Uttar Pradesh Journal of Zoology

Volume 45, Issue 20, Page 237-247, 2024; Article no.UPJOZ.4260
ISSN: 0256-971X (P)

Assessment of Heavy Metal (Zinc, Copper, Lead and Cadmium) Contamination in Common Edible Shellfish Species of the Hooghly River and Sunderbans, India

Kausiki Chakrabarti [a] and Sobhana Palit (Paul) [b*]

[a] Department of Zoology, Charuchandra College, Kolkata, West Bengal, India.
[b] Department of Zoology, Sree Chaitanya College, Habra, West Bengal, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i204581

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4260

Original Research Article

Received: 22/08/2024
Accepted: 25/10/2024
Published: 31/10/2024

ABSTRACT

The content of four heavy metals (Zinc, Copper, Lead and Cadmium) in muscles of three shell fish species - prawns -*Penaeus monodon* and *Penaeus indicus* and crabs-*Scylla serrata*, the most prime shellfish food resources of human in the domestic and international market were studied at four stations in the estuarine belt near Bay-of-Bengal which receives wastes of various types from industries and various anthropogenic activities. The samples were digested by acid digestion. The metal contents were quantified using an AAS (Atomic absorption spectrophotometer). The distribution of trace metals accumulated in the muscle tissues of the above mentioned species was

**Corresponding author: Email: sobhanapaul70@gmail.com;*

***Cite as:** Chakrabarti, Kausiki, and Sobhana Palit (Paul). 2024. "Assessment of Heavy Metal (Zinc, Copper, Lead and Cadmium) Contamination in Common Edible Shellfish Species of the Hooghly River and Sunderbans, India". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (20):237-47. https://doi.org/10.56557/upjoz/2024/v45i204581.*

in the order- Zn > Cu> Pb> Cd. In the present study, levels of Zinc in the shell fishes ranged from 07.44±0.65 to 128.10 ±2.96 ; Cu ranged from 5 ±0.15 to 99.14 ±1.31; Pb varied from 1.67± 0.14 to 23.99 ± 0.79 and Cd ranged from 1.29± 0.35 to 7.09 ± 0.80.The concentration of the four metals exhibited spatial variation as station 1 >station 2 > station 3 > station 4, which could be a reflection of different bioavailability of metal concentrations in the water body at different stations. The results obtained showed that the metal concentrations in most of the cases were above the threshold value recommended by WHO. Metals such as Cu, Zn, Pb, Cd, can affect various aquatic organisms as well as human being either directly or indirectly as they can enter into the food chain and may be biomagnified at higher trophic levels which is one of the top concerns for human health. Essential heavy metals such as Zinc and Copper are relatively less harmful at low concentration while non-essential metals such as Cd and Pb are highly toxic even at low concentrations.

Keywords: Heavy metals; Shellfish muscle; prawns; crabs.

1. INTRODUCTION

Large water bodies experience various kinds of contaminations as majority of the industrial, chemical, urban waste, agricultural runoff, etc. are discharged into these open water areas. India is a river based country and river Ganga is one of the most important rivers which originates in Gamukh cave and travels through many states such as Uttarakhand, Uttar Pradesh, Bihar, Jharkhand, and West Bengal. In West Bengal, it is named Hooghly River that runs through many areas and ultimately reaches the Bay of Bengal. Almost all the religious practices and anthropogenic activities happen at the banks of the river. Although metals or trace elements can occur naturally in the environment from erosion, rock weathering, etc., human activities such as mining, use of fertilizers, municipal sewage sludge, different types of agricultural and industrial practices also contribute to the presence of metallic trace elements or heavy metals in the aquatic environment. In recent years, there has been an increasing public health concern associated with environmental contamination by these metals. Heavy metals can enter various ecosystems in many ways. Aquatic invertebrates such as the shellfishes feed through filter feeding and metal pollutants enter these shell fishes mainly via food, gills, oral consumption of water, sediment particles and the skin. Metals such as Cu, Zn, Cr, Pb, Cd, Hg can affect various aquatic organisms as well as human being either directly or indirectly as these can enter into the food chain and may be biomagnified at higher trophic levels (Hossain et al., 2018; Zhang et al., 2016; Ahmed et al., 2015; Kibria et al., 2016). As these metals are accumulated in the bodies of the aquatic organisms, monitoring of heavy metals need to be done to ascertain the pollution level of the aquatic ecosystems and their effects on the human body. Essential heavy metals such as Zn and Cu are relatively less harmful at low concentration while non-essential metals such as Cd and Pb are highly toxic even at low concentration (Kim et al., 2019; Lee et al., 2022). The lower part of the Hooghly estuary has various industries such as paper, textiles, chemicals, pharmaceuticals, plastic, food, leather, jute, etc. These units are point sources of heavy metals in the estuarine water (Mitra et al., 2010; Paul & Sinha, 2013; Mondal et al., 2021).

