



Analysis of Trace Metals in Commercially Important Crustaceans Collected from UNESCO Protected World Heritage Site of Indian Sundarbans

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Abstract

The study, which measured the concentrations of Zn, Cu, Pb and Cd, in muscle tissue of 5 commercially important crustacean species (*Penaeus monodon*, *Penaeus indicus*, *Penaeus semisulcatus*, *Penaeus marguensis* and *Metapenaeus brevicornis*) collected from the UNESCO declared world heritage site Indian Sundarbans. The Indian Sundarbans at the apex of Bay of Bengal is recognized as one of the most diversified and productive ecosystems in the world located at the confluence of Hooghli- Matla estuarine complex. However, due to intense industrialization, urbanization and increase of anthropogenic activities in recent era, Indian Sundarbans have been contaminated with heavy metals which vary with seasons and often exhibit pronounced monsoonal effect. Significant variation of heavy metals in muscle tissue of 5 commercially important crustaceans or shrimp species collected from four different sampling stations (2 each in central and western sector) of Indian Sundarbans were observed. The distribution of trace metals accumulated in all the muscle tissues of shrimp species followed the order Zn > Cu > Pb > Cd. The concentration of metals exhibited significant spatial variation and followed the order station 1 > station 2 > station 3 > station 4, which may be related to different degree of contamination in different location ($p < 0.01$) that indicated the adverse impact of industrialization and urbanization on the edible crustaceans community. Although the concentration of selected heavy metals were within the normal range in all stations, but at station 1 the metal level has exceeded in the muscle of shrimp species as a food source for human consumption.

Keywords: Indian Sundarbans, Hooghli-Matla estuarine complex; *Penaeus monodon*, *Penaeus indicus*, *Penaeus semisulcatus*, *Penaeus marguensis*, *Metapenaeus brevicornis*.

Introduction

Metals are a group of the most important pollutants which cause environmental degradation in coastal areas. Trace metals are introduced into the aquatic ecosystems in a number of ways. These chemicals accumulate in the tissues of aquatic organisms at concentrations many times higher than concentrations in water and may be biomagnified in the food chain to levels that cause physiological impairment at higher trophic levels and in human consumers (Raposo *et al.*, 2009). Heavy metals are natural constituents of the Earth's crust and are present in varying concentrations in all ecosystems. During the past two decades, high levels of metals and their compounds, both inorganic and organic, have been released to the environment as a result of a variety of anthropogenic activities (Komarnicki, 2005).

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). Coastal pollution has been increasing significantly over the recent years and found expanding environmental problems in many developing countries. Urban and industrial activities in coastal areas introduce significant amount of trace metals into the marine environment, causing permanent disturbances in marine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to a number of flora and fauna species, including humans, through food chains (Boran and Altınok, 2010).

Heavy metals were chosen as suitable pollutants because they are widespread environmental contaminants, from either natural or anthropogenic sources, and are widely believed to be a threat to the health and survival of many marine or aquatic animals, including crustaceans (Lorenzon *et al.*, 2001). The contaminated fish and crustaceans from

aquatic environment may become a public health concern. Hence, it is important to determine the concentrations of heavy metals in commercial fish and shrimps in order to evaluate the possible risk of human consumption (Cid *et al.* 2001). Pollution enters fin and shell fishes through five main routes: via food or non-food particles, gills, oral consumption of water and the skin. The use of fin and shell fishes as bio-indicators of metal pollution of aquatic environments and suitability for human use from toxicological point has been documented (Amin *et al.*, 2011). Apart from that, the sensitivity of crustaceans to heavy metals is well documented and for all these reasons, the importance of marine shrimp for environmental monitoring studies as bio-indicators of heavy metal pollution has been emphasized by several investigators (Yilmaz and Yilmaz, 2007).

The concentration of trace metals by seafood is a potential problem to man. Aquatic organisms accumulate metals to concentrations many times higher than present in water. Hence, estimation of heavy metal accumulation is of utmost importance in this sector of biotic community. Increased circulation of hazardous heavy metals in soil, water and air has raised considerable concern for environmental protection and human health. The Indian Sundarban regions are no exception to this usual trend. The rapid industrialization and urbanization of the city of Kolkata, 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, 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. 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. Thus crustacean products can be used for monitoring potential risk to humans because these are directly consumed by a large population (Subramanian and Sukumar, 1988). Reports on metal concentration in shrimps under natural conditions for coastal waters of India are limited (Qasim *et al.*, 1988; Mitra *et al.*, 2011). The growing rate of anthropogenic waste input into the creeks and estuaries leads to bioaccumulation of heavy metals in biota and their increased levels in economically important crustaceans have become a

matter of concern. 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).

Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the levels of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern. There is an increasing concern regarding the roles and fates of trace metals in aquatic ecosystem of Indian Sundarbans. Much of this concern arises from the low level of available information on the concentration of these metals within the environment. The present paper aims to highlight the level of selective trace metals (Zn, Cu, Pb and Cd) in the muscle tissue of five commercially important species of shrimps collected from the aquatic subsystem of four stations distributed in two sectors of the UNESCO (United Nations Education, Scientific and Cultural Organization) declared world heritage site Indian Sundarbans

Materials and Methods

Description of the Study Site

The Sundarbans is bestowed with a wide range of natural forest and aquatic resources, offering the largest mangrove concentration with a great biodiversity and the only mangrove tiger-land in the world. Being a group of estuarine islands, Sundarban region is located at the delta-face of Hooghly-Matla estuarine system in India. Indian Sundarbans comprises of 9630 sq. kms out of the total 71885 sq.kms., covering six and 13 police station areas of districts of North and South 24 Parganas, respectively, of the State of West Bengal, demarcated by Dampier & Hodges Line towards North, Bay of Bengal towards South, river Hooghly towards West and Ichhamati- Kalindi- Raimangal rivers towards East. Its geodetic location stretches from 21° 30' N to 22° 39' N latitudes and from 88° 5' E to 89° 09' E longitudes. The tidal action of the sea inundates the whole of Sundarbans to varying depths, pushing back silt to the channels and depositing there. Sundarbans delta is one of the dynamic estuarine deltas of the world. It is crisscrossed by water channels that bring with them tons of sediments from terrestrial sources and play a major role in the eroding and accreting nature of this deltaic estuary (Gurmeet, 2009; Mitra, 1998).

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. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel in the late 15th century (Chaudhuri and Choudhury, 1994). The western sector also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the upstream zone. On this background four sampling stations (two each in western and central sectors)

were selected (Table 1) to analyze the concentrations of heavy metals in the muscles of common edible shellfishes (Figure 1).

Sampling of Specimen

Five species of crustaceans, namely *Penaeus monodon*, *Penaeus indicus*, *Penaeus semisulcatus*, *Penaeus marguensis* and *Metapenaeus brevicornis* were collected during high tide condition from the

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 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 on 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.

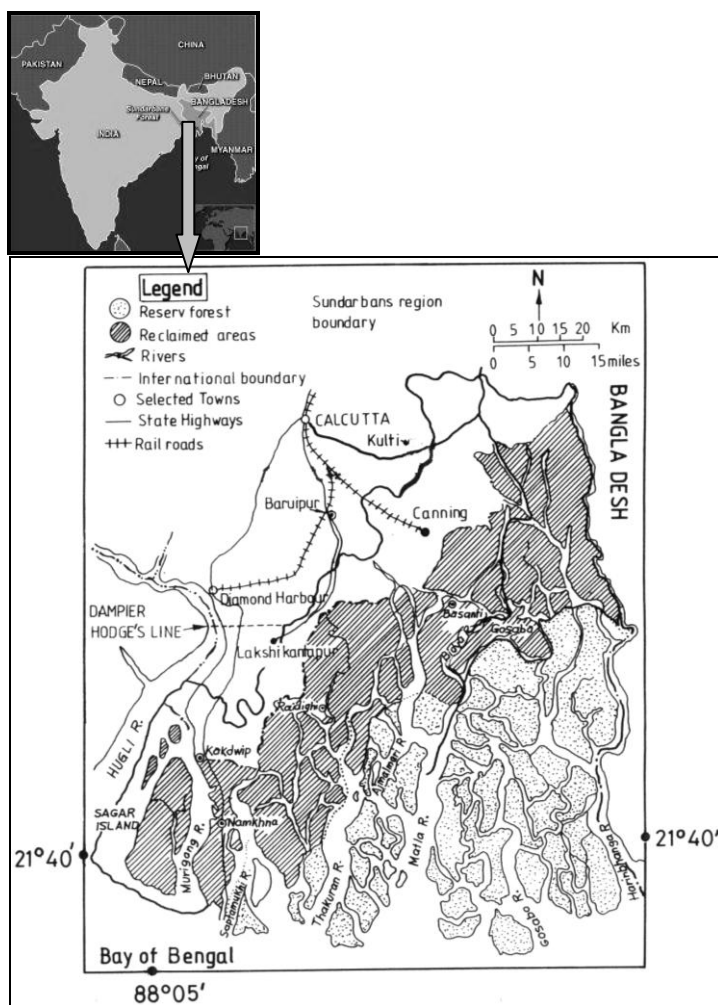


Figure 1. Map of the Study area at Indian Sundarbans

selected stations were collected during low tide condition from the selected stations during a rapid EIA study from 5th March April to 25th March April, 2011. (Table 1). 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.

Trace Metals 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 shellfish. 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 20 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 10 individuals of each species of shell fishes 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. 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 analysed 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. Possibilities less than 0.01 ($p < 0.01$) were considered statistically significant. All statistical calculations were performed with SPSS 9.0 for Windows. Statistical methods applied include correlation analysis ($P < 0.01$) was done for find out the relationship between 5 commercially important shrimp species for bioaccumulation of trace metals in all the selective stations.

Results

A number of studies have shown that various factors such as season, length and weight, physical and chemical status of water can play a role in the tissue accumulation of metals. The precision of technique was tested by replicate analysis of heavy metals (Table 2), using DORM-2 (dogfish muscle). Average recoveries were ranged from 89 to 97%. Number of sample, scientific, common name and feeding habits of each sample are summarized in Table 3. The mean concentrations of trace metals Zn, Cu Pb and Cd concentrations of commercially important shrimps as $\mu\text{g/g}$ dry weight for all the species studied are presented in Figures 2, 3, 4, 5.

