Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2024; 12(3): 54-61

Received: 25-03-2024
Accepted: 26-04-2024

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Assessment of molluscan diversity and their responses to heavy metal deposition in a river ecosystem of Bangladesh

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DOI: https://doi.org/10.22271/j.ento.2024.v12.i3a.9319

Abstract
The key challenge of bio-monitoring is to explore comprehensive knowledge of bio-indicator organisms and to understand their responses to environmental alterations such as heavy metal pollution. The present study aimed for assessing the diversity of sediment-dwelling molluscs and their responses to different heavy metals in the sediment and water of the Shitalakshya River. Classical taxonomic analysis of Molluscan fauna revealed 16 species under 8 families of two major classes, Gastropoda and Bivalvia. There was a clear variation in the species-wise abundance of both molluscs but the overall abundance of both invertebrate groups did not vary significantly across the sites ($p>0.05$). Among ten heavy metals (Cr, Cd, As, Ni, Pb, Mn, Cu, Zn, Co, and Se), the concentration of Mn was relatively higher than other metals in both water and sediment samples. In contrast, the lowest concentration was also observed for Cd and Se. Mollusca abundance showed positive correlation with heavy metals except for chromium. Furthermore, GLM model confirmed the strong negative relationship ($p<0.05$) of Pb) with Molluscs, respectively. Overall, these results have implications for the biodiversity assessment of aquatic macro-invertebrates as well as their uses in monitoring the health of river ecosystems of Bangladesh.

Keywords: Heavy metals, pollution, bioindicator, river ecosystems, Mollusca, Bangladesh

Introduction
Bangladesh is a densely populated, developing country with a large network of about seven hundred rivers running across its landmass. These river ecosystems have a significant influence on transportation, fishing, and industrial activities and support diverge aquatic fauna (fish, aquatic insects, annelids molluscs etc.). Therefore, any man-made or anthropogenic disturbances can cause changes to riverine fauna especially aquatic vertebrates and invertebrate organisms. The recent rising of urbanization and industrialization in Bangladesh has a disastrous influence on the water quality and the life of aquatic biota because industrial effluents and urban runoff are being dumped directly into adjacent water bodies with little regard for the environment [6]. Among many point and non-point sources of water pollution, metal and metalloid pollution from different industries and factories poses a significant impact on river biodiversity and ecosystems [21] which are ultimately causing significant hazards to the local human population. Untreated industrial effluents are the primary source of heavy metals and metalloids in river water [3]. In addition, heavy metals in riverine environments may be added by mining and from sewage treatment plant effluent and urban runoff. Moreover, inter-connected river networks and rainfall have a significant role in the regional and cross-regional dissemination of heavy metals and metalloids [17]. Therefore, river pollution has been a critical concern regarding the contamination of water, sediments, and water bodies in and around the country's industrial and urban areas [10]. This critical situation necessitates the urgent assessments of ecological consequences caused by human-induced pollution for the management and conservation of aquatic environment. To address the biomonitoring issue, the usage of bioindicator especially the macroinvertebrates could be a right choice for to monitor the river health through diversity assessment and their responses to different alteration in the river ecosystems.

Macroinvertebrate groups broadly include molluscs, oligochatets, polychaetes, aquatic insects, and crustaceans who are mainly bottom dwelling organism commonly called benthic invertebrates.

Generally, they are highly diverse, and widespread, and contribute to various ecosystem functioning including nutrient cycling between trophic levels of the food chain of an aquatic body. Benthic macro-invertebrate species responds to varying sensitivity to pollutants and have been frequently utilized to assess the environmental consequences of metal pollution in streams [11, 18]. Their activities create a dynamic mosaic of sediment in aquatic ecosystems, efficiently transfer solutes into burrows, promote sediment oxygenation, stimulate microflora, and speed up disintegration rates [16]. Consequently, these benthic species serve as ecosystem engineers [12] and play crucial functions in the detoxification of contaminants, dissemination, decomposition, and secondary production. All of these attributes make benthic organisms the best biomarkers to indicate the ecosystem's past and current environmental conditions than physical and chemical indices of water and sediment [19].

