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RESEARCH ARTICLE

**BIOACCUMULATION OF HEAVY METALS (Zn, Pb, Cd, AND Ni) IN TISSUES OF
Penaeus monodon (Fabricius, 1798) FROM INDIA**

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Abstract

Penaeus monodon (Fabricius), giant tiger shrimp, is commercially important seafood species. Shrimp export from India earned foreign exchange of US\$1261.81 million in 2010-11. However, rapid industrialization and urbanization have contaminated the coastal ecosystems to a great extent, which could lead to bioaccumulation of heavy metals in tissues of the tiger shrimp. Consumption of such shrimps may lead to the harmful effect on the health of human. This necessitates establishing the levels of heavy metals in the shrimp. Thus, the study was undertaken with the aim to determine the extent of bioaccumulation of four heavy metals (Zn, Pb, Cd and Ni) in hepatopancreas, gill, carapace, and muscle of the tiger shrimp from Uran coast, Panvel, Raigad district, Maharashtra using a standard atomic absorption Spectrophotometer. The greatest metal accumulation was found to occur in the hepatopancreas, followed by gills, muscle and carapace. Concentration levels of non-essential metals such as Pb, Cd, and Ni were very low compared to essential metals such as Zn. The observed mean concentration of Zn, Pb, Cd, and Ni in the hepatopancreas of tiger shrimp was 48.978 ± 5.603 ppm, 0.4345 ± 0.0841 ppm, 0.381 ± 0.0593 ppm, and 0.3905 ± 0.0148 ppm $\mu\text{g g}^{-1}$ respectively. Gill accumulated largest amount of Zn (23.6945 ± 0.1492 ppm), followed by Pb, Cd, and Ni as 0.49 ± 0.0424 ppm, 0.1685 ± 0.2001 ppm, and 0.0866 ± 0.0057 ppm respectively. In muscle, mean concentration of Zn, Pb, Cd, and Ni was 21.881 ± 1.81 ppm, 1.5443 ± 1.1086 ppm, 0.1243 ± 0.1749 ppm, and 0.0623 ± 0.0070 ppm respectively. In the case of Carapace, mean concentration of Zn, Pb, Cd, and Ni was 8.454 ± 0.523 ppm, 0.211 ± 0.1299 ppm, 0.0116 ± 0.0115 ppm, and 0.0410 ± 0.010 ppm respectively. All the metal concentrations were found to be below their acceptable international standards, and thus safe for human consumption.

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Introduction

India earned \$2.84 billion from seafood exports in 2010-11 and Associated Chambers of Commerce and Industry of India has projected seafood exports are likely to touch \$4.7 billion by 2013-14. In terms of seafood export earnings, frozen squid is the largest export item (60.11 per cent in value terms) followed by frozen shrimp (36.21 per cent), frozen fish (28.03 per cent) and fresh cuttle fish (18.45 per cent) in 2010-11. Shrimp export earned foreign exchange of US\$1261.81 million in 2010-11. There is increasing demand of shrimps from Indian water, especially of *Penaeus monodon*, the giant tiger shrimp, in world market due to its large size, unique taste, high nutritive value and persistent consumers' demand. However, aquatic pollution could be one of the major hurdles in meeting world demand of seafood from India.

Heavy metals are an important category of aquatic pollutants and have major detrimental impact on human and environmental health (Streit, 1992, Saavedra et al., 2004). Heavy metals may enter aquatic systems from different natural and anthropogenic sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbour activities, geological weathering of the earth crust and atmospheric deposition (Reddy et al., 2007).

