

Bioaccumulation Pattern of Heavy Metals in the Shrimps of the lower stretch of the River Ganga

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Abstract - Rapid industrialization and urbanization have contaminated the riverine and estuarine ecosystems to a great extent. To evaluate such kind of contamination, we undertook a research programme to analyze the concentrations of zinc, copper, lead and cadmium in muscle tissue of five commonly edible shrimp species, namely *Penaeus monodon*, *Penaeus indicus*, *Penaeus semisulcatus*, *Penaeus marguensis* and *Metapenaeus brevicornis* collected from the lower stretch of the River Ganga (in the Sundarbans delta complex). The heavy metals in tissue samples were estimated using a Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer. Concentrations in shrimp species ranged as follows: Zn: $4.11 \pm 0.13 - 353.45 \pm 2.98$; Cu: $3.43 \pm 0.10 - 140.49 \pm 1.81$; Pb: BDL - 8.21 ± 0.63 and Cd: BDL - $3.66 \pm 0.12 \text{ mg kg}^{-1}$ dry weight. Irrespective of species, heavy metals accumulated in the shrimp muscle in the order Zn > Cu > Pb > Cd. The concentration of heavy metals in the tissues varied significantly depending upon the locations from where the species were collected. Although the concentration of selected heavy metals were within the normal range in all stations, but at station 1 (Nayachar Island) the metal level has exceeded in the muscle of shrimp species as a food source for human consumption. The shrimp samples were collected from different locations of lower Gangetic region with different degree of industrial and anthropogenic activities and station 1 is exposed to maximum stress (in terms of pollution) from the adjacent port-cum-industrial zone of Haldia and industrial discharge of multifarious industries situated in and around the city of Calcutta (upstream to station 1) along the bank of the River Ganga.

Keywords: Heavy metals, edible shrimp, Indian Sundarban.

Introduction

Metals generally enter the aquatic environment through atmospheric deposition, erosion of geological matrix or due to anthropogenic activities caused by industrial effluents, domestic sewage and mining wastes (Reddy *et al.*, 2007). From an environmental point of view, coastal zones can be considered as the geographic space of interaction between terrestrial and marine ecosystems that is of great importance for the survival of a large variety of plants, animals and marine species (Castro *et al.*, 1999). Adverse anthropogenic effects on the coastal environment include eutrophication, heavy metals, organic and microbial pollution and oil spills (Boudouresque

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and Verlaque, 2002). The discharge of these wastes without adequate treatment often contaminate the estuarine and coastal water with conservative pollutants (like heavy metals), many of which bioaccumulate in the tissues of resident organisms like fishes, oysters, crabs, shrimps, seaweeds *etc.* In many parts of the world, especially in coastal areas and on smaller islands, shellfish is a major part of food, which supplies all essential elements required for life processes in a balanced manner (Iyengar, 1991). The shellfish (particularly the shrimp) is also the major exportable item of countries like India and therefore keenly related to economy of the country. Hence, it is important to investigate the levels of heavy metals in these organisms to assess whether the concentration is within the permissible level and will not pose any hazard to the consumers (Krishnamurti and Nair, 1999).

The Gangetic delta, formed by the depositional activity of the River Ganga is located at the apex of Bay of Bengal. The deltaic lobe is unique for its wilderness, mangrove gene pool and tiger habitat. However due to intense industrial activities in the upstream zone, and several anthropogenic factors, the western part of the deltaic complex is exposed to pollution from domestic sewage and industrial effluents leading to serious impacts on biota. The presence of Haldia port-cum-industrial complex in the downstream region of the River Ganga (also known as the Hooghly River) has accelerated the pollution problem with a much greater dimension. The organic and inorganic wastes released from industries and urban units contain substantial concentrations of heavy metals. The central part of the delta (encompassing the surroundings of Matla River) is relatively less stressful in terms of industrial discharge. Due to siltation of the Bidyadhari channel the area does not receive any water supply from the Hooghly River in the western sector and is therefore tide-fed in nature receiving the tidal flux from the Bay of Bengal (average salinity = ~32 psu). The present paper aims to highlight the level of selective heavy metals (Zn, Cu, Pb and Cd) in the muscle of five commercially important species of shrimp collected from the aquatic subsystem of four stations distributed in two sectors (western and central Indian Sundarbans) of the lower Gangetic region.