People are very fond of shell fishes almost throughout the world. Shellfishes have high economic value as a source of protein and minerals and are able to meet the food need of the people. In particular, shellfishes rarely migrate and have longer lifespan. Therefore, they can act as an useful indicator for identifying the pollutants (Oosterom et al., 2010).

To prevent the bioaccumulation of heavy metals in the human body, a study is needed to analyse the heavy metal content of the concerned environment. In this study, heavy metal (Zinc-Zn, Copper-Cu, Lead-Pb and Cadmium-Cd) contents were analysed using the AAS (Atomic absorption spectrophotometer) method, on three different species of shell fishes obtained from the lower stretches of the river Hooghly, around Bakkhali and Sunderban areas of West Bengal, India, at the apex of Bay of Bengal. This study aims to analyse the concentrations of the metals Zn, Cu, Pb and Cd in the muscle tissue of the commonly edible and commercially important crustacean species like prawns -*Penaeus monodon* and *Penaeus indicus* and crabs-*Scylla serrata*. These shell fishes are commercially very important and available easily in the local markets for human consumption. This research work will emphasize the need for monitoring heavy metals in these widely consumed seafood species to ensure consumer safety.

2. MATERIALS AND METHODS

2.1 Description of the Study Site

The Hooghly estuarine system which is constituted by the first offshoot of the river Ganga - the Bhagirathi, flows southwards through the lower Ganga deltaic plane and joins the Bay of Bengal in Sunderbans. A total of four sampling sites were selected (Fig. 1) to collect the edible shellfishes inhabiting the zone.

Two sampling sites namely, Diamond Harbour (station 1) and Kakdwip (station 2) were in the vicinity of Hooghly estuary and two sampling sites namely, Sagar South (station 3) and Chemaguri (station 4) were selected in the western sector of Indian Sundarbans, a Gangetic delta at the apex of the Bay of Bengal.

2.2 Brief Description of the Sampling Stations

Diamond Harbour (station 1) is located at 22º11´4.2´´N Latitude 88º11´22.2´´E longitude in the Hooghly estuarine region. The station is very near to Haldia Port cum Industrial complex.

Kakdwip (station 2) located at 21º52'35.7"N latitude; 88º11'55.0″E longitude in the Hooghly estuarine region. It is located at the southern part of Gangetic delta. A considerable migration of people occurred from countryside to the areas of Diamond Harbour and Kakdwip in search of farming, fishery or agriculture-based livelihood.

Sagar South (Stn.3) located at 21º 39′ 04''N latitude and 88º 01′ 47''E longitude is situated at the confluence of the River Hooghly and the Bay of Bengal in the western sector of Indian Sundarbans. The station is an important navigational channel for the major ports of the area. It has also tourism units and many fishing ventures.

Chemaguri (Stn.4) located at 21º39′ 49''N latitude and 88º 09′ 11''E longitude is situated at the western sector of Indian Sundarbans and faces river Muriganga at the eastern side. The western sector receives wastes and effluents from various industries concentrated mainly in the upstream zone.