Species richness, composition and abundances of different group of macroinvertebrates varies with the extent and nature of environmental alterations by habitat destruction, heavy metal contamination and other anthropogenic disturbances. The sensitivity and tolerance responses also varied within and between group of macroinvertebrates. For example, mayflies, caddisflies, and stoneflies are highly sensitive bioindicator to any sort of pressure whereas molluscs and odonates are included under sensitive to moderately tolerant groups. Most of the dipterans, oligochaete and polychaetes are known to highly pollution tolerant bioindicator organisms. Hence metal pollution in aquatic habitats increases sediment-bound metal concentrations, posing a threat to some groups of benthic bioindicator organisms (e.g., mayflies, stoneflies and caddisflies etc.). In contrast, some pollution-tolerant bioindicator organisms (e.g., Polychaetes) get benefits from heavy metal deposition to expand their population excluding other groups of invertebrates. Therefore, macro-invertebrates are the most frequently used indicator group for monitoring the health of river ecosystems [24] and macro-invertebrate-based indices and metrics have been established and systematically applied in various regions of the world, including Europe [27].

Freshwater ecosystems especially the rivers of Bangladesh are experiencing various man-made and natural pressures. Therefore, biological monitoring using benthic macro-invertebrates is one of the primary needs for the protection and conservation of riverine ecosystems. However, the diversity and density/abundance data of macoinvertebrates are the prerequisites for understanding their responses to various pollutants, especially to varying concentrations of heavy metals deposited in the sediments. Globally, the key challenges of a biomonitoring programme are the diversity assessment of benthic macroinvertebrates and the understanding of the way of responses to existing stressors in a particular ecosystem. Likewise, Bangladesh has no adequate updated aquatic macroinvertebrate diversity data except for some sporadic studies on the diversity of benthic fauna, aquatic insects, and molluscs [1, 5, 20, 22]. Unfortunately, the studies concerning the impact of water pollution on macroinvertebrate fauna is nearly absent in the country though many studies were conducted on heavy metal assessment in water, soil, fish and other organisms of freshwater ecosystems [2, 9, 13, 15]. Therefore, it is imperative to assess the diversity, density of all groups of bioindicators consecutively and their responses to human induced aquatic pollution.

To address these critical issues and considering the knowledge gap, the present study firstly aims for exploring the diversity of molluscs in the Shitalakshya River of the country which flows nearby Dhaka, the capital city of Bangladesh. As there is no information on the mechanisms of responses of these benthic invertebrates to pollution, the abundance of molluscan species was also estimated to be correlated with the arrays of heavy metal concentration in river-bed sediments. The results of the present study will have great implications for monitoring river health providing the current status of molluscan diversity and their response mechanism to different heavy metals in river ecosystems.

Materials and Methods

Study area

In the present study, the benthic macro-invertebrates, mollusc samples were collected from the Shitalakshya River, which passes by four districts namely Gazipur, Narshindi, Narayanganj and Dhaka of Bangladesh. This year-round navigable river is 110 km long having a maximum width of 300m with an average depth of 10m. In total, 18 benthic macro-invertebrate samples were collected from six sites (locally named as Narayanpur, Nandipur, Bibadia, Singhasree, Bagherhat, and Dardaria) along the 4 to 5-kilometres sampling reach of the river near Kaligonj and Kapashia Upazilla of Gazipur district (Fig 1). Sampling sites were at approximately equal distances from each other, as much as logistically possible in the river.

Sample collection

At each of the six sites, three benthic samples were obtained using an Ekman grab sampler (10x8 inch), and samples were preliminary processed with a sieve net (mesh size 1.0 mm) in the field and preserved in absolute ethanol after manually removing large debris. In addition, to assess the heavy metal concentration, 18 sediment and 12 water samples were also collected in plastic containers and preserved in an ice box to transfer into the laboratory. The retrieved macro-invertebrate sediment samples were finally sieved using a 1.0 mm meshed net in the laboratory of the Department of Zoology, University of Dhaka. After washing away the alcohol, Mollusca organisms were sorted from the detritus using a compound light microscope if necessary. The sorted organisms were then preserved in glass containers/vials with absolute ethanol for subsequent morphological and molecular analysis.