Materials and Methods

Description of the study site

Two sampling sites were selected each in the western and central sectors of Indian Sundarbans, a Gangetic delta at the apex of the Bay of Bengal. The deltaic complex has an area of 9630 sq. Km and houses 102 islands. The western sector of the deltaic lobe receives the snowmelt water of mighty Himalayan glaciers after being regulated through several barrages on the way. It also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the upstream zone. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel since the late 15th century (Chaudhuri and Choudhury, 1994). The present geographical locale

thus offers a unique test bed to study the effect of pollution on biological species. On this background four sampling stations (two each in western and central sectors) were selected (Table 1 and Fig. 1) to analyze the concentrations of heavy metals in the muscles of five commonly edible shrimp species.

Sampling of Specimen

Five species of shrimps, namely *Penaeus monodon*, *Penaeus indicus*, *Penaeus semisulcatus*, *Penaeus marguensis* and *Metapenaeus brevicornis* were collected during high tide condition from the selected stations (Table 1) during a scientific trip from 15th to 25th April, 2009. 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 metal level in the muscle.

Table 1. Sampling stations with coordinates and salient features

Station	Coordinates	Salient Features
Nayachar Island (Stn.1)	88° 15' 24" E 21° 45' 24" N	It is located in the Hooghly estuary in the western sector of lower Gangetic delta and faces the Haldia port-cum-industrial complex that houses a variety of industrial units.
Sagar South (Stn.2)	88° 01' 47" E 21° 39' 04" N	Situated at the confluence of the River Hooghly and the Bay of Bengal in the western sector of Indian Sundarbans.
Gosaba (Stn. 3)	88° 39' 46" E 22° 15' 45" N	Located in the Matla Riverine stretch in the central sector of Indian Sundarbans.
Annpur in Satjelia Island (Stn. 4)	88° 50' 43" E 22° 11' 52" N	Located in the central sector of Indian Sundarbans. Noted for its wilderness and mangrove diversity; selected as our control zone.