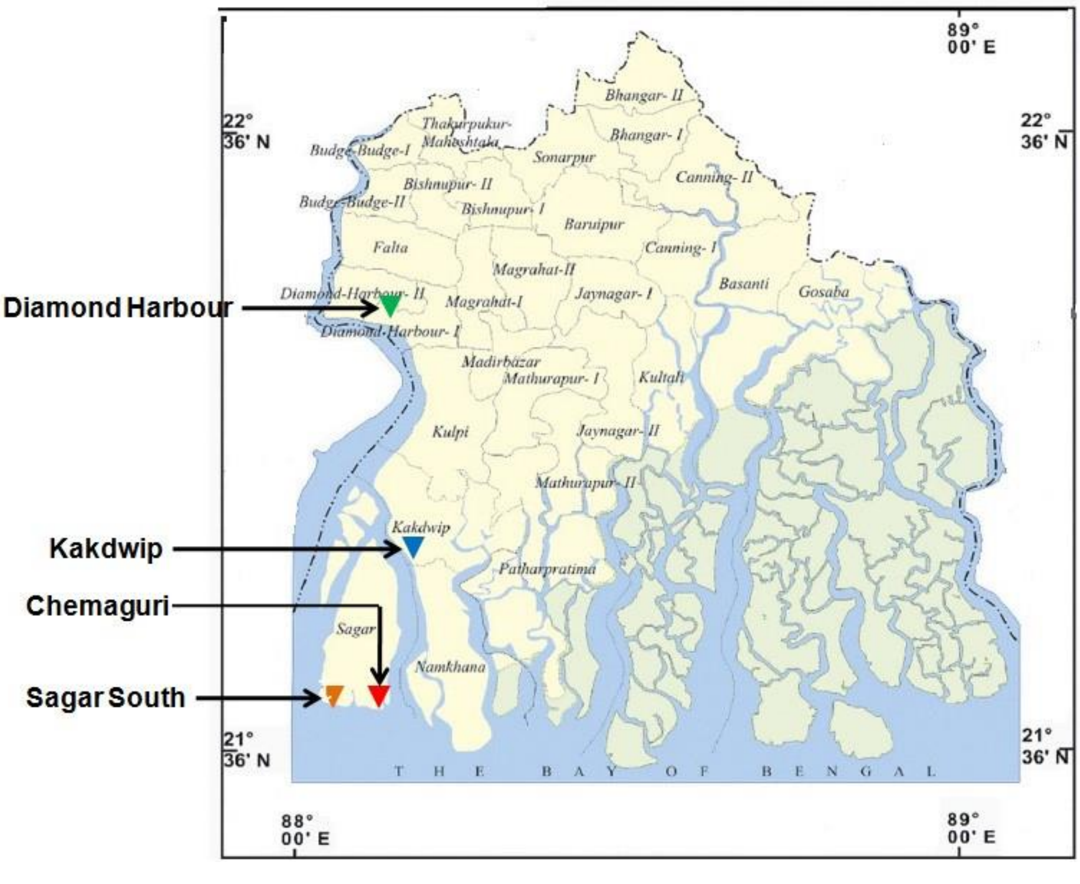

Fig. 1. Map of Gangetic delta showing four sampling stations

2.3 Sampling of Specimens

Two commonly edible prawn species (*P. monodon* and *P. indicus*) and one crab species (*S. serrata*) were collected from the selected stations (Fig. 1). The sampling was performed randomly from the markets and temporary markets. The collected samples were stored in a container, preserved in crushed ice, and brought to the laboratory for further analysis. Similar sized specimens of each species were sorted out for analyzing the heavy metal level in the muscle of prawns and crabs.

2.4 Analysis of Heavy Metal Content

The muscle tissues of the samples were first subjected to dry ashing. Dried samples were digested in nitric acid using a microwave digester and then transferred to a 50 ml volumetric flask. Then the solution was made up to the marking line of the flask with demineralized water and filtered using Whatman filter paper. This filtrate was used as a sample solution for heavy metal analysis. The absorbance of the sample solution was measured using an atomic absorption spectrophotometer (Model Perkin Elmer Type 2380). Concentrations of the selected metals (Zn, Cu, Pb and Cd) were expressed in ppm (mg/kg dry wt. basis).