Morphometric analysis of Mollusca

So far 150 Molluscan individual organisms were sorted from six sites. The morphological analysis of the isolated organisms especially the identification molluscs was done to their lowest possible taxonomic level (up to family and genus) using the available published literature [26, 25]. Photos of small Mollusca organisms were taken using a stereotype dissecting microscope (Leica EZ4 E, Wetzlar, Germany).

Heavy metal analysis

The concentration of 10 heavy metals (Cr, Cd, Pb, As, Ni, Mn, Cu, Zn, Se, Co) in water and sediments was measured using the IPC-MS spectrometer (NexION®2000, USA). The wet sediment samples (n = 18) were properly air-dried. After drying, the sediment samples were mashed with a porcelain mortar and pestle and weighed 1g in a weight balance (Bel engineering, Italy), and stored in a 1.5-mL MCT tube for later examination. A pre-cleaned 50 ml digestion beaker was filled with 1.0 g of sieved and well-mixed soil samples; then 10 ml of concentrated HNO3 acid (70%) was added in the samples with blank beaker (ultra-pure deionized water) and stirred for 2 minutes. These samples were retained overnight for free digestion. The solutions were then covered with convex glass and put in a digesting chamber for 1 hour of heating at 100 °C, and then continuously heated at 120 °C for 1 hour and at 150 °C until no more brown vapours were produced by the mixture. The liquid was then cooled and filtered through filter paper (Whatman, Grade 44 pore size - 3 µm) into a 50 ml flask before being marked with distilled water (Figure 3.8). To control for the matrix effect, the same preparation procedures were used to make reagent blanks. The same procedure was followed for the 200 ml of water (n = 12) except the samples were filtered using Whatman filter paper (No. 1).

For each element in the samples, at least one standard was prepared. This was done so that the elements could be analyzed. First, 10 ppm of metal standard solution was prepared from 1000 ppm, and from 10 ppm, three different standards (e.g., 0.5 ppm, 1 ppm, and 2 ppm) were prepared. After that, these three standards were run through the ICP-MS spectrometer. All sediment samples were analyzed in triplicate measurement whereas the water sample was duplicate for the measurement of 10 heavy metals. CRM016 Sigma-Aldrich was used as certified reference material to assure the accuracy, sensitivity and precision of the analytical procedure of ICP-MS. Metal concentrations were calculated using the following formula: The concentration of heavy metals = (ICP-MS reading-blank reading) x Primary dilution factor (PDF) (10) x Volume of extract in ml /weight of sample (for sediment/ water) was taken.

Statistical analysis

All statistical analyses for measuring diversity measures (e.g., richness and abundance) with the construction of relevant graphs/figures are performed using different packages of 'R' statistical software (Version 4.1.2). A one-way ANOVA was performed to observe the mean differences of the abundance of Mollusca (total count) among six locations (Nondipur, Bibadia, Singhasree, Dardaria, Bagherhat, Narayanpur). Pearson's correlations were used to test for relationships between all heavy metals (Cr, Cd, Pb, As, Ni, Mn, Cu, Zn, Se, Co) and the abundance of Mollusca. The relationship between the abundance of molluscs and the concentration of different heavy metals (ppm) was assessed using a correlation matrix and a Generalized Linear Model (GLM) was fitted with negative binomial distribution log link function in the same statistical programme 'R' [23]. The abundance of Mollusca was fitted as response variables, with 10 heavy metals as predictor variables.