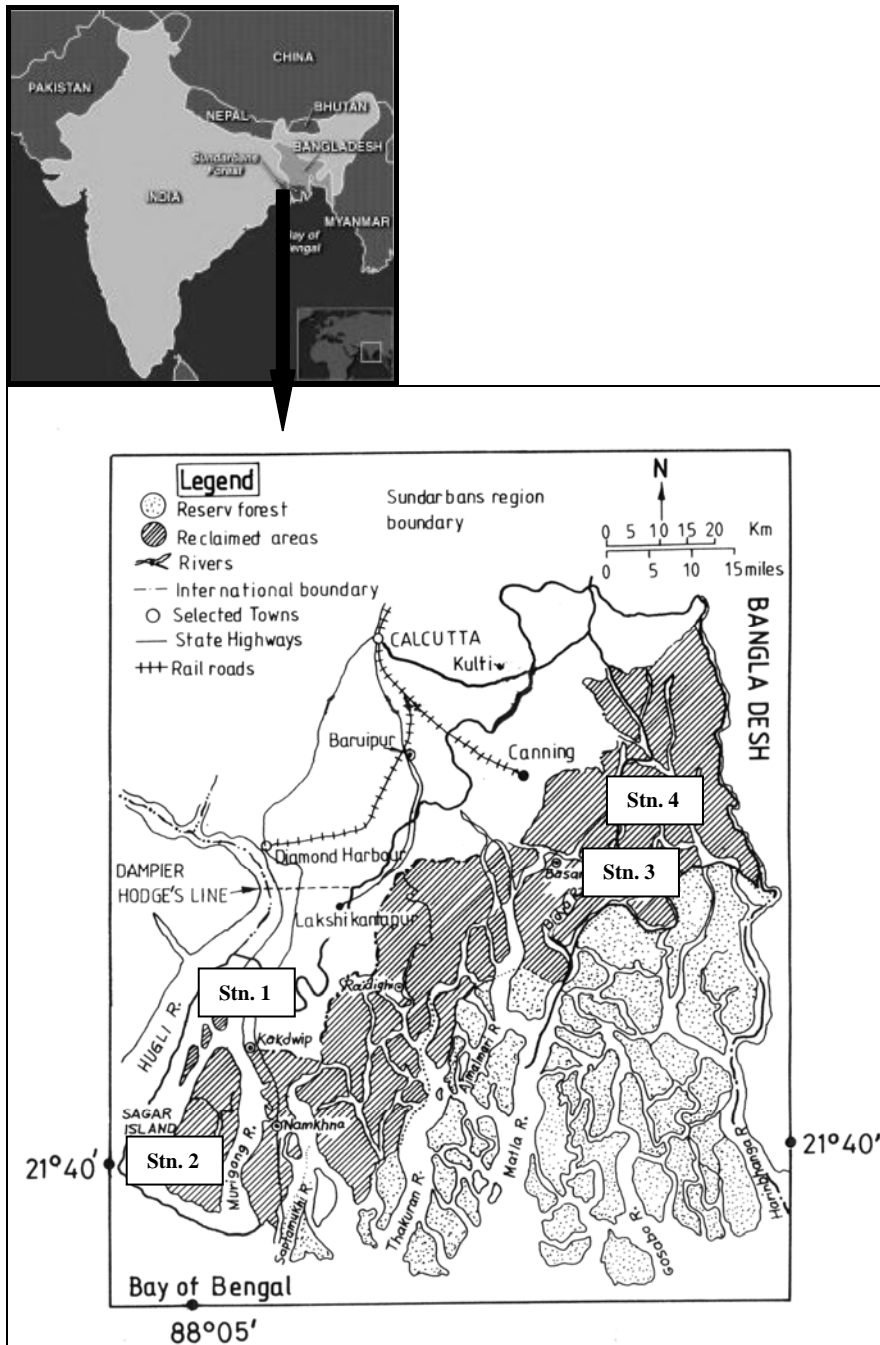
MAP OF INDIA

Figure 1. Location of sampling stations

Laboratory analysis

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is now - a - day accepted as a fast, reliable means of multi-elemental analysis for a wide variety of sample types (Date and Gray, 1988). A Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer was used for the present analysis. A standard torch for this instrument was used with an outer argon gas flow rate of 15 L/min and an intermediate gas flow of 0.9 L/min. The applied power was 1.0 kW. The ion settings were standard settings recommended, when a conventional nebulizer/spray is used with a liquid sample uptake rate of 1.0 ml/min. A Moulinex Super Crousty microwave oven of 2450 MHz frequency magnetron and 1100 W maximum power Polytetrafluoroethylene (PTFE) reactor of 115 ml volume, 1 cm wall thickness with hermetic screw caps, were used for the digestion of the muscle samples of the shrimps. All reagents used were of high purity available and of analytical reagent grade. High purity water was obtained with a Barnstead Nanopure II water-purification system. All glasswares were soaked in 10% (v/v) nitric acid for 24 h and washed with deionised water prior to use.

The analyses were carried out on composite samples of 10 specimens of each species having uniform size. This is a measure to reduce possible variations in metal concentrations due to size and age. 20 mg composite muscle samples from each species of shrimps were weighed and successively treated with 4 ml aqua regia, 1.5 mL HF and 3 ml H₂O₂ in a hermetically sealed PIFE reactor, inside a microwave oven, at power levels between 330-550 W, for 12 min to obtain a clear solution. The use of microwave-assisted digestion appears to be very relevant for sample dissolution, especially because it is very fast (Nadkarni, 1984; Matusiewicz and sturgeon, 1989; De la Guardia, 1990). After digestion, 4 ml H₂BO₃ was added and kept in a hot water bath for 10 min, diluted with distilled water to make up the volume to 50 ml. Taking distilled water in place of muscle samples and following all the treatment steps described above the blank process was prepared. The final volume was made up to 50 ml. Finally, the samples and process blank solutions were analyzed by ICP-MS. All analyses were done in triplicate and the results were expressed with standard deviation.