2.5 Statistical Analysis

Two way analysis of variance (ANOVA) was performed to assess whether heavy metal concentrations varied significantly between sites and the species. Possibilities less than 0.01 ($P < .01$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

3. RESULTS

The results obtained with respect to Zn, Cu, Pb and Cd concentrations in the shellfishes were as follows: 7.44ppm/ dry wt.– 128.1ppm/dry wt. (Zn), 5 ppm/dry wt.– 99.14 ppm/dry wt. (Cu), 0 – 23.99 ppm/dry wt. (Pb) and 0-7.09ppm/dry wt. (Cd). The results of Zn, Cu, Pb and Cd contents in the shellfishes are revealed in the Tables 1,2,3,4 and the Figs. 2a,b,c,d respectively.

The results showed that the highest heavy metal content was found in the crab *Scylla serrata* with Zn content of 128.10ppm /dry weight at station 1 (Fig. 3). The value of lead and cadmium were found to be below detectable limit at stations 3 and 4. However, the Figs. 2a,2b,2c,2d, showed that most of the samples at four stations gave positive results for Zn, Cu, Pb and Cd metals with different levels.

Table 1. Zn concentrations (in ppm /dry weight) in shell fish muscles

Species	station 1 (Diamond Harbour)	station 2 (Kakdwip)	station 3 (Sagar South)	station 4 (Chemaguri)
Penaeus monodon	99.8±2.30	44.42±1.47	31.45±1.0	9.1±0.98
Penaeus indicus	95.5±2.41	39.65±1.49	24.6±1.35	7.44±0.65
Scylla serrata	128.1±2.96	85.09±1.80	66.23±1.68	23.12±1.02

Table 2. Cu concentrations (in ppm /dry weight) in shell fish muscles

Species	station 1 (Diamond Harbour)	station 2 (Kakdwip)	station 3 (Sagar South)	station 4 (Chemaguri)
Penaeus monodon	71.18±1.12	36.69±0.51	8.06±0.26	7.12±0.19
Penaeus indicus	62.99±1.16	28.11±0.57	7.05±0.29	5±0.15
Scylla serrata	99.14±1.31	50.44±0.66	35.77±0.61	27.99±0.41

Table 3. Pb concentrations (in ppm /dry weight) in shell fish muscle

Species	station 1 (Diamond Harbour)	station 2 (Kakdwip)	station 3 (Sagar South)	station 4 (Chemaguri)
Penaeus monodon	4.18±0.38	2.56±0.25	BDL	BDL
Penaeus indicus	3.76±0.43	1.67±0.14	BDL	BDL
Scylla serrata	23.99±0.79	17.85±0.50	7.44	3.79

(BDL -below detectable limit)

Table 4. Cd concentrations (in ppm /dry weight) in shell fish muscles

Species	station 1 (Diamond Harbour)	station 2 (Kakdwip)	station 3 (Sagar South)	station 4 (Chemaguri)
Penaeus monodon	2.3±0.55	1.4±0.33	BDL	BDL
Penaeus indicus	1.89±0.62	1.29±0.35	BDL	BDL
Scylla serrata	7.09±0.80	2.12±0.49	BDL	BDL

(BDL -below detectable limit)