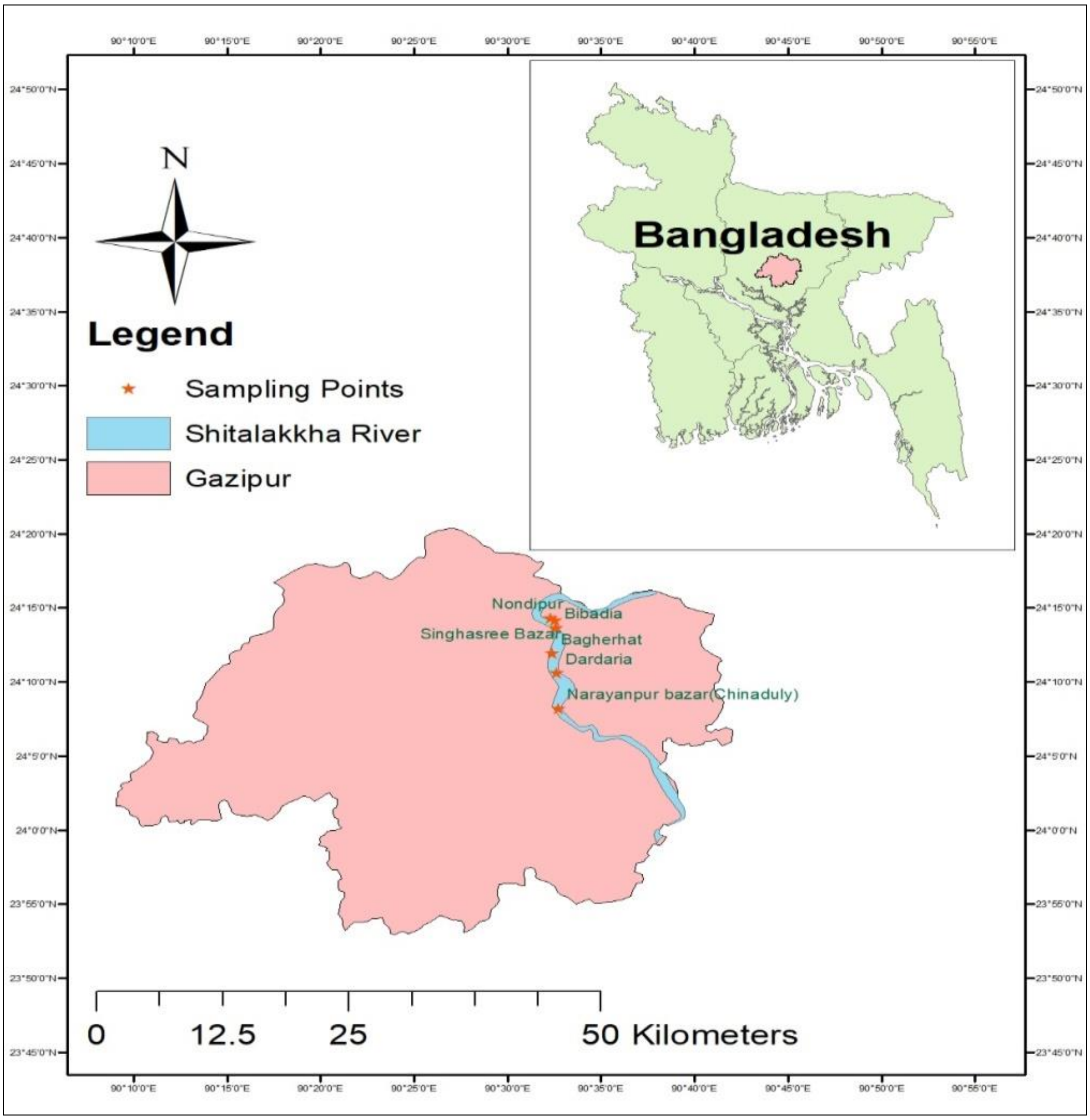

Fig 1: Study area on the map showing the six sampling sites (Narayanpur, Nandipur, Bibadia, Singhasree, Bagherhat, and Dardaria) spanning around the 5000 m river section in the Shitalakshya River

Results

Diversity and density of Mollusca

In the study present study, morphology-based taxonomic analysis estimated 16 species of Mollusca under 8 families (Thiaridae, Bithyniidae, Cyrenidae, Pharidae, Unionidae, Viviparidae, Planorbidae, Pachychilidae) of two major classes, Gastropoda and Bivalvia. These sediment dwelling gastropods and bivalve mollusc included the genus *Melanoides* (1species), *Bellamya* (3 species), *Tarebia* (2 species), *Brotia* (1 species), *Bethynia* (2 species), *Indoplanorbis* (1 species) under the class Gastropoda (Fig 2). Bivalve molluscs included *Lamellidens* (2 species), *Parreysia* (2 species), *Corbicula* (1 species), *Novaculina* (1 species) (Fig 3).