The accuracy and precision of our results were checked by analyzing standard reference material (SRM, Dorm-2). The results indicated good agreement between the certified and the analytical values (Table 2).

Statistical analysis

A logarithmic transformation was done on the data to improve normality. Analysis of variance (ANOVA) was performed to assess whether heavy metal concentrations varied significantly between sites and species; possibilities less than 0.01 ($p < 0.01$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows.

Table 2. Concentrations of metals found in Standard Reference Material DORM-2 (dogfish muscle) from the National Research Council, Canada (all data as means \pm standard errors, in mg kg⁻¹ dry wt).

Value	Zn	Cu	Pb	Cd
Certified	26.8	2.34	0.065	0.043
SE	2.41	0.18	0.009	0.005
Observed*	23.9	2.29	0.060	0.040
SE	1.99	0.17	0.006	0.006
Recovery (%)	89.2	97.8	92.3	93.0

*Each value is the average of 5 determinations.

Results and Discussion

The coastal zone receives a large amount of metal pollution from agricultural and industrial activity (Usero *et al.*, 2005). Pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biota (Islam and Tanaka, 2004). There is still a general concern about the impact of metals in the aquatic environment (Grosell and Brix, 2005). Heavy metals have contaminated the aquatic environment in the present century due to intense industrialization and urbanization. Heavy metal contamination of the environment has been occurring for centuries, but its extent has increased markedly in the last fifty years due to technological developments and increased consumer use of materials containing these metals. The Gangetic delta is no exception to this usual trend. The rapid industrialization and urbanization of the city of Kolkata (formerly known as Calcutta), Howrah and the newly emerging Haldia complex in the maritime state of West Bengal has caused considerable ecological imbalance in the adjacent coastal zone (Mitra and Choudhury, 1992; Mitra, 1998). The Hooghly estuary, situated on the western sector of the Gangetic delta receives drainage from these adjacent cities, which have sewage outlets into the estuarine system. The chain of factories and industries situated on the western bank of the Hooghly estuary is a major cause behind the gradual transformation of this beautiful ecotone into stinking cesspools of the megapolis (Mitra and Choudhury, 1992). The lower part of the estuary has multifarious industries such as paper, textiles, chemicals, pharmaceuticals, plastic, shellac, food, leather, jute, tyres and cycle rims (UNEP, 1982). These units are point sources of heavy metals in the estuarine water. Heavy metals such as zinc, copper, lead and cadmium are normal constituents of marine and estuarine environments, but when additional quantities are introduced through industrial wastes or sewage, they enter the biogeochemical cycle and pose adverse impact on the biotic community. According to Hashmi *et al.* (2002) continental sources (river runoff and atmospheric transport), oceanic sources (upwelling) and diagenetic exchanges at water-sediment interface have been identified as the factors that influence the heavy metals in aquatic organisms. Due to toxic nature of certain heavy metals, these chemical constituents interfere with the ecology of a particular environment and on entering into the food chain they cause potential health hazards,

mainly to human beings. Reports on metal concentration in shrimps and crabs under natural conditions for coastal waters of India are limited (Zingde *et al.*, 1976; Matkar *et al.*, 1981; Qasim *et al.*, 1988). It was reported by several workers that the discharge of heavy metals into the sea through rivers and streams results in the accumulation of pollutants in the marine environment especially within shrimps (Yusof *et al.*, 1994). Thus shellfish and shellfish products can be used for monitoring potential risk to humans because these are directly consumed by a large population (Subramanian and Sukumar, 1988). Bioaccumulation patterns of metals in shellfish muscle can be utilized as effective indicators of environmental metal contamination (Atchison *et al.*, 1977; Larsson *et al.*, 1985). According to many researchers, some shellfishes by virtue of their mobile nature are not fair indicator of aquatic contamination, but their regular consumption by human beings makes it absolutely necessary to monitor their different organs, particularly the muscles. The present study is therefore important not only from the safety point of view of human health, but also from the quality point of view as many of these shellfish species have high export value.