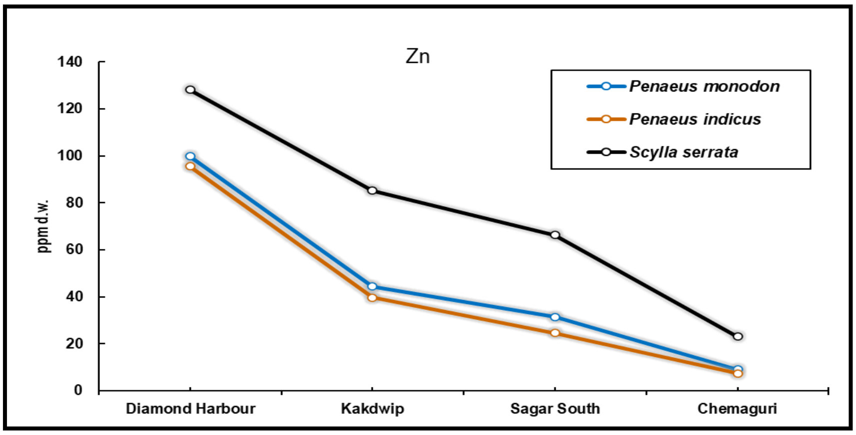

Fig. 2a. Concentration of Zn (ppm dry wt.) accumulated in selected shell fishes in the study sites

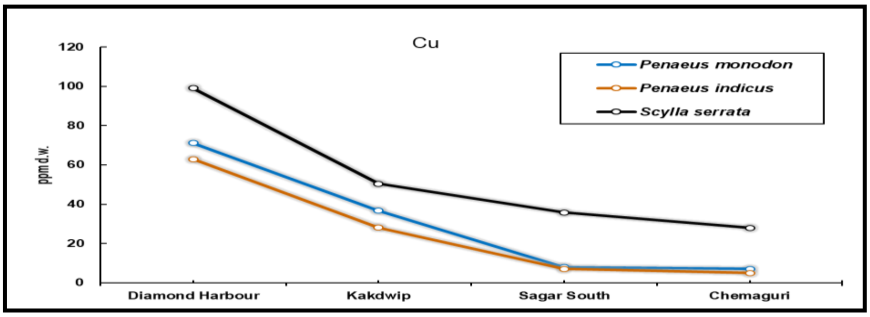

Fig. 2b. Concentration of Cu (ppm dry wt.) accumulated in selected shell fishes in the study sites

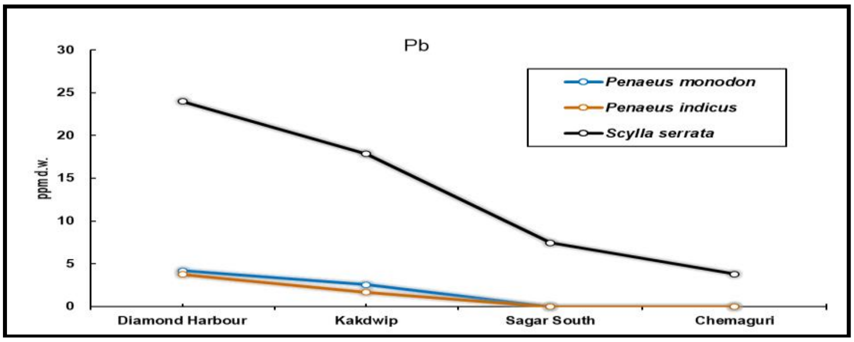

Fig. 2c. Concentration of Pb (ppm dry wt.) accumulated in selected shell fishes in the study sites

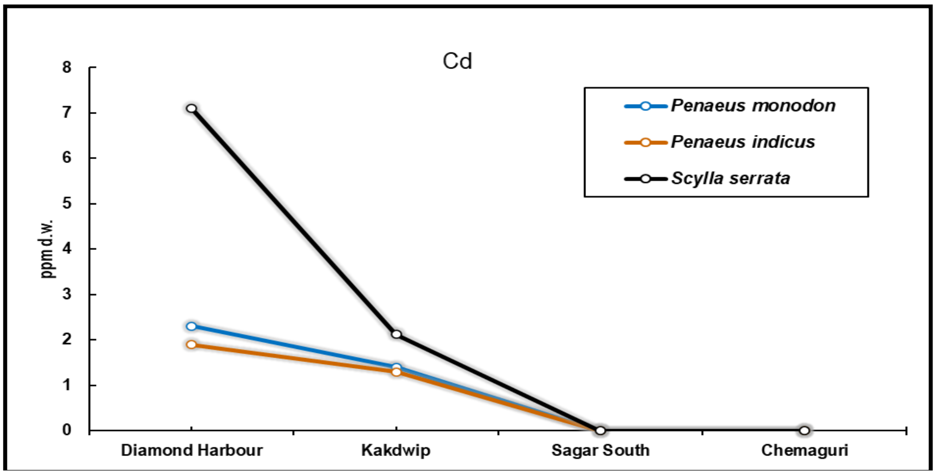

Fig. 2d. Concentration of Cd (ppm dry wt.) accumulated in selected shell fishes in the study sites