Due to their toxicity and accumulation in biota, determination of the levels of heavy metals in commercial fin fish and shell fish species has received considerable attention in different countries in the region and around the world (Krishnamurti and Nair 1999, Girija et al., 2007, Barrento et al 2009, Akpu et al., 2010, Zhao et al., 2011). Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazard effect on human health. However, reports on heavy metal concentration in shrimps from the Indian coastal waters in general and from the Uran coast, Panvel in particular are scanty. The growing rate of anthropogenic waste input into the Uran coast due to heavy industrialisation of the region leads to bioaccumulation of heavy metals in biota and their levels in economically important crustaceans and fishes have become a matter of great concern. Hence, it is important to establish 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). Thus, the aim of this study was to evaluate most common heavy metal concentrations, zinc (Zn), lead (Pb), cadmium (Cd), and nickel (Ni) in hepatopancreas, carapace, muscle and gill of *P. monodon* from the Uran coast, Panvel, Raigad district, Maharashtra and to find out whether the tiger shrimp has elevated concentrations of these heavy metals in their tissues that could render them dangerous for human consumption, by comparing the results generated in the present study with the maximum permissible limits of these heavy metals of International standards. Also being the first report, the concentration levels in commercially important tiger shrimp will serve the baseline for future studies.

Materials and Methods

20 individuals of the tiger shrimp were collected by trawling in the month of September, 2012 from the Uran coastal water (18.98° N and 73.11° E), Panvel, India. The collected samples were stored in a container, preserved in crushed ice, and brought to the laboratory for further analysis. The samples were identified in the laboratory. Similar sized 10 specimens were sorted out for analyzing the metal levels in the hepatopancreas, gill, muscle and carapace. All reagents used during analysis were of analytical grade and deionized water was used throughout the study. All the glassware were soaked in nitric acid for 3 days and rinsed with deionized water before use. The instrument was calibrated with chemical standard solutions prepared from commercially available chemicals. The shrimps were washed with double distilled water and dissected to collect hepatopancreas, gills, muscles and carapace. These tissues were kept in hot oven at 110°C for 24 h for drying. After drying these tissues were stored in clean tissue paper. Then these tissues were weighed and powdered. 0.5 g each of samples were taken into clean dried beaker (100 mL), 5 mL of aqua regia HCl and HNO₃ (3:1) was then added to the sample for digestion. Few drops of H₂O₂ were then added to clear the solution to pale yellow. The samples were allowed to be evenly distributed in the acid by stirring with a glass rod and then the beaker was placed on the heater. The digested sample was filtered through whatman filter paper No-40 into a graduating cylinder and the filtrate was made up to 50 mL using deionized water. A reagent blank was also run simultaneously. Standard atomic absorption Spectrophotometer (Perkin Elmer model - 373) was used to estimate the concentration of Zn, Cd, Pd, and Ni in the samples of shrimp. Heavy metals concentrations were expressed as mean±standard error (X±SE) ppm/dry weight.

Results

The concentration of heavy metals such as Zinc, lead, cadmium, and nickel in the hepatopancreas, carapace, gill, and muscle of the tiger shrimp is shown in Table 1 and is graphically represented in Fig. 1 and 2. Hepatopancreas was the most accumulating organ among four organs for all the four heavy metals whereas the carapace is the least heavy metal accumulating organ in the shrimp (table 1). A level of Zn was higher than those of other metals in the hepatopancreas, gill, muscle and carapace. Concentration levels of non-essential heavy metals such as Pb, Cd, and Ni were very low compared to essential metals such as Zn. Trace metal levels varied significantly in different tissues of the species.

The observed mean concentration of Zn, Pb, Cd, and Ni in the hepatopancreas of tiger shrimp was 48.978 ± 5.603 ppm, 0.4345 ± 0.0841 ppm, 0.381 ± 0.0593 ppm, and 0.3905 ± 0.0148 ppm $\mu\text{g g}^{-1}$, respectively. In the case of Carapace, mean concentration of Zn, Pb, Cd, and Ni was 8.454 ± 0.523 ppm, 0.211 ± 0.1299 ppm, 0.0116 ± 0.0115 ppm, and 0.0410 ± 0.010 ppm respectively. In muscle, mean concentration of Zn, Pb, Cd, and Ni was 21.881 ± 1.81

ppm, 1.5443 ± 1.1086 ppm, 0.1243 ± 0.1749 ppm, and 0.0623 ± 0.0070 ppm respectively. Gill accumulated largest amount of Zn (23.6945 ± 0.1492 ppm), followed by Pb, Cd, and Ni as 0.49 ± 0.0424 ppm, 0.1685 ± 0.2001 ppm, and 0.0866 ± 0.0057 ppm respectively.