In the present study heavy metals accumulated in the shrimp muscle in the order $Zn > Cu > Pb > Cd$. Significant spatial variations of heavy metal concentrations in shrimp muscles were observed between the selected stations, which reflects the adverse impact of industrialization and urbanization on the biotic community.

Of the four metals studied in the present work, Zn and Cu are essential elements while Pb and Cd are non-essential element for most of the living organisms. The concentrations of zinc and copper in all the shrimp samples were also relatively higher, compared to the concentrations of other metals in same shrimp samples. It can be explained because these metals (Cu and Zn) are essential elements required by animals for metabolic process. Zinc and copper appear to diffuse passively (probably as a soluble complex) by the gradients created through adsorption of membrane surfaces and are found in blood proteins metallothioneins. Carbonell and Tarazona (1994) concluded that different tissues of aquatic animals provide and/or synthesize nonexchangeable binding sites resulting in different accumulation levels. Romeo *et al.*, (1999), point out that the affiance of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism, and the contamination gradients of water, food and sediment, as well as other factors, such a salinity, temperature and interacting agents.

Zn being an essential element for normal growth and metabolism of animals, exhibited highest accumulation in the shrimp muscle when compared with the other three metals. According to the results obtained, the zinc levels in the muscle of shrimp species from station 1 (Nayachar Island) facing the Haldia port-cum-industrial complex were highest (Table 3 and Fig. 2), which were higher than the permissible level, *i.e.*, 400 ppm in crustacean tissue (Franklin, 1987). However the values were much lower than the permissible limit for human consumption, which is 1000 ppm for prawn (FAO, 1992).

Significant differences were observed in Zn concentrations between the stations and also between the species ($p < 0.01$). Zn accumulated as per the order *Penaeus semisulcatus* > *Penaeus marguensis* > *Penaeus indicus* > *Penaeus monodon* > *Metapenaeus brevicornis* in all the stations. Except for the station 1 sample, the concentrations of Zn were within the permissible level (values ranged from 4.11 ± 0.13 to 63.47 ± 1.08 in three other stations). The main sources of Zn in the present geographical locale are the galvanization units, paint manufacturing units and pharmaceutical processes, which are mainly concentrated in the Haldia industrial sector (opposite to station 1).

Table 3. Zn concentrations (in ppm/dry wt.) in shrimp muscles.

Species	Stn. 1	Stn. 2	Stn. 3	Stn. 4
<i>Penaeus monodon</i>	333.88 ± 2.91	48.40 ± 1.06	27.33 ± 1.13	7.71 ± 0.15
<i>Penaeus indicus</i>	342.50 ± 2.97	62.13 ± 1.16	34.66 ± 1.15	11.34 ± 0.20
<i>Penaeus semisulcatus</i>	353.45 ± 2.98	63.47 ± 1.08	40.20 ± 1.12	18.39 ± 0.26
<i>Penaeus marguensis</i>	349.61 ± 2.99	59.08 ± 1.07	33.14 ± 1.00	13.44 ± 0.20
<i>Metapenaeus brevicornis</i>	326.19 ± 2.76	19.22 ± 1.00	6.29 ± 0.55	4.11 ± 0.13

