage, and industrial pollution (Yao *et al*., 2014).

3.5 Statistical analysis and source determination of studied heavy metals

The correlation matrix was designed to examine relationships in order to determine the element's point of origin along with its dispersion pattern given in **Table 7** (Suresh *et al*., 2012, Wang *et al*., 2012). This investigated result confirmed that Zn exhibits a significant positive relationships ($p < 0.01$) with Cu ($r = 0.797$). It was also perceived that, Cd has strong negative correlations with Zn, Cu ($r = -0.673, -0.538$, respectively) in **Table 6**. Furthermore, a significant relationship has been established between heavy metals, demonstrating their ability to possess a longer persistence in the environment via the formation of integrated compounds with water (Yang *et al*., 2015). There is also a significant relationship between these variables, indicating that they are derived from the same sources. Those factors with negative connections imply that those metals possess similar pollution points, as their sources of origin differ (Ewa *et al*., 2013).

Table 6. Correlation coefficient matrix of the investigated heavy metals

	Zn	Pb	Cu	Cd	Fe	Cr	Ni
Zn	1						
Pb	0.223	1					
Cu	0.797**	0.132	1				
Cd	-0.673**	-0.388	-0.538*	1			
Fe	0.484	0.292	0.320	-0.484	1		
Cr	0.426	-0.289	0.293	-0.254	0.492	1	
Ni	0.506	0.512	0.513	-0.201	-0.017	-0.024	1

N.B: ** indicates that correlation is 1% level; * reveals that correlation is 5% level

Principal Component Analysis is a compressed collection of variance components for every data set by (Singh *et al*., 2005) that is utilized for qualitative assessment and clustering behavior (Ma *et al*., 2016), as displayed in **Table 6** results of the PCA of the investigated heavy metals with three components. The PCA findings were consistent with loading values and eigenvalues that were corresponding to or greater than 1. Furthermore, the deviation of 100% and the cumulative deviation for each component were calculated from the eigenvalue results. PCA 1 participates in 46.36% of the total deviation through a higher loading of Zn (r = 0.510) given in **Table 7** and **Figure 5**.

Table 7. PCA of three elements using suitable metal loadings

Heavy metals	PCA 1	PCA 2	PCA 3
Zn	**0.510**	-0.053	0.193
Pb	0.237	**0.575**	-0.487
Cu	0.454	0.007	0.391
Cd	-0.435	0.012	0.284
Fe	0.353	-0.295	**-0.556**
Cr	0.256	**-0.593**	0.125
Ni	0.312	0.476	0.407
Eigenvalues	3.246	1.545	1.010
Variance (%)	46.36	22.07	14.44
Cumulative Variance (%)	46.36	68.44	82.88

N.B: Bolds indicate that are significant relationships ($P \leq 0.05$)

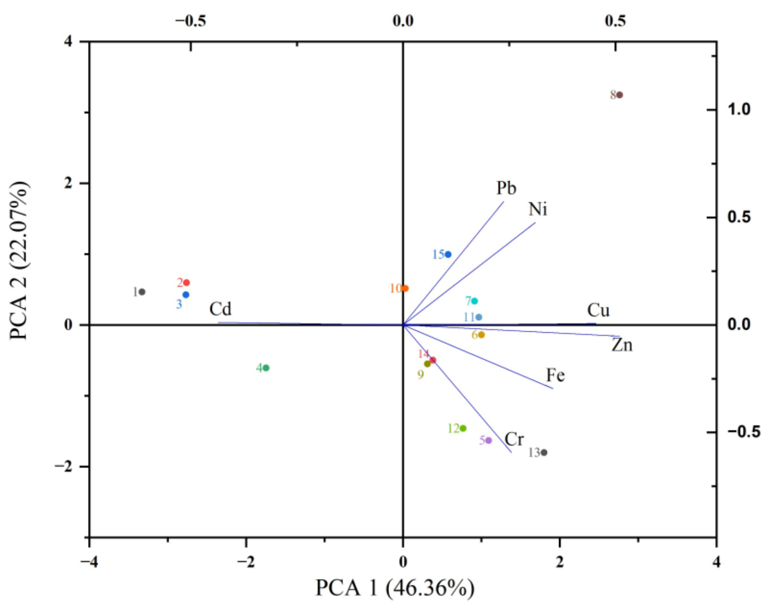

Figure 5. PCA of two elements with appropriate metal loadings

By contrast, PCA 2 provided 22.07% of the overall deviation related to the loadings of Pb (r = 0.575) and (r = -0.593). Lastly, PCA 3 appeared in 14.44% of the overall variance related to the loading of Fe (r = -0.556). The outcomes explored the internal connection of the metals and indicated component sources, which might include untreated industrial and urban pollutants as well as other geogenic processes (Croteau *et al*. (2005). As a result, Industrial and agricultural compounds and other man-made achievements can be utilized to determine the element's origin (Martín *et al*., 2006, Shikazono *et al*., 2012). The element appears to have come from both natural and man-made sources (Croteau *et al*. (2005).

The statistical comparison of studied heavy metals at various sampling sites is illustrated in **Table 8**. The ANOVA results revealed that the dispersion pattern of investigated heavy metal reading in the various sampling sites was significantly diverse ($P \leq 0.05$) for Zn, Cu, and Ni. However, no significant variation was identified for Fe, Pb, Cd, and Cr in the studied samples. This clearly demonstrates that different sites contribute to the mean heavy metal contents in the study area in different ways. Variations in this aspect probably arise from changes in similar anthropogenic activities, such as sand scraping, industrial waste, paper factories, and neighboring power plants, which might raise the accumulation of metal concentrations in the surrounding environment (Varol and enB, 2012).