Pattern of accumulation of heavy metals in the hepatopancreas, carapace, muscle and gill follow the sequence: Zn>> Pb> Ni > Cd, Zn> Pb> Ni > Cd, Zn>> Pb> Cd> Ni and Zn>> Pb> Cd> Ni respectively. The distribution patterns of Zn, Pb, Cd and Ni in tissues of *P. monodon* follow the order: hepatopancreas>gill> muscle>carapace, muscle>gill> hepatopancreas>carapace; hepatopancreas> gill> muscle> carapace and hepatopancreas>gill> muscle>carapace respectively.

Table 1. Heavy metal concentrations (in ppm dry weight) in tissues of *Penaeus monodon*

Tissues	Zn	Pb	Cd	Ni
Hepatopancreas	48.978 ± 5.603	0.4345 ± 0.0841	0.381 ± 0.0593	0.3905 ± 0.0148
Carapace	8.454 ± 0.523	0.211 ± 0.1299	0.0116 ± 0.0115	0.0410 ± 0.010
Muscle	21.881 ± 1.818	1.5443 ± 1.1086	0.1243 ± 0.1749	0.0623 ± 0.0070
Gill	23.694 ± 0.1492	0.49 ± 0.0424	0.1685 ± 0.2001	0.0866 ± 0.0057