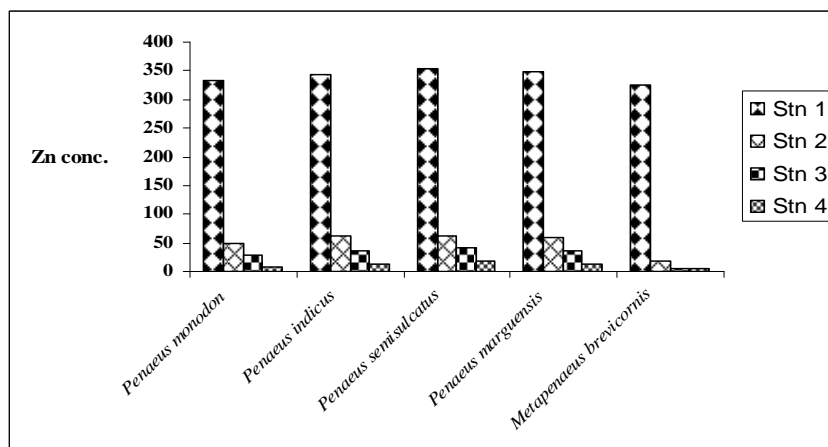


Figure 2. Zn conc. (in ppm/dry weight) in shrimp muscles.

In case of Cu concentrations significant differences were observed between the stations and also between the species ($p < 0.01$). Cu accumulated in the shrimp muscle as per the order *Penaeus semisulcatus* > *Penaeus marguensis* > *Penaeus indicus* > *Penaeus monodon* > *Metapenaeus brevicornis*. Levels of Copper in shrimps from the selected

stations (except for station 1) ranged from 3.67 ± 0.10 to 42.91 ± 0.60 $\mu\text{g/g}$, far below the normal permissible range, *i.e.*, 120 ppm as recommended for the crustacean tissue (Franklin, 1987). In case of station 1 the value ranged from 102.35 ± 2.00 to 140.49 ± 1.81 $\mu\text{g/g}$ (Table 4 and Fig. 3), which was higher than the maximum limit of 10 ppm for prawn consumption as recommended by the Food and Agricultural Organization (FAO, 1992).

Table 4. Cu concentrations (in ppm/dry wt.) in shrimp muscles.

Species	Stn.1	Stn.2	Stn.3	Stn.4
<i>Penaeus monodon</i>	111.30 ± 1.18	28.09 ± 0.34	9.10 ± 0.11	3.67 ± 0.10
<i>Penaeus indicus</i>	119.66 ± 1.17	39.85 ± 0.56	13.80 ± 0.16	4.93 ± 0.11
<i>Penaeus semisulcatus</i>	140.49 ± 1.81	42.91 ± 0.60	18.46 ± 0.17	7.48 ± 0.13
<i>Penaeus marguensis</i>	130.26 ± 1.63	39.75 ± 0.49	15.10 ± 0.14	5.25 ± 0.10
<i>Metapenaeus brevicornis</i>	102.35 ± 2.00	9.81 ± 0.14	4.85 ± 0.10	3.77 ± 0.12

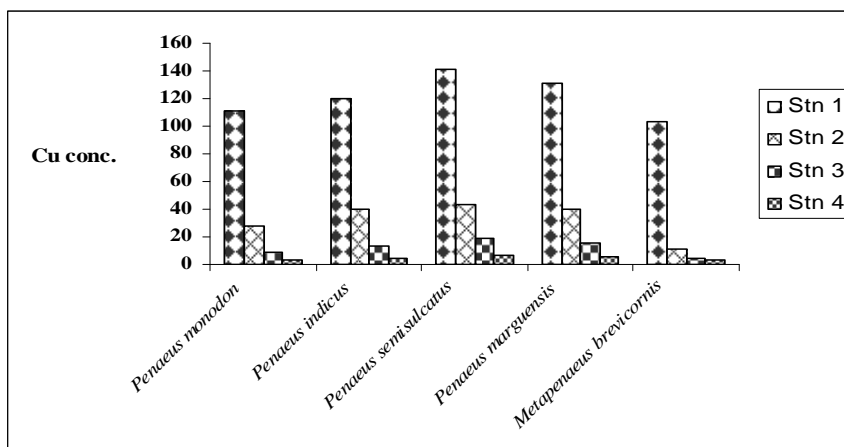


Figure 3. Cu conc. (in ppm/dry weight) in shrimp muscles.