In the present study, highest concentration of Zn was observed in all the species of shellfish followed by Cu, Pb and Pb (Figures 2, 3, 4, 5). The range of Zn varied from 31.23 to 98.10 ppm while that of Cu, Pb and Cd were from 12.98 to 65.80 ppm, 3.67 to 15.88 ppm and BDL to 8.40 ppm respectively. The species wise variation was not uniform for all the metals. Significant differences were observed in Zn concentrations between the stations and also between the species ($p < 0.01$). Zn accumulated as per the order

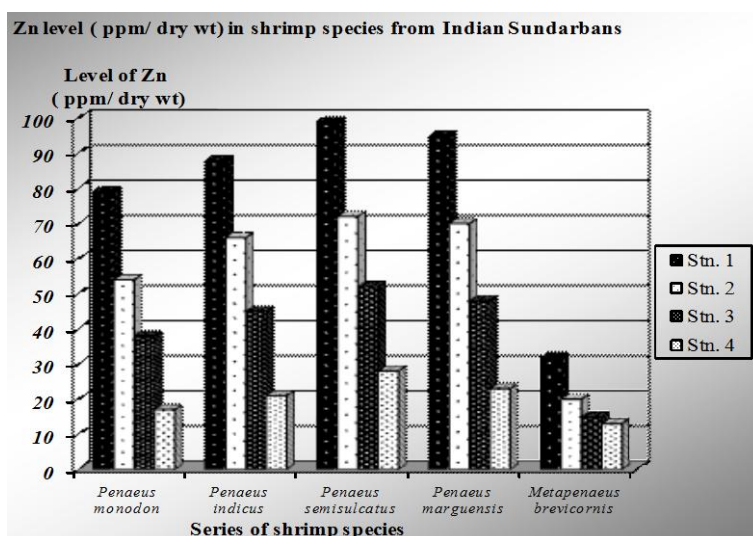
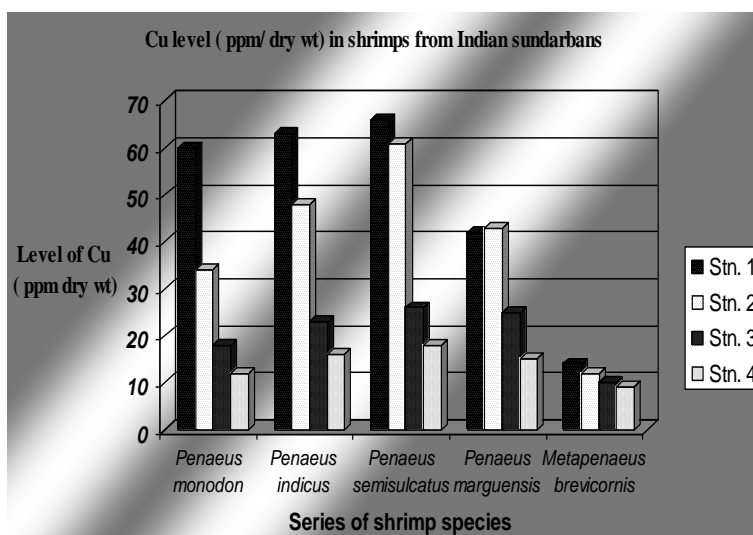
Table 2. Concentrations of metals found in Standard Reference Material DORM-2 (dogfish muscle) from the National Research Council, Canada (all data as means \pm standard errors, in ppm dry wt)

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

Table 3. Number of samples and its common name and feeding habit of studied shrimps

Sample species	Common Name	Feeding Habit	No.
<i>Penaeus monodon</i>	Black-tiger shrimp	Crustaceans, fishes, mollusks and polychaetes.	10
<i>Penaeus indicus</i>	Indian White shrimp	diatoms, copepoda, ostracods, amphipods, small crustaceans, molluscan larvae, polychaetes and detritus	10
<i>Penaeus merguensis</i>	Banana shrimp	Crustaceans, polychaetes, mollusks, fishes, Organic detritus, diatoms.	10
<i>Penaeus semisulcatus</i>	Green tiger shrimp	polyohaetes, crustaceans, molluscs diatoms, foraminiferousm radiolarians, detritus, fishes	10
<i>Metapenaeus brevicornis</i>	Yellow shrimp	vegetable matter, small crustacea, echiurid setae; large crustacea, remains of fishes, polychaeta and Sand grains	10

**Figure 2.** Zn concentration (in ppm/dry weight) in shrimp muscles.**Figure 3.** Cu concentration (in ppm/dry weight) in shrimp muscles.