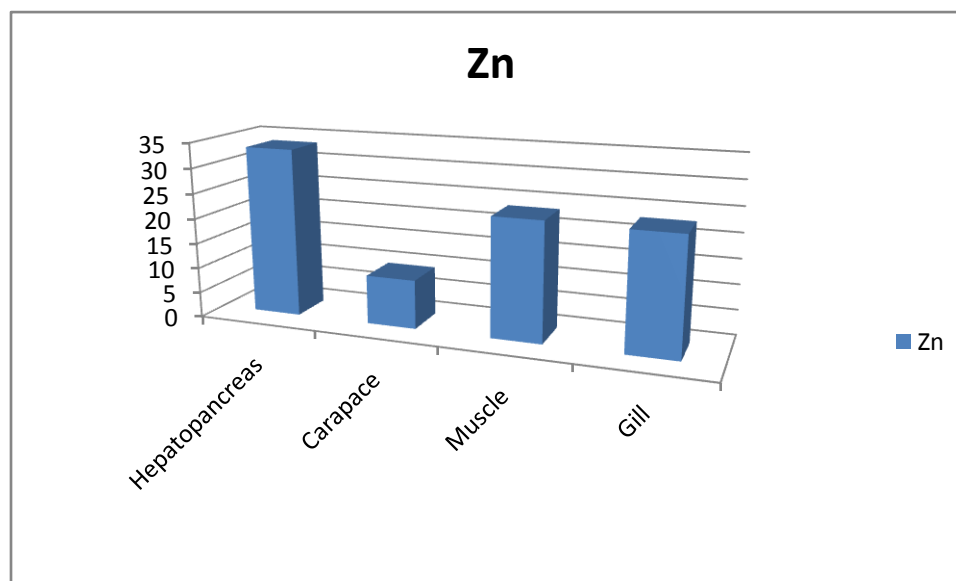


Fig. 1. Bioaccumulation of Zn (ppm dry weight) in tissues of *Penaeus monodon*

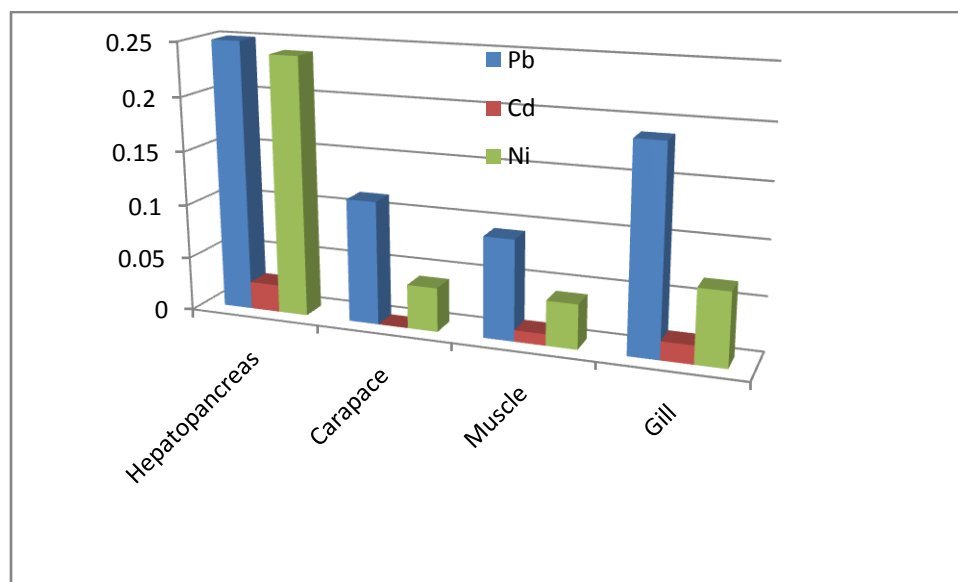


Fig. 2. Bioaccumulation of Pb, Cd and Ni (ppm dry weight) in tissues of *Penaeus monodon*

Discussion

Heavy metals have the potential to cause toxic effects in organisms above a threshold bioavailability (Rainbow, 1993). Various studies have shown that aquatic invertebrates tend to accumulate high level of heavy metals. Thus, determination of harmful and toxic substances in water sediments and biota gives direct information on the significance of pollution in the aquatic environment. The importance of marine shrimp for environmental monitoring studies as bioindicators of heavy metal pollution has been emphasized by several investigators (Guhathakurta & Kaviraj, 2000; Hashmia et al, 2002; Kargin et al., 2001; Pourang et al, 2004).

Metal accumulation in crustaceans can be realized via two routes; either via hepatopancreas during feeding or via gills (Silverstone et al., 2004). In crustaceans, hepatopancreas has an important role in uptaking, detoxification and elimination of metals (Barrento et al., 2009). Many studies have shown that, metal elimination in hepatopancreas takes place more slowly beside muscle tissue (Kalay and Canli, 1999). In general, the metal concentrations tend to be higher in the hepatopancreas than in muscle tissue (Pourang et al., 2004). Muscle tissue in aquatic organisms is less active in metal accumulation depends on low metabolic activity and thus metal accumulation in muscles of decapods was less in comparison to other tissues (Madigosky et al. 1991). Accumulation of metals in aquatic organism tissues changes according to the exposure time and environmental concentrations. In the present study, hepatopancreas was the most accumulating organ among four organs for all the four heavy metals whereas the carapace is the least heavy metal accumulating organ in the shrimp (table 1). It was possible that periodic molting could have helped the shrimp in eliminating Pb and Cd levels from the exoskeleton or less availability of food sometimes might have led to uptake of heavy metal through drinking of water (Soundarapandian et al., 2010). Moulting has often been considered as one of the main excretory mechanisms of crustaceans since large amounts of metals may be lost with the moulted carapace (Viswanathan et al., 2013). Results of the present study indicate similarity with other studies (Canli and Stagg, 1996; Barrento et al., 2009, Soundarapandian et al., 2010).

Crustaceans take up and accumulate metals from a wide range of sources and the trace metal concentrations within their tissues and bodies show great variability (Mitra et al., 1999). Mitra et al (1999) observed Zn and Ni accumulated in the body tissues in the order gill >hepatopancreas>muscle>exoskeleton. In case of Pb and Cd the tissue accumulation is in the order gill >hepatopancreas>exoskeleton >muscle. Trace metals such as Zn, Ni, Pb, Cd and Fe were found to bioaccumulate in liver followed by gills and muscles in fish (Ni *et al.*, 2005). Darmono and Denton, 1990 reported Zn and Cd is more bioaccumulated in hepatopancreas than muscle tissues of *P. monodon*. Similar observations were made in the present study. The distribution patterns of Zn, Pb, Cd and Ni in tissues of *P.*

monodon follow the order: hepatopancreas>gill>muscle>carapace, muscle>gill>hepatopancreas>carapace, hepatopancreas> gill> muscle> carapace and hepatopancreas>gill> muscle>carapace respectively.