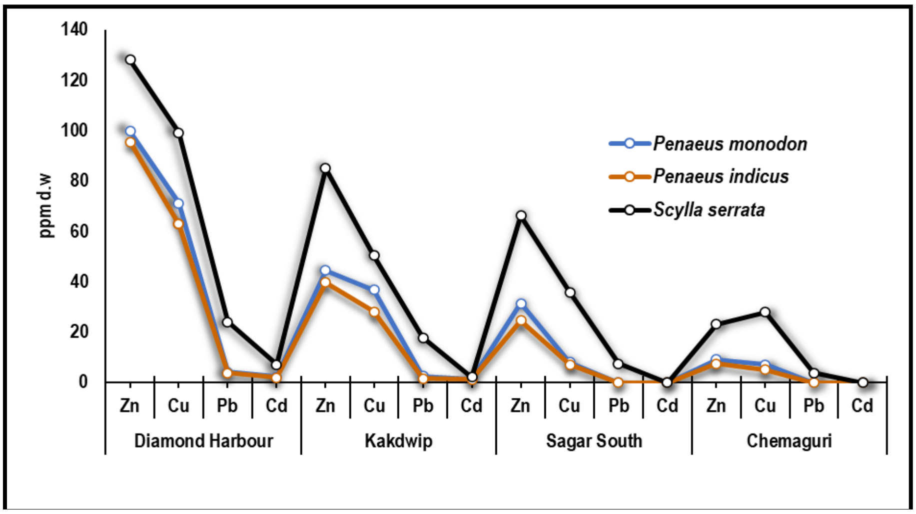

Fig. 3. Concentration of selected heavy metals (ppm dry wt.) accumulated in selected shell fishes at the four study sites

Table 5. ANOVA for Zn

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Species	2708.2043	2	1354.1022	26.0982	0.001096	5.1433
Between Stations	14211.8057	3	4737.2686	91.3034	0.000021	4.7571
Error	311.3095	6	51.8849			
Total	17231.32	11				

Table 6. ANOVA for Cu

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Species	1724.1775	2	862.0888	50.5905	0.000175	5.1433
Between Stations	7870.8491	3	2623.6164	153.9634	0.000005	4.7571
Error	102.2432	6	17.0405			
Total	9697.27	11				

Table 7. ANOVA for Pb

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	F_{crit}
Between Species	368.1462	2	184.0731	9.9430	0.012453	5.1433
Between Stations	170.9015	3	56.9672	3.0772	0.112077	4.7571
Error	111.0769	6	18.5128			
Total	650.1247	11				

Table 8. ANOVA for Cd

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	F_{crit}
Between Species	5.5826	2	2.7913	1.4511	0.306163	5.1433
Between Stations	28.5508	3	9.5169	4.9476	0.046179	4.7571
Error	11.5413	6	1.9235			
Total	45.67469	11				

Table 9. Maximum permitted concentration of metals as per WHO compared with the concentrations of metalsobtained in the sampling sites

Metals	**Range (ppm/dry wt,)**	**WHO,1989 level in food**
Zn	07.44±0.65 - 128.10 ±2.96	100 ppm
Cu	05 ±0.15 - 99.14 ±1.31	30 ppm
Pb	BDL - 23.99 ± 0.79	2 ppm
Cd	BDL - 7.09 ± 0.80	1 ppm