Penaeus semisulcatus > *Penaeus marguensis* > *Penaeus indicus* > *Penaeus monodon* > *Metapenaeus brevicornis* (Fig. 2). In case of Cu concentrations significant differences were observed between the stations and also between the species ($p < 0.01$). Cu accumulated as per the order *Penaeus semisulcatus* >

Penaeus indicus > *Penaeus monodon* > *Penaeus marguensis* > *Metapenaeus brevicornis* (Figure 3). Pb accumulated as per the order *Penaeus semisulcatus* > *Penaeus marguensis* > *Penaeus indicus* > *Penaeus monodon* > *Metapenaeus brevicornis* (Fig 4). On the other hand, Cd accumulation of shrimp species

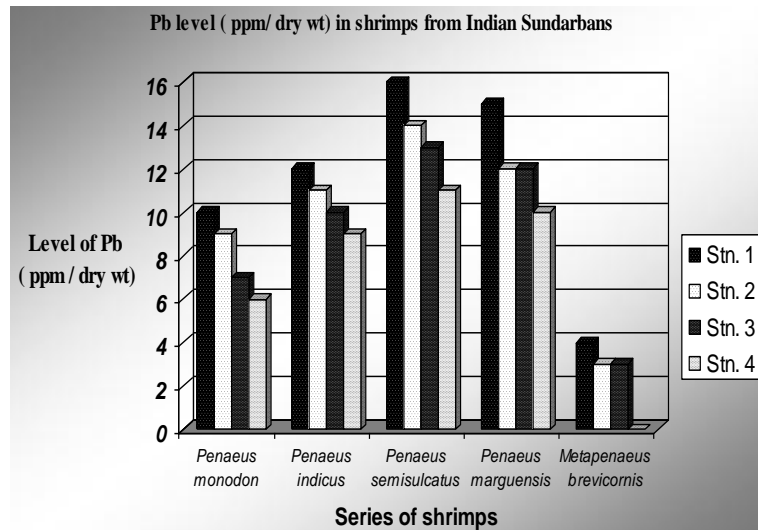


Figure 4. Pb concentration (in ppm/dry weight) in shrimp muscles.

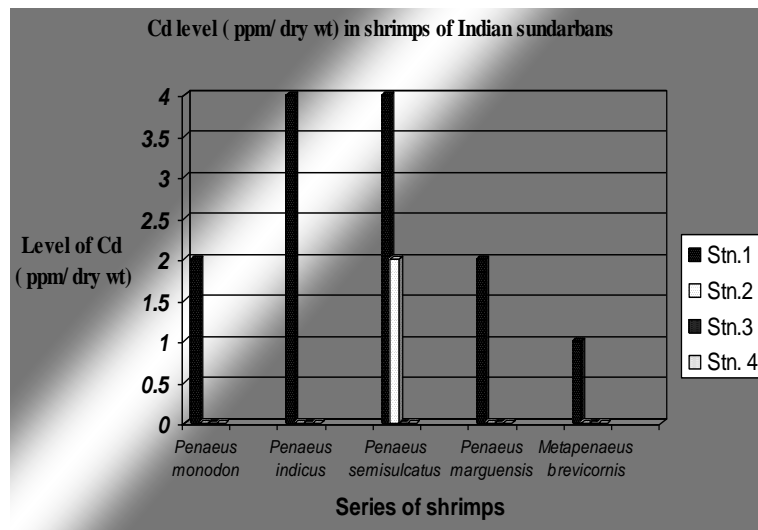


Figure 5. Cd concentration (in ppm/dry weight) in shrimp muscles

followed the ordered *Penaeus semisulcatus* > *Penaeus indicus* > *Penaeus marguensis* > *Penaeus monodon* > *Metapenaeus brevicornis* (Figure 5).

The levels of different metals showed variations among species as well as between stations (Figure 2, 3, 4 and 5). The station wise concentrations of Zn, Cu, Pb and Cd were in the order of Stn 1 > Stn 2 > Stn 3 > Stn 4. The highest concentrations of all the three heavy metals in shellfish muscles were found at station 1, i.e., Nayachar Island (proximal to the Haldia industrial belt) and the lowest concentrations were observed at Satjelia Island (far away from the industrial and anthropogenic stresses) in the Matla estuarine stretch.

Comparing heavy metal contents of shrimp species with one another, their coefficients of correlation (r) were calculated and the correlation between the metals was determined (Table 3). For the levels of Zn, Cu, Pb and Cd, inter-metal relationships appear to be different and positively correlated in the

environmental matrices.

Table 4 presented the maximum residue limit and maximum permitted concentration of metals in marine biota muscle especially for crustaceans (ppm dry weight) from various countries and organizations. Almost all metals measured in this study are relatively very close to the values recorded in aquatic animals from other regions of the world. The findings of other studies are summarized in Table 5, and are compared with the concentrations reported in this study and elsewhere in the world. In the present study, the pattern of trace metal concentration of five shrimp species were found as order Zn > Cu > Pb > Cd. Table 6 presents a general view on results of several studies regarding the order of heavy metals bioaccumulation in edible tissues or whole body of *Penaeus* shrimps

Discussion

The levels of heavy metal in aquatic animals

Table 4. Maximum residue limit and maximum permitted concentration of metals in marine biota muscle (ppm dry weight) from various countries and organizations

Contaminant	U.K. ¹	Australia ²	Hong Kong ¹	European Regulation 466/200t/EC ³	Indonesia ⁴	NHMRC ⁵	Turkey ⁶	US ⁷
Cd	--	0.80	8	0.4	--	0.05	0.05	1.5 ppm
Cu	80	--	--	--	80	--	20	60
Zn	200	--	--	--	400	150	250	200
Pb		6.0	24	1.6	8	1.5	0.2	3 ppm