The main sources of Cu in the coastal waters are antifouling paints (Goldberg, 1975), particular type of algaecides used in different aquaculture farms, paint manufacturing units, pipe line corrosion and oil sludges (32 to 120 ppm). Ship bottom paint has been found to produce very high concentration of Cu in sea water and sediment in harbours of Great Britain and southern California (Bellinger and Benham, 1978; Young *et al.*, 1979). In the present study area, the major source of Cu is the antifouling paints used for conditioning fishing vessels and trawlers apart from industrial discharges (that is predominant around station 1). This is the reason why Cu

was detected in the shrimp samples of stations 3 and 4, even there is no existence of industries. The complete siltation of the Bidyadhari River also does not permit the industrial effluents released in the Hooghly River to mix with the rivers in the central sector of the deltaic complex (location zone of stations 3 and 4).

Pb is a toxic heavy metal, which finds its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries *etc.* Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain lead as an important component. These paints are designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately is transported to the sediment and aquatic compartments. Lead also enters the oceans and coastal waters both from terrestrial sources and atmosphere and the atmospheric input of lead aerosols can be substantial. Station 1 is exposed to all these activities being proximal to the highly urbanized city of Kolkata, Howrah and the newly emerging Haldia port - cum - industrial complex, which may be attributed to high Pb concentrations in the shrimp muscle (4.29 ± 0.23 in *Penaeus monodon*, 7.48 ± 0.60 in *Penaeus indicus*, 8.21 ± 0.63 in *Penaeus semisulcatus*, 7.11 ± 0.58 in *Penaeus marguensis* and 2.07 ± 0.19 in *Metapenaeus brevicornis*). Station 2 falls in the navigational route of the ships and tankers in the Hooghly channel through which the wastes of the upstream region find way to Bay of Bengal. Hence shrimps sampled from these stations exhibited considerable concentrations of Pb in the muscles. Stations 3 (Gosaba) and 4 (Satjelia island) are in the central sector of Gangetic delta, almost without any industrial activities which may be attributed to low concentrations lead in the shrimp samples collected from these areas (Table 5 and Fig. 4). We also observed significant statistical differences in Pb concentrations between the stations and also between the species ($p < 0.01$). Except for station 1 levels of lead in shrimp muscle from three locations were below the permissible level which is $4.0 \mu\text{g/g}$ for crustacean tissue (Franklin, 1987). When compared with the recommended value of WHO (1989) in context to consumption of prawn (2 ppm for Pb), the concentrations in all the shrimp species from stations 1 and 2 were above this level.

Table 5. Pb concentrations (in ppm/dry wt.) in shrimp muscles.

Species	Stn.1	Stn.2	Stn.3	Stn. 4
<i>Penaeus monodon</i>	4.29 ± 0.23	2.18 ± 0.13	BDL	BDL
<i>Penaeus indicus</i>	7.48 ± 0.60	2.49 ± 0.10	BDL	BDL
<i>Penaeus semisulcatus</i>	8.21 ± 0.63	3.10 ± 0.17	BDL	BDL
<i>Penaeus marguensis</i>	7.11 ± 0.58	3.70 ± 0.15	BDL	BDL
<i>Metapenaeus brevicornis</i>	2.07 ± 0.19	2.01 ± 0.07	BDL	BDL