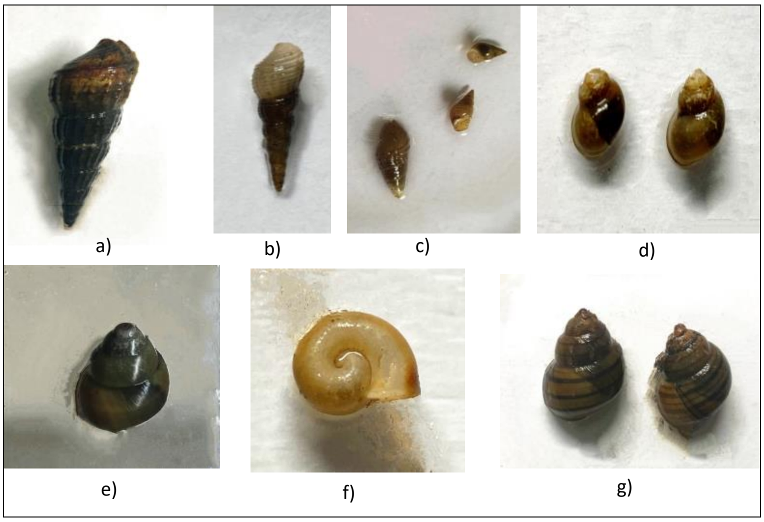

Fig 2: a) *Brotia costula*, b) *Melanoides tuberculata* , c) *Terebia lineata* , d) *Bethynia* sp (e) *Bellamya crassa*. , (f) *Indoplanorbis* sp (g) *Bellamya bengalensis*

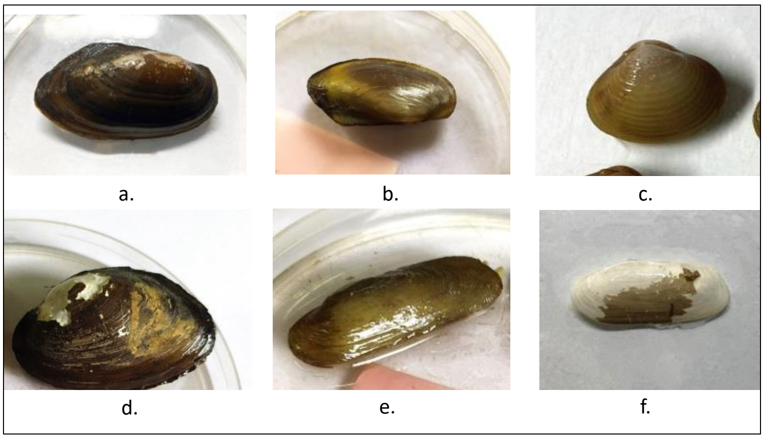

Fig 3: (a) *Lamellidens marginalis* , (b) *Lamellidens corrianus* , (c) *Corbicula* sp. , (d) *Parreysia corrugata* (e) *Novaculina gangetica* , (f) *Novaculina* sp.

The species-wise and site-wise abundance of molluscan species showed variation in the collected sediment samples in the study area though this difference did not vary significantly among six locations for the abundance of Mollusca species (p>0.05). The abundance of *Bethynia* and *Corbicula* species was higher across the sampling sites (Fig 4A). The mean abundance of Molluscan species was highest in Singhasree (47.33 ± 18.62) site and lowest was in Narayanpur (21.33 ± 1.0) (Fig 4B).