4. DISCUSSION

In the present study, level of Zinc in the shell fishes ranged from 07.44±0.65 (station 4) to 128.10 ±2.96 (station 1) in the stations under study (Fig. 2a). Highest level of Zn concentration was found in the crab -*Scylla serrata* at station 1 (Fig. 3). It was found that the concentration of Zn in all the shell fish species were relatively higher compared to the concentration of other metals under study in the same animals. The similar findings were also recorded in fishes caught from different water bodies (Yap & Al-Mutairi, 2022; Dione et al., 2021; El-Khatib et al., 2020). Significant variations of Zn were observed between the stations and also between the species, *P*< .01(Table 5). Zn is an essential element for normal growth of animals. It also plays a role in the physiological processes of aquatic animals as it is important as a catalyst for proper functioning of different enzymes. So it exhibited highest accumulation in the shellfish muscle when compared with the other three metals. However, at station 1, the concentration of Zinc exceeded the recommended maximum level of Zn (Table 9) allowed in food by World Health Organization which is 100 ppm (World Health Organization, 1989).

Cu is also essential for animal metabolism but may be toxic at high levels. Levels of Copper in the shell fishes ranged from 5 ±0.15 (station 4) to 99.14 ±1.31(station 1) in the stations under study (Fig. 2b). Significant variations of Cu were observed between the stations and also between the species, *P*<.01(Table 6). In the present geographical locale, the antifouling paints used for conditioning fishing vessels and trawlers seems to be one of the important contributors in raising Cu levels in these areas (Mitra et al., 2010). The results obtained revealed that the Cu concentrations were higher in most of the stations than the recommended value of Cu (Table 9) in sea food, which is 30 ppm as prescribed by WHO (1989). Highest level of Cu concentration was again found in the crab -*Scylla serrata* at station 1 (Fig. 3).

Lead is one of the most toxic metals coming from the industries and factories which can make its way into the water body and affect the aquatic organisms. It is a neurotoxin that hampers the survival and growth of the aquatic organisms (Burger et al., 2002). The concentrations of lead however, were below detectable limit in the prawns at stations 3 and 4 only but a low concentration of 3.79 to 7.44 ppm/dry wt. was found in *Scylla* in the same stations (Fig. 2c). At stations 1 and 2 the concentration of Pb varied from 1.67± 0.14 to 23.99 ± 0.79 in *Scylla* at station 1. The differences in metal accumulation between species and stations were not significant for Pb (Table 7). When compared with the recommended value of WHO (1989) (Table 9) in context to consumption as food (2 mg/kg for Pb), the concentrations found in the shell fishes were mostly above this level at stations 1 and 2.

Table 10. Patterns of trace metal occurrence in muscle of several shellfish species from different studies

Species	Tissues	Order	Reference
Penaeus monodon	muscle	Zn>Fe>Pb>Cd	Guhathakurta & Kaviraj (2000)
Scylla serrata	muscle	Zn > Cu> Pb> Cd	Rudra et al. (2016)
S. glomerata	muscle	Fe > Cu > Zn > As > Mn > Al > Pb > Cr	Jahan & Strezov (2019)
M. rosenbergii	muscle	Fe > Zn > Cu > Cr > Ni > Pb > As > Cd	Chakma et al. (2024)
M. rosenbergii	muscle	Zn > Ni > Cu > As > Pb > Cd	Ahmed et al. (2020)

Except for stations 1 and 2, levels of Cadmium in shell fish muscle were below detectable level at the other stations. When Cd is absorbed through the sea foods it tends to accumulate in the human body in places such as liver and kidney (Mol et al., 2010). In the present study, Cd concentrations in the crustacean species obtained from stations 1 and 2 ranged from 1.29± 0.35 to 7.09 ± 0.80 (Fig. 2d), all found to be above the threshold value of 1ppm as recommended by WHO,1989 (Table 9). The differences in metal accumulation between species and stations were not significant for Cd (Table 8).

Heavy metals have the tendency to accumulate in various organs of marine organisms, especially fishes and shell fishes which in turn may enter into the human metabolism through food causing serious health hazards. Each organ of the shellfish body is different in metabolic function. Also the metal detoxification process in the body of shellfish affects the distribution of metals in different tissues of shellfishes (Al-Weher, 2008).