Table 8. One-way ANOVA comparison of studied heavy metals for different sites in the Padma River

		SS[a]	df[b]	MS[c]	F	Significance
Zn	Between Groups	84.139	4	21.035	12.172	**.001**
	Within Groups	17.281	10	1.728		
	Total	101.421	14			
Pb	Between Groups	2434.228	4	608.557	.977	.462
	Within Groups	6230.663	10	623.066		
	Total	8664.891	14			
Cu	Between Groups	208.923	4	52.231	3.541	**.048**
	Within Groups	147.505	10	14.750		
	Total	356.428	14			
Cd	Between Groups	1.834	4	.458	2.442	.115
	Within Groups	1.877	10	.188		
	Total	3.711	14			
Fe	Between Groups	43677.397	4	10919.349	.792	.556
	Within Groups	137864.305	10	13786.431		
	Total	181541.703	14			
Cr	Between Groups	1.996	4	.499	2.394	.120
	Within Groups	2.084	10	.208		
	Total	4.079	14			
Ni	Between Groups	20.975	4	5.244	3.748	**.041**
	Within Groups	13.991	10	1.399		
	Total	34.966	14			

N.B: Bold indicates that are significant association ($P \leq 0.05$) level; [a] Square sum, [b] Degrees of freedom, and, [c] Mean square

Furthermore, by using artificial mussels for detecting metals in rivers waters in Bangladesh, commercial and agricultural/aquaculture releases are accountable for greater amounts of Cr, Ni, and Pb (Islam *et al*., 2015, Kibria *et al*., 2012, Kibria *et al*., 2016). Hierarchical cluster analysis is an approach utilized for categorizing components of a system into clusters according to data similarities. HCA with a centralized correlation strategy is utilized in this investigation to evaluate the connection between heavy metals in **Figure 6**. There are two distinct clusters: the first cluster is entirely made of Cd. Second cluster is made up of Zn, Cu, Fe, Cr, Pb, and Ni. This analysis demonstrates that Zn, Cu, are mostly related to Fe, Cr, and probably Pb and Ni by one point of origin. Furthermore, it implies that the principal sources of Cd emission into river water vary from one another.

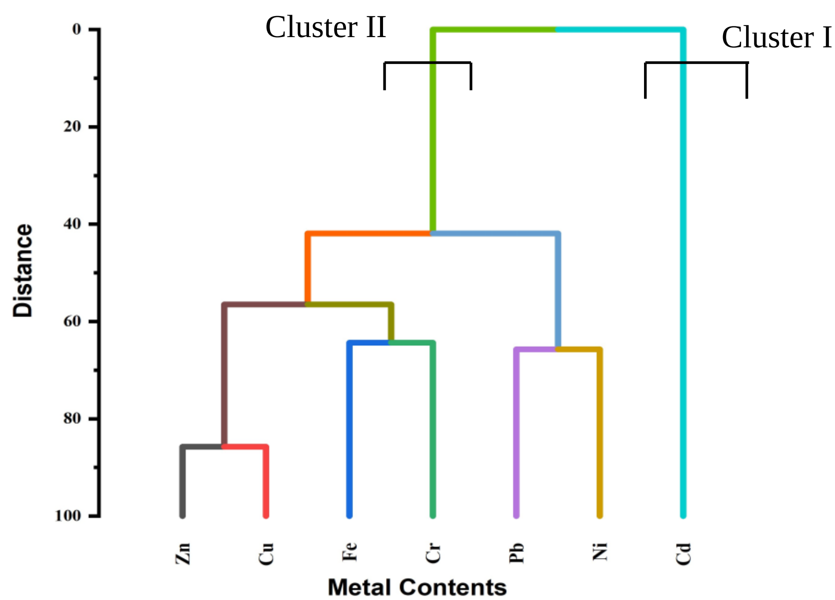

Figure 6. Hierarchical cluster analysis among studied heavy metals at sampling sites

Conclusion

The current study examines the Padma River's water quality condition in terms of physicochemical parameters and heavy metal pollution. Significant contamination was observed at the Padma River concerning recognized parameters such as pH, temperature, TDS, EC, DO, BOD, Alkalinity and TH. This study also investigated the status of relevant heavy metals like Zn, Pb, Cd, Cr, Cd, Fe and Ni pollution in Padma river water to understand ecological risk and bioaccumulation factor. Heavy metal pollution indices Ii, MI, and HPI show that Padma River water quality is unfit for drinking water. The bioaccumulation factor (BAF) was higher among all sites for Cu, Cd. Although the ecological risk index (ER) classification implies a low degree of contamination, an increasing degree of pollution may make the Padma River ecology more concerning. The hierarchy of heavy metal toxicity according to mean concentrations found in the collected water sample from the studied sites was Fe>Cu>Ni>Pb>Zn>Cr>Cd. In addition to natural sources, the study demonstrated man-made activities such as chemical attribution, agricultural waste disposal, and burning coal also constitute contributing factors for metal enrichment. However, the current investigation is to assess surface water heavy metal concentration; thus, further research is required to adequately evaluate the implications of our findings. Future research might focus on identifying heavy metal transport mechanisms and how they facilitate metal accumulation in humans via ingestion. Again, this study was undertaken during the dry season; it would have been better appreciated if the time period had been extended. The current study will be quite helpful for residents and government authorities in terms of policy formulation and taking additional actions to reduce the presence of heavy metals in the Padma River. Heavy metals in the study region must be treated and monitored continuously.

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