Note: 1. Parsons(1998); 2. Otway(1992); 3. Tyrrell et al. (2005); 4. Decree of General Director of Food and Drug Supervision No.0 3725/B/SK/VII/89 concerning maximum limit of metals in food; 5. Australian National Health and Medical Research Council (Darmono and Denton (1990), 6. Turkey's food codex, 2008, 7. U.S- FDA, 2003

vary in various species and different aquatic environments (Canli and Atli, 2003). Trace element concentrations varied markedly among species. These variations are presumably due to individual samples being of different size categories, from different ecological niches, and from different trophic levels. Possibly, species also have different metabolic requirements for specific trace element (Soegianto *et al.*, 2008). The present study exhibited significant spatial variation in metal level amongst the species, which may be due to variations in environmental conditions. The western part of the Gangetic delta is connected to Himalayan glacier through Bhagirathi River. Researchers pointed out that the glaciers in the Himalayan range are melting at the rate of 23 m/yr (Hasnain 2002). This along with Farraka discharge has resulted in gradual freshening of the system, which has role in elevation of dissolved metal level in the system by way lowering of pH. The presence of chain of factories and industries along the bank of Hooghly estuary is another major cause of increased metal level in the aquatic phase of Hooghly estuary that have been reflected in the shellfish muscles of stations 1 and 2. Around these stations multifarious industries such as paper, textiles, chemicals, pharmaceuticals, plastic, shellac, food, leather, jute, tyres and cycle rims exist. These units are point sources of heavy metals in the estuarine water. The central sector on contrary is deprived from freshwater supply of Ganga-Bhagirathi system on account of siltation of Bidyadhari River in the 15th century. The Matla River, in the central sector is now tide fed with an increasing trend of salinity (Mitra *et al.*, 2009; Barua *et al.*, 2011). This results in the precipitation of dissolved metals on the sediment showing low metal level in the muscles of shellfish thriving in the system. The absence of factories and industries around Matla estuary is another cause for low concentrations of metals in the shellfish muscles.

Among the four metals studied in the present programme, Zn and Cu are essential elements while Pb and Cd are non-essential element for most of the living organisms. 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 (Mitra *et al.*, 2011). 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 Zn levels varied from 31.23 to 98.10 ppm in the muscle of shrimp species from station 1 (Nayachar Island) facing the Haldia port-cum-industrial complex were highest (Figure 2), which were lower than the permissible level, *i.e.* 400 ppm and 200 ppm in crustacean tissue for human consumption (Franklin, 1987; Parsons, 1999). However the values were much lower than the permissible limit for human consumption, which is 1000 ppm for prawn and 100 ppm for marine sea food (FAO, 1992; WHO, 1989).

In the present study, it was found that concentration of Zn in all the shrimp species were relatively higher compared to concentration of other metals in the same animals. The similar findings were also recorded in fishes (Parsons, 1999; Miramand *et al.*, 2001; Zehra *et al.*, 2003; Tyrrell *et al.*, 2005) and crustaceans (Ridout *et al.*, 1985; Swaileh and Adelung, 1995; Parsons, 1998; Hossain and Khan, 2001; Miramand *et al.*, 2001; Tynelell *et al.*, 2005; Mitra *et al.*, 2010) caught from other waters of the world. We observed significant variations of Zn between the stations and also between the shrimp species ($p < 0.01$). It is generally believed that fin and shell fishes are actively regulate Zn concentrations in tissues and that therefore Zn tissue levels do not reflect the changes in Zn concentrations in the environment (Rejamon *et al.*, 2010).

Levels of Zn and Cu have been found to be higher than Pb and Cd and it could be explained because of these metals play a role in the enzymatic and respiratory processes of in aquatic animals (Cogun *et al.*, 2005). Cu is an essential trace metal for animal metabolism but at high levels is a very toxic substance to aquatic life (Bryan *et al.*, 1983). The main sources of Cu in the coastal waters are antifouling paints (Goldberg, 1975) and this metal entered into the water body through industrial effluents containing CuSO₄ used in metal plating and fishing operations (Goldberg, 1975; Mitra and Chowdhury, 1993). Contaminated food probably

Table 5. A comparison of heavy metals concentrations (ppm in dry weight) in crustaceans collected from different parts of the world