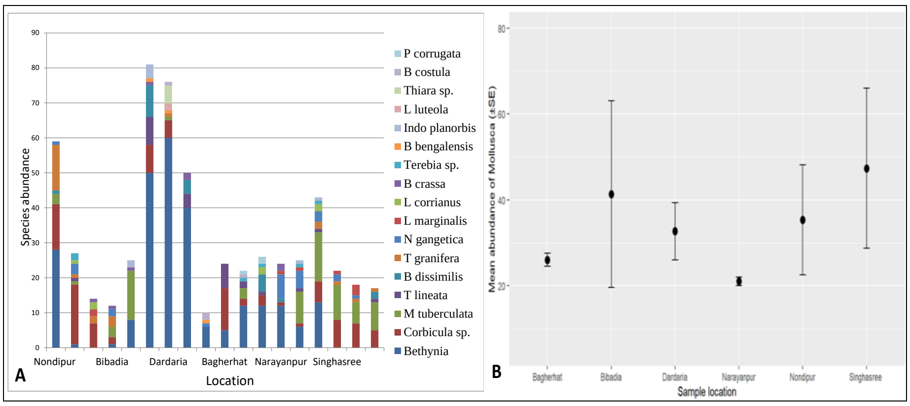

Fig 4: Species (A) and site (B) wise abundance of Mollusca species in six sampling locations of Shitalakshya River.

Heavy metal concentration in water and sediment samples of six locations

The concentration of heavy metals such as Cromium (Cr), Cadmium (Cd), Arsenic (As), Nickel (Ni), Lead (Pb), Manganese (Mn), Copper (Cu), Zinc (Zn), Cobalt (Co) and Selenium (Se) varied in water and sediment of six sampling locations and was mostly exceed the recommended level of WHO (0.05ppm). Among ten heavy metals, the concentration of Mn was relatively higher than other metals in both water and sediment samples. The concentration of Cd, Co and Se were lowest in water samples of all locations (Fig 5A). In sediment samples, the lowest concentration was also observed for Cd and Se among six locations (Fig. 5B).

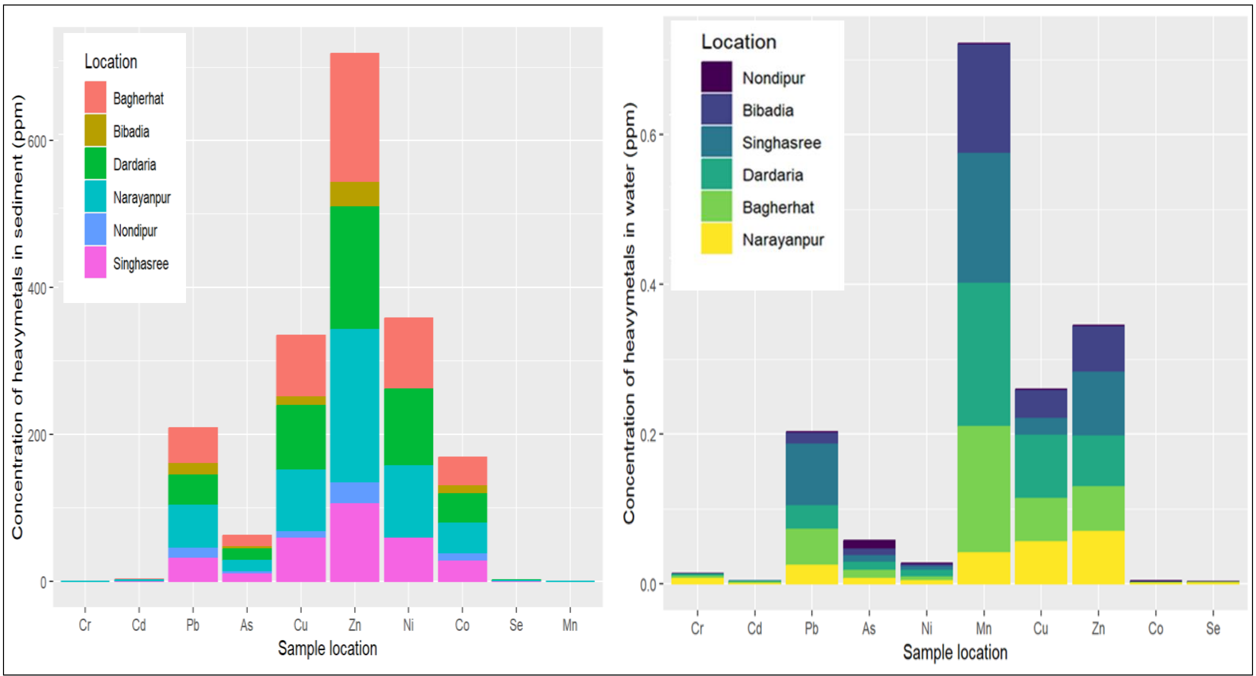

Fig 5: The relative concentration of 10 heavy metals in water (A) and sediment (B) of six sampling locations of Shitalakshya River.