Zn being an essential element for normal growth and metabolism of animals, exhibited highest accumulation in the shrimp samples when compared with the other three metals. According to the results obtained, the zinc levels in the hepatopancreas, muscle, carapace and gill are 48.978 ppm, 21.881 ppm, 8.454 ppm and 23.6945 ppm in *P. monodon* respectively, which were much lower than the permissible level, *i.e.*, 400 ppm in crustacean tissue (Franklin, 1987) and the permissible limit for human consumption, which is 1000 ppm for prawn (FAO, 1992). The similar findings were also recorded in fishes (Tyrrell *et al.*, 2005) and crustaceans (Hossain and Khan, 2001; Tynelell *et al.*, 2005 ; Mitra *et al.*, 2012) caught from other waters of the world. All the body parts of invertebrates have a high tendency to accumulate higher level of zinc possibly due to the presence of a sulphide-transporting protein with zinc at its active site (Flores *et al.*, 2005) and also due to acting as a precursor in most enzymatic activities.

Krishnamurti and Nair (1999) reported Pb and Zn concentration in *Penaeus indicus* from Thane Creek, Maharashtra as 0.02-0.09 ppm and 41.9-72.0 ppm respectively. Hossain and Khan (2001) reported levels of Zn, Pb, Cd and Ni in *Penaeus monodon* from Bengal Bay as 24.2-35.7 ppm, 0.8-1.3 ppm, 0.2-0.3 and 2.9-5.9 ppm respectively. Chitrarasu, *et al.* (2013) reported concentration of Zn, Pb, Cd and Ni in *Penaeus monodon* as 52.0 ± 1.7 , 2.3 ± 0.3 , 1.7 ± 0.1 and 7.3 ± 0.6 ppm respectively. Several other authors have reported similar results in crustaceans from different water bodies (Awaluddin *et al.*, 1992; Patimah, I and A.T. Dainal, 1993, Viswanathan *et al.*, 2013). As result indicated, the lead levels in the hepatopancreas, muscle, carapace and gill are 0.4345 ppm, 1.5443 ppm, 0.211 ppm and 0.49 ppm in *P. monodon* respectively, which 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 the shrimp species was below this level. According to the Seafood Standards, maximum allowable limits for Pb accumulation of shrimps are 0.5 ppm (The Seafood Standards, 2003). The nickel levels in the hepatopancreas, muscle, carapace and gill are 0.3905 ppm, 0.0623 ppm, 0.0410 ppm and 0.0866 ppm in *P. monodon* respectively. According to the WHO (1989), maximum permitted level of Ni in shrimps is 0.5-1.0 ppm. The cadmium levels in the hepatopancreas, muscle, carapace and gill are 0.381 ppm, 0.1243 ppm, 0.0116 ppm and 0.1685 ppm in *P. monodon* respectively. The values are lower 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). Cadmium and zinc concentrations found in the muscle tissue of *P. monodon* were similar to those found in the shrimps reported by Eisler (1981) and in Pacific shrimps by Harding and Goyette (1989). Pattern of accumulation of heavy metals in the hepatopancreas, carapace, muscle and gill follow the sequence: Zn> Pb> Ni > Cd, Zn> Pb> Ni > Cd, Zn> Pb> Cd> Ni and Zn> Pb> Cd> Ni respectively. Same pattern of accumulation is observed in shrimp and other crustaceans and is in agreement with that of the previous studies (Krishnamurti, 1998; Mitra *et al.*, 2012).

Conclusion

The results revealed that the heavy metals concentrations in the shrimp are below the threshold levels associated with the toxicological effects and the regulatory limits. *P. monodon* does not appear to accumulate high concentrations of lead, cadmium, zinc, and nickel in its edible tissues and therefore appears to be an acceptable food item with respect to these metals. However, it needs to be monitoring the industrial discharges from the surrounding factories because untreated industrial effluents lead to bioaccumulation in living organisms and finally biomagnifications to human beings. The aquatic organisms usually exhibit high degree of variability in the bioaccumulation of different metals suggest the need for detailed studies involving more species of economic importance in evaluating the general background and toxic levels for utilizing them as indices of pollution.

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