Table 10 presents a general view on the results of several studies regarding the order of the heavy metal content in the edible tissues or whole body of a shellfish species. In the present study, the distribution of the trace metals accumulated in the muscle tissues of all the species followed the order Zn > Cu> Pb> Cd. With respect to the results presented in Table 10, it can be concluded that in most of the cases (except in limited cases) concentrations of zinc and copper were higher than the other studied elements.

Differences in the metal levels in shellfishes at different stations studied here also indicate differences in the amount of harmful metals received in the water bodies. Mitra et al. (2018) analysed that the industrial effluents polluted the water and increased heavy metal deposition into the river. It seems that stations 1 and 2 are facing more anthropogenic pressure due to the chain of factories located near the river bank as highest amount of metal accumulation was recorded from there. Station 1 is basically in the Gangetic delta region which is exposed to all sorts of industrial and anthropogenic activities as it is in proximity to the highly urbanized city of Kolkata and the Haldia port-cum-industrial complex, which may be attributed to the high concentration of most metals in the shell fish muscle. Station 2 falls in the navigational route of the ships in the Hooghly channel through which the wastes of the upstream region find their way to Bay of Bengal. Hence shellfish sampled from these two stations exhibited considerable concentrations of metals in their muscles. The concentrations of the four metals exhibited spatial variation as station 1 >station 2 > station 3 > station 4, which could be a reflection of different bioavailability of metal concentrations in the water body at different stations. However many other factors such as season, body weight, nature of water, etc. affect the accumulation of metals in the muscle tissues and also species have different metabolic requirements for specific trace elements as indicated by Soegianto et al. (2008).

Shrimps are rich source of protein and omega-3 polyunsaturated fatty acids and can reduce the risk of cardiovascular disease, stroke, and diabetes (Smith & Guentzel, 2010). But they can accumulate toxins and pollutants from their environment, raising potential health concerns. In our study, the difference in the pattern of heavy metal distribution in the three shellfish species might be a result of their difference in feeding habits, nature of their habitats, ecological needs, metabolism, etc. The nature of the shellfishes is sessile for which it is easy for the heavy metals to concentrate and accumulate in their muscles. Metal accumulation levels in crabs were high in all the cases compared to the prawns. They pick up particulate matter from surrounding water and in particular sediments while feeding. Crabs are bottom feeder and are generally expected to concentrate more metals than surface feeders like prawn. In the present study it was observed that highest level of metal accumulation occurred in the crab – *Scylla serrata* - the Mud crab, one of the prime shellfish food resources of human in the domestic and international market. This is in agreement with Kilgour (1991) who indicated that the animals in close relationship with the sediment, showed a relatively high concentration of metals. So they can also be good indicators for biomonitoring of metal pollution in the aquatic environment.

Heavy metals at certain concentrations are required for different physiological processes in a human body but they are harmful at certain concentrations as well. So wastewater, industrial efluents, etc. must be treated prior to their discharge into the water bodies. Industries must use the heavy metal detection methods before discharging their wastes. Contaminations of aquatic resources by heavy metals coming from different anthropogenic activities and their toxic effect on aquaculture and human health have been reported by many workers (Lee et al., 2022; Sonone et al., 2021; Mukherjee et al., 2022).

5. CONCLUSION

The results showed that the highest value of heavy metal content was found in the crab *Scylla serrata* with respect to all the heavy metals studied. The content of Zn, Cu, Pb and Cd found in the shell fishes showed different levels for each metal where the Zn content of the crab muscle was higher than the levels of other metals. The discharges of urban and industrial wastewater into the waterbody get the shell fishes exposed to the heavy metals. Many metal concentrations in many places also crossed the threshold level mentioned by WHO. The accumulation of heavy metals in the muscles of the shellfishes is one of the top concerns for the health of the human being.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Principal of the college for encouraging research work of the faculties. Also heartily thank Professor Dr. Abhijit Mitra for his valuable guidance and continuous support. Without his guidance it would not be possible to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4260