Relationship of heavy metals with the abundance of Mollusca

The correlations among all measured heavy metals (Cr, Cd, Pb, As, Ni, Mn, Cu, Zn, Se, Co) and Molluscan abundance showed that molluscs responded negatively to all metals except for chromium which support the tolerance capacity of some molluscs to chromium. However, overall molluscan density showed a wide range of sensitivity to most of the heavy metals measured in this study. in an opposite manner (Fig 6). All measured heavy metals were strongly correlated (positive) with each other (Pearson's correlation, r>0.5 (Fig 6). Among all metals, Mollusca showed positive correlation with the chromium indicating their increasing trend in chromium rich sediments. Simple linear regression analysis also supported this relationship for most of the metals. Furthermore, GLM with negative binomial distribution and log link shows that there was a significant correlation of Mollusca abundance with Pb concentration ($F = 4.91$, $df = 1, 17$, $p = 0.04$) (Fig 6B). However, GLM analysis did not reveal significant relationship for Cr as well as other metals such as Ni, Cu, Mn, Zn, Co and Se ($p>0.05$) with Mollusca abundance in sediment samples.

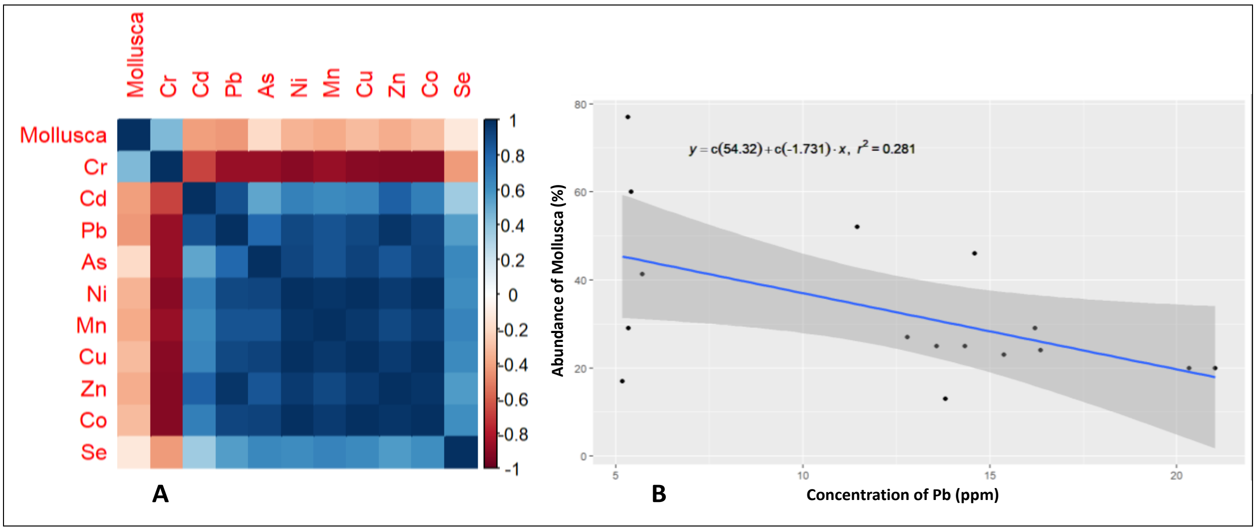

Fig 6: A. Correlogram showing correlations among all measured heavy metals and benthic organisms (Mollusca) in sediment samples. Blue colour denotes positive correlations where shade of the square box indicates the strength of the correlation. The association of Pb concentration with the abundance Mollusca species, across the sediment samples of six locations.