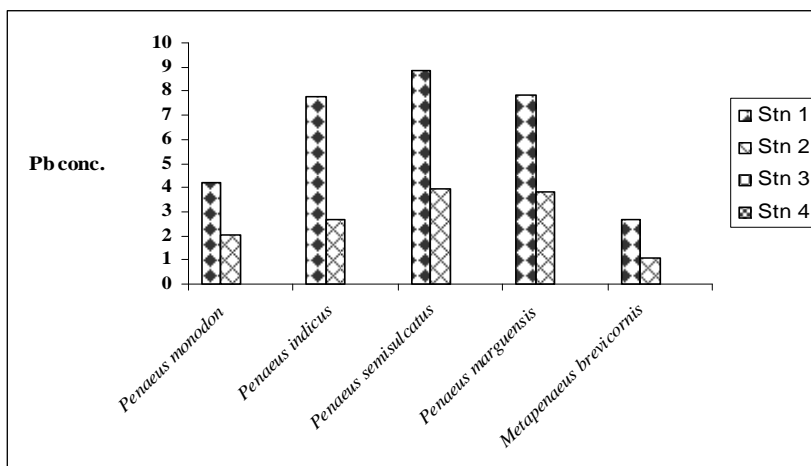


Figure 4. Pb conc. (in ppm/dry weight) in shrimp muscles.

In the present study the concentration of Cd ranged from BDL to 3.66 ± 0.12 ppm (Table 6 and Fig. 5). Cadmium levels in all the shrimp species from station 1 and *Penaeus semisulcatus* from station 2 were much above the normal range ($0.02 \mu\text{g/g}$). The values were also higher than the WHO (1989) recommended value for prawn consumption which is 1 ppm. The main sources of Cd in the present geographical location are electroplating, manufacturing of Cd alloys and of pigments and plastic stabilizers, production of Ni-Cd batteries and welding. No trace of Cd was recorded in the shrimp muscle from stations 3 and 4. Significant differences were observed in Cd concentrations between the stations and also between the species ($p < 0.01$).

Table 6. Cd concentrations (in ppm/dry wt.) in shrimp muscles.

Species	Stn.1	Stn.2	Stn.3	Stn. 4
<i>Penaeus monodon</i>	1.14 ± 0.09	BDL	BDL	BDL
<i>Penaeus indicus</i>	2.40 ± 0.05	BDL	BDL	BDL
<i>Penaeus semisulcatus</i>	3.66 ± 0.12	1.10 ± 0.06	BDL	BDL
<i>Penaeus marguensis</i>	1.09 ± 0.07	BDL	BDL	BDL
<i>Metapenaeus brevicornis</i>	BDL	BDL	BDL	BDL

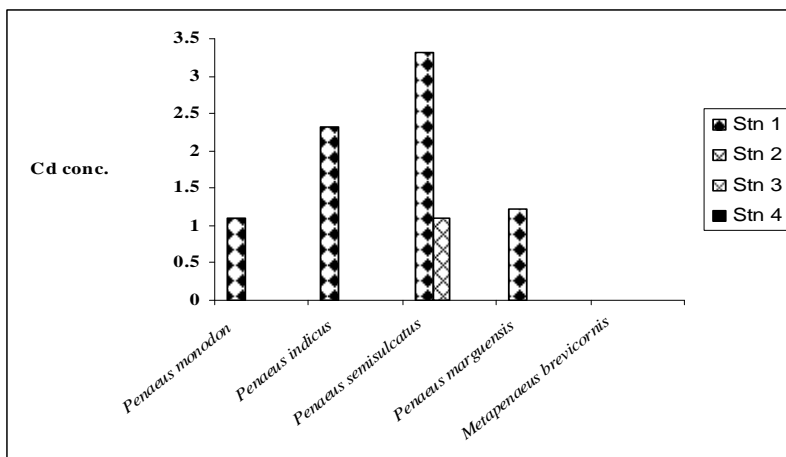


Figure 5. Cd conc. (in ppm/dry weight) in shrimp muscles.

Conclusions

The River Ganga in the Indian sub-continent is the lifeline of millions in terms of livelihood and natural resources. However due to rapid industrialization, urbanization and unplanned tourism, a negative impact has been exerted on the positive health of the aquatic system. The contamination of water is also transmitted in the biological compartment, many of which are consumed as food by the local people. The present study is important not only from the human health point of view, but it also presents a comparative account of heavy metals in shrimps from two different sectors of Gangetic delta that are physico-chemically different.

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