Location	Species	Zn	Cu	Pb	Cd	References
Indonesia	<i>Penaeus merguensis</i>	2.13	1.166	0.022	0.0002± 0.0001	Agoes Soegianto et al, 2008
Bangladesh	<i>Penaeus monodon</i>	24.2-35.7	12.2-21.3	0.8-1.3	0.2-0.4	Hossain and Khan (2001)
Pakistan	<i>Palaemon longirostris</i>	79-89	67.5-100.7	0.31-1.0	0.05-0.23	Mirarnand et al, (2001)
Malaysia	<i>Penaeus monodon</i>	13.030	3.567	ND	0.002	Ismael et al. , 1995
India	<i>Polynemus paradise us</i>	B.D.L.	1.0	62.5	--	Mitra et al, 2000
Egypt	<i>Penaeus indicus</i>	19-27	20-45	0.03-0.14	0.04-1.47	Ahdy et al., 2007
India	<i>Metapenaeus brevicornis</i>	42.5-85.8	30.2-50.6	0.01-0.02	0.1-0.4	Krishnamurti and Nair, 1998
	<i>Penaeus indicus</i>	41.9-72.0	16.9-37.4	0.02-0.09	0.04-0.1	Krishnamurti and Nair, 1998
Nigeria	<i>Macrobrachium felicinum</i>	2.516	12.4-20.5	0.350±0.08	0.08±0.1 0	Opuene and Agbozu, 2008
China	<i>Metapenaeus ensis</i>	15.8	28.0 ± 11.0	0.135 ± 0.064	0.001- 0.003	Ip et al, 2005
Mexico	<i>Penaeus californiensis</i>	2-17	3.7-8.8	--	0.01-0.3	Paez-Osuna and Ruiz- Fernandez, 1995
Gulf of Fonseca	<i>Penaeus monodon</i>	19-30	2.1-6.9	0.035-0.5	0.002- 0.03	Carbonell et al. 1998
Gulf of California	<i>Litopenaeus stylirostris</i>	57.8	25.4	5.3	0.66	Frias- Espericueta et al, 2007
Iskenderun Gulf, Turkey	<i>Penaeus semisulcatus</i>	53.7	32.2	19.1	3.47	Cog'un et al. (2005)
Persian Gulf	<i>P. merguensis</i>	47.3	20.3	--	0.31	Pourang and Dennis (2005)
Gulf of Mexico	<i>P. setiferus</i>	107	17.3	7.73	6.11	Vazquez et al. (2001)
Sundarban, India	<i>P. monodon</i>	1184	--	32.1	0.74	Guhathakurta and Kaviraj (2000)
Coast of England	<i>Palaemon elegans</i>	113-180	105-162	--	-	Abdenmour, 1997
Algeria	<i>Parapenaeus longirostris</i>	81-100	100-125	0.5-2.6	0.4-0.9	Abdenmour et al, 2000
Coast Malay Peninsula,	<i>Peneaus sp</i>	60-85	60-130	0.7-3.4	0.2-0.9	Everaarts et al, 1989
Coastal zone, Kenya	<i>Peneaus sp</i>	49-102	45-90	0.1-0.6	1.1-8.5	Everaarts and Nieuwenhuize, 1995
Java Sea	<i>P. merguensis</i>	26-109	5-120	--	0.6-13.9	Everaarts and Swennan, 1987
Coastal zone NE Pacific	<i>Penaeus sp.</i>	48-53	14-20	0.7-1.3	0.1	Harding and Goyette D, 1989
Indian Sundarbans	<i>Penaeus monodon</i>	16-80	10-60	5.50-10	BDL- 2.50	
	<i>Penaeus indicus</i>	20.50- 87.50	14.50-63	8.5-12	BDL- 4.50	
	<i>Penaeus semisulcatus</i>	27-99	17.50-66.50	10.50-16.50	BDL- 8.40	Present Study
	<i>Penaeus marguensis</i>	22-95	13-42	9-15	BDL- 3.90	
	<i>Metapenaeus brevicornis</i>	12.50-32.50	8.50-13.50	0-4	BDL-1.40	

Table 6. Patterns of trace metals occurrence in muscle of several shrimp species belonging to genus *Penaeus* from different parts of the world

Species	Tissue	Order	Reference
<i>Penaeus notialis</i>	Muscle	Zn>Fe>Cu>Hg>Cd	Biney and Ameyibor (1992)
<i>Penaeus monodon</i>	Muscle	Zn>Fe>Pb>Cd	Guhathakurta and Kaviraj (2000)
<i>Penaeus californiensis</i>	Muscle	Zn>Fe>Cu>Mn	Paez-Osuna and Tron-Mayen (1995)
<i>Penaeus indicus</i>	Muscle	Zn>Cu>Cr>Pb>Ni>Cd	Joseph and Srivastava (1992)
<i>Penaeus merguensis</i>	Muscle	Zn>Cu>Mn>Fe>Hg>Cd	Darmono and Denton (1990)
<i>Penaeus monodon</i>	Muscle	Zn>Cu>Fe>Mn>Hg>Cd	Darmono and Denton (1990)
<i>Penaeus latisulcatus</i>	Muscle	Zn>Cu>Pb>Cd	Maher (1986)
<i>Penaeus vannamei</i>	Muscle	Zn>Fe>Cu>Mn>Cr>Ni>Cd	Paez-Osuna and Ruiz - Fernandez (1995)
<i>Penaeus semisulcatus</i>	Muscle	As>Pb>Hg>Cd	Madany et al. (1996)
<i>Penaeus kerathurus</i>	Muscle	Zn>Cu>Fe>Ni>Pb>Cr>Cd	Balkas et al. (1982)
<i>Penaeus semisulcatus</i>	Muscle	Zn>Cu>Co>Pb>Ni>Cd>Cr	Sadiq et al. (1982)
<i>Penaeus semisulcatus</i>	Muscle	Cu>Pb>Ni>Cd>Co>Hg	Kureishy (1993)
<i>Penaeus kerathurus</i>	Muscle	Hg>Pb>Cd	Pastor et al. (1994)
<i>Penaeus merguensis</i>	Muscle	Zn>Cu>Fe>Cr>Ni>Mn>Cd	Pourang and Amini (2001)
<i>Penaeus Semisulcatus</i>	Muscle	Zn > Cu > Cd	Pourang et al, 2005
<i>Litopenaeus stylirostris</i>	Muscle	Zn > Cu > Pb > Cd	Frias-Espericueta et al, 2007
<i>Penaeus monodon</i>	Muscle	Zn > Cu > Pb > Cd	Mitra et al, 2011
<i>Penaeus monodon</i>	Muscle	Zn > Cu > Pb > Cd	Present Study
<i>Penaeus indicus</i>	Muscle		
<i>Penaeus semisulcatus</i>	Muscle		
<i>Penaeus marguensis</i>	Muscle		
<i>Metapenaeus brevicornis</i>	Muscle		