Discussions

Globally freshwater ecosystems are being endangered by various sources of pollution mainly from man-made factors including overpopulation, overexploitation of aquatic resources and pollution of river waters. Likewise, most of the river systems (nearby urban area) in Bangladesh are facing severe threats caused by industrial pollution, pollutants from aquatic vessels, various effluents from household activities. Therefore, this is an urgent plea of the concern scientific and policy making community to address this issue through practicing proper measures for management of the river ecosystems. Bio-monitoring or biological monitoring using bioindicators is one of the primary components to assess the ecological health of an aquatic body. To address the biomonitoring issues in the river systems of Bangladesh, the present study has attempted an assessment of molluscan fauna and the determination of heavy metal concentrations in water and sediments in the Shitalakshya river near by Dhaka city. In addition, the abundance of explored molluscan species were correlated with the sediment's metals and whether these bioindicator organisms are being impacted to what extent in the six sampling sites of the selected river.

Morpho-taxonomy based technique identified 16 molluscan species from this study, of which some are common (e.g., *Bellamya*, *Brotia*, *Lamellidens* spp.) and also supported by other explorative studies in other rivers like the River Buriganga, Turag and Brahmaputra [4, 14, 7]. Some rare species of bivalves such as *Corbicula fluminea*, *Lymnaea luteola* and *Novaculina* sp. were also recorded that require further taxonomic, biological and evolutionary research in future. This study also recorded some rare bivalves and gastropods which has added their occurrence, composition, abundance and distributional data to the existing biodiversity knowledge. DNA barcoding-based molecular studies could be further improved option to supplement these studies Molluscan fauna of Bangladesh.

The estimation of heavy metal concentration in sediment and water samples provided the variation of ten heavy metals in both samples which was supported by other studies conducted in this river and other adjacent rivers in the country [8, 9, 13, 15, 2]. Although there are many studies concerning the heavy metal determination in sediment, water, plant and animal tissues but there is a paucity of research dealing with their impact on the diversity, distribution and abundance of various bioindicator invertebrates in freshwater ecosystems, especially in river systems in Bangladesh.

A study on heavy metal contamination in soil of the Shitalakshya River reported that the highest concentration of Cr as 0.16 mg/kg, and Zn as 0.04 mg/kg during the monsoon season in Shitalakshya. During the summer, Pb levels in the Shitalakshya river were detected to be higher than the permissible level (0.16 mg/l) [15]. Study of on the heavy metals conducted in the Shitalakshya river [2] found that metals concentration varied from 4.31 to 7.83 µg/L for Ni, 41.24 to 63.15 µg/L for pb, 7.12 to 10.11 µg/L for Cd, 192.18 to 234.32 µg/L for Cr, and 156.38 to 254.07 µg/L for Cu. This current study determined the highest concentration for Mn and Zn for almost all sampling sites (e.g., Mn: 0.136 ppm and Zn: 78.282 ppm). The lowest concentration was observed for Cr, Cd, Se, and Co in water samples, while in sediment samples the lowest levels were found for Cr, Cd, Se, and Mn across the study area in the selected river.

This study also established a correlation among the heavy metals both in water and samples where there was a strong positive relation among them. Molluscan species negatively correlated with most of the heavy metal depositions except for Cr where the abundance of Mollusca decreased with increased concentration of metal deposition in the sediments. These significant findings were also supported by the generalized linear model (GLM) in particular for Pb. As expected, this result clearly indicates that are molluscan organisms are facing threats from a polluted environment and the populations of Molluscs are declining through responding to heavy metal pollution. As diverse Mollusca groups are also known as pollution tolerant group, its abundance was found to increase with the higher degree of chromium concentration. Therefore, these significant findings would be very useful information for pollution reduction considering the sources of those metals as well as for selecting potential bioindicators in the regular monitoring of the ecological health of the river ecosystems in Bangladesh.

Heavy metal concentration measured in sediments and water estimated in this study would help the concerned authority to prioritize the reduction of pollution sources as well as to make the public awareness of the potential health hazards of those metals. Furthermore, this study established the responses of Molluscan species to various heavy metals which could be used as baseline information in river monitoring using bioindicator macro-invertebrates. As this study was carried out in a single stretch of a long river (The River Shitalakshya), further extensive research should be carried out in many other rivers to explore the comprehensive biodiversity information of these understudied fauna and the impact of various pollution/alterations on the aquatic organisms in the river ecosystems in Bangladesh.

Acknowledgements

The authors thank to the Ministry of Science and Technology for grating a special allocation fund to conduct this research project. We also acknowledge the facilities provided by the Dhaka Laboratory of Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh.

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