represents a more important source of copper than water and thus burdens in fish and shell fishes can not be consistently related to ambient pollution levels in water (Moore and Ramamoorthy, 1981). Despite the existence of a number of detoxifying and storage systems for Cu, it is the most toxic metal after mercury and silver, to a wide spectrum of marine life, hence its value in antifouling preparations (Clark, 1997). In the present geographical locale, the major source of Cu is the antifouling paints used for conditioning fishing vessels and trawlers apart from industrial discharges. Levels of Copper in shrimps from the selected stations ranged from 8.50 ± 0.43 to 65.80 ± 1.14 ppm, far below the normal permissible range, i.e. 120 ppm as recommended for the crustacean tissue (Franklin, 1987). In case of station 1 and station 2, Cu accumulation for all the shrimp species excluding *Metapenaeus brevicornis* ranged from 33.09 ± 1.42 to 65.80 ± 1.14 are higher than the recommended value of Cu in sea food, which is 30 ppm prescribed by WHO (1989) and 10 ppm as prawn consumption as recommended by the Food and Agricultural Organization (FAO, 1992). According to the Seafood Standards, maximum allowable limits for shrimps for Cu as 20 ppm (The Seafood Standards 2003) also indicated Cu accumulation of shrimp species in western sector of Indian Sundarbans relatively higher than seafood standards bench mark. From the different investigation, it was found that western sector of Indian sundarbans exhibited maximum Cu concentrations in the surface water and aquatic living organisms which can't only be attributed to the conditioning of huge number of authorized and unauthorized fishing vessels, trawlers,

traveler boat and cargo ships in the creeks and bay regions, but also to the leaching from several aquacultural farms in the area that use Cu compounds as algicide (Mitra, 1998; Chakraborty et al., 2009).

Pb is a neurotoxin that cause behavioral deficits in aquatic organisms and an cause decreases in survival, growth rates and metabolism (Burger et al., 2000). There is often little accumulation of Pb in marine and freshwater species. Consequently lead is not a threat to fisheries resources except at extreme pollution (Clark, 1997). The most toxic of the heavy metals is Pb, 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. When compared with the recommended value of World health Organization (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. 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. Station 2 falls in the navigational route of the ships and tankers for Haldia port, and also in the Hooghly channel, through which the wastes of the upstream region find way to Bay of Bengal. Hence shellfishes sampled from this station exhibited

considerable concentrations of Pb in the muscles. The values of Pb in shrimp samples were compared with maximum allowable limits of the Seafood Standards and also with available literatures. According to the Seafood Standards, maximum allowable limits for Pb accumulation of shrimps are 0.5 ppm (The Seafood Standards, 2003). In the present study, Pb contents in shrimp species obtained from all stations were all found to be above the threshold values.

Cadmium is regarded as a priority pollutant because of its toxicity to organisms in the aquatic environment (Saddiq, 1992). When excess Cd is absorbed through sea food it tends to accumulate in the liver and in the kidneys of the human body (Mol et al., 2010). The main sources of Cd in the present geographical location are electroplating and an important metal with many industrial applications. Also, Cd is a by-product of Zn and Pb mining and smelting (Mitra, 1998). No trace of Cd was recorded in the shrimp muscle from stations 3 and 4 that are located almost in industry-free zone surrounded by mangrove vegetation. Codex Alimentarius Commission (2002) has standards or proposed standards for Cd in mollusks (1.0 µg/g) and crustacean (0.5 µg/g). For Cd, the joint Monitoring Programmed established under the Oslo and Paris Commissions set a guideline of 0.2 µg/g in crustaceans and below 2 µg/g in mussels. In the present study the concentration of Cd ranged from BDL to 4.50 ppm (Figure 5). Cadmium levels in all the shrimp species from station 1 and *Penaeus semisulcatus* from station 2 were much above the maximum limits for prawns/shrimps for human consumption as indicated by the Food and Agricultural Organization (FAO 1992). The values were also higher than the WHO (1989) recommended value for prawn consumption which is 1 ppm. The values of Cd in shrimp samples also compared with maximum allowable limits of the Seafood values are 0.5 ppm (The Seafood Standards 2003). In the present study, Cd contents in shrimp species obtained from station 1 and 2 (only *Penaeus semisulcatus*) were all found to be above the threshold values. Significant differences were observed in Cd concentrations between the stations and also between the species ($p < 0.01$).

Almost all metals measured in this study are relatively higher than the values recorded in shrimp species from other regions of the world. The findings of other studies are summarized in the Table 4, and are compared with the concentrations reported in this study and elsewhere in the world, which reflects the adverse impact of industrialization and urbanization on the biotic community. It is interesting to note that in some cases, mean levels of trace elements in edible tissues of shrimps from a selected region may be different under different storage conditions (Pourang et al., 2004). In this regard Pourang and Amini (2001) reported that mean Cu and Zn levels in edible parts of *Metapenaeus affinis* stored at -10 °C exceeded some

existing guidelines, while the concentrations at -30 °C were somewhat lower than them.

Table 6 presents a general view on results of several studies regarding the order of heavy metals bioaccumulation in edible tissues or whole body of *Penaeus* shrimps. In the present study, The distribution of trace metals accumulated in all the muscle tissues of shrimp species followed the order Zn > Cu > Pb > Cd. With respect to the results presented in the Table, it can be concluded that in almost all cases (except in limited cases) concentrations of zinc and copper were higher than the other studied elements. The widest and narrowest range of variations can be observed in the case of Zn and Cd, respectively, Zn and Cu are essential elements and play important roles in growth, cell metabolism and survival of most animals including crustaceans. Hence, the relatively high levels of these metals can be attributed to their essentiality. In the case of Pb and Cd, the reverse case can be observed, namely their levels were lower relative to the other studied elements. The relatively low accumulation of these elements may be due to existence of developed systems to excrete toxic metals in crustaceans (Pourang et al., 2004). Comparing the present data with guidelines and limits (Table 4), it can be seen that most of metal concentrations found in the tissues of aquatic animals proved to be below (excluding Pb) the tolerance levels for human consumption.

A good correlation between groups of metals has been suggested as indicative of particular biochemical pathways (Mason and Simkiss 1983). In Table 3, positive correlations were observed for: Cu: Cd, Pb: Cu, Cu: Zn, Zn: Pb and Cd: Zn. Correlation coefficients between metal pairs in crustaceans show species and location dependent differences. Muscle contains the highest load of both metals (Páez-Osuna and Tron-Mayer 1995); thus, the correlation in muscle should also be observed in peeled shrimps. In the present study, inter-metal relationships exist ($P < 0.01$) between Zn-Cu ($r = 0.98$) and considerable correlation between Cu- Pb ($r = 0.97$) and Pb- Cd ($r = 0.95$) for the crustaceans collected from four stations of the Indian Sundarbans. This result showed that the same factors were effective on the accumulation of both metals

Most shrimps are benthic organisms living on the bottom of oceans or seas. They generally live among algae and sea grass, under stones and shells, in the cracks of rocks and corals on hard surfaces, and in shallow holes on soft surfaces and they are omnivores that consume foraminifer, polychaeta, crustacean, algae species, and detritus. It could be said that sediment geochemistry has a very important role in the accumulation of metal in shrimps because of feeding behaviors of shrimps besides creating a living environment for shrimps. When the metal contents of shrimps are examined, it is seen that there are differences among species. It is thought that these differences are caused by the genetic variations

among species, different feeding habits of species and differences in their living environments (Pourang *et al.*, 2005). In addition, it is observed that the differences in the lengths of the species in some stations play role in these differences. It is seen in many similar studies on this subject that small animals generally include more metal compared to large animals (Pourang *et al.*, 2005).

Bioaccumulation patterns of metals in shellfish muscle can be utilized as effective indicators of environmental metal contamination (Larsson *et al.*, 1985). Comparing the present data with guidelines and limits (Table 4), it can be seen that most of metal concentrations found in the tissues of aquatic animals proved to be below the tolerance levels for human consumption. 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.

Conclusion

The shrimp species *Penaeus monodon*, *Penaeus indicus*, *Penaeus semisulcatus*, *Penaeus marguensis* and *Metapenaeus brevicornis* are commonly available in the mangrove dominated Indian Sundarbans region at the apex of Bay of Bengal. The knowledge of heavy metal concentrations in crustaceans are very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species and the most polluted area. Information on the distribution pattern of toxic heavy metal pollutants in aquatic environment becomes important so as to know the accumulation of such pollutants in the organisms and final transfer to man through sea foods. The International official regulatory agencies have set limits for heavy metal concentrations above which the fish is considered unsuitable for human consumption. However in the Indian sub-continent there is no safety level of heavy metals in fish and shrimp tissues. The present zone of investigation situated in and around Indian Sundarbans, a world Heritage Site, demands regular monitoring of metal status for effective management and conservation of this famous mangrove gene pool. 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 edible shellfishes from two different sectors of Gangetic delta that are physico-chemically different. The high concentrations of heavy metals in commercially important crustaceans sampled from Nayachar Island (station 1) is a cause of concern, and requires regular monitoring of water quality around the point sources

present opposite to the western bank of the island, in combination with the fact that shrimp consumption is the main source of heavy metal intake in people not occupationally exposed, amplifies the need for preventive measures to safeguard public health

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