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Bioaccumulation of Heavy Metals in Horseshoe Crabs (*Tachypleus gigas*) from Pekan, Pahang, Malaysia

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ABSTRACT

An attempt was made to determine the various heavy metal accumulative concentrations in different body parts of horseshoe crab [*Tachypleus gigas* (Muller, 1785)]. Heavy metal accumulation levels were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Highest mean concentration (μg g^{-1} wet weight) of iron (Fe), Zinc (Zn), Copper (Cu) and Cadmium (Cd) was observed in gill tissue (1336.73±2.08 ppm), apodeme (921.11±8.12 ppm), Gut (129.94±13.8 ppm) and apodeme (4.16±0.54 ppm) samples, respectively while lowest concentrations of metals were observed in gut (Fe = 556.61 ppm), mouth (Zn = 605.36 ppm), leg tissue (Cu = 60.85 ppm) and leg tissues (Cd = 2.12 ppm). Results clearly showed that bioaccumulation of essential metal concentration in all the analyzed body parts were higher than non-essential heavy metals with the flow of metals in Fe>Zn>Cu>Cd order. Statistical predictions revealed that bioaccumulation of metals were not significantly influenced by weight, total length and carapace width of the animal. The heavy metal accumulations in samples were higher than the national and international permissible limit range hence not suitable for human consumption.

Key words: Bioaccumulation, pekan, *Tachypleus gigas*, living fossil, chelicerate

INTRODUCTION

Horseshoe crabs are marine chelicerate arthropods remarkably retaining their genetic makeup and morphologically unchanged for more than 200 million years (Walls *et al*., 2002; Hurton and Berkson, 2004). Out of four known species of horseshoe crab, three species (*Trachypleus gigas, T. tridentatus* and *Carcinoscorpius rotundicauda*) inhabits Malaysian waters (Kassim *et al*., 2008; John *et al*., 2010) where they spawn throughout the year (Hajeb *et al*., 2005) with the peak spawning during May and June (John *et al*., 2010). Field observation showed that *T. gigas* and *C. rotundicauda* usually nest along the sandy and muddy beaches of Malaysia respectively while *T. tridentatus* nests only in East Malaysian coasts (Borneo). The utilization of horseshoe crab blood in pharmaceutical industries to detect the bacterial endotoxin level in biologicals including injectable drugs pose severe threats to its population. Earlier studies proved that the stress undergone by *Limulus polyphemus* due to blood extraction leads to 15-30% of mortality after being released in the ocean (Hurton and Berkson, 2004). However, It is also evident that the environmental contaminants has direct influence on limiting horseshoe crab population (Zaleha *et al.*, 2010). A series of experiments by Botton (2000), Botton *et al.* (1998) and Itow *et al.* (1998a, b) have shown that horseshoe crab eggs are vulnerable to heavy metals, with mercury, organotin and cadmium which is being the most toxic. There were mortality and developmental defects were observed in developing embryos at the laboratory rearing at 39.5 mg L^{-1} (LC_{50}) levels of cadmium and 3.2 mg L^{-1} (LC_{50}) levels of mercury for continuous exposure. However, Itow *et al.* (1998a) did not found a high rate of abnormalities in the eggs or larvae of horseshoe crabs from delaware Bay. Further, horseshoe crab eggs were remarkably tolerant of heavy metal pollution in comparison to the tolerances of similar developmental stages of other marine crustacean. The relatively high tolerance of horseshoe crab eggs to heavy metals suggests that eggs might pose a problem for consumers (Botton *et al.*, 1998).

Various studies had been carried out to determine the toxic heavy metal level in different parts of *L. polyphemus* (Botton, 2000; Burger *et al.*, 2002). The studies on heavy metal bioaccumulation in *T. gigas* from Malaysian waters are still scanty except a finding on metal concentration in nesting grounds of horseshoe crabs (Zaleha *et al.*, 2010). Hence present study was initiated to determine some heavy metal bioaccumulation in different parts of shore reaching matured horseshoe crab (*T. gigas*) from Pekan, Pahang, Malaysia.

MATERIALS AND METHODS

Sample collection and preparation: Matured horseshoe crab (*T. gigas*) samples were collected alive from Pekan coast (East Coast of peninsular Malaysia) nesting grounds (N 03^0 30' 0.00" E 103^0 25' 1.20") on May 2010 during Full moon days and immediately transferred to INOCEM laboratory (Fig. 1). Data such as sex, total length (mm), carapace width (mm), weight of the samples (g) were noted after removing the symbionts attached to their body. Samples were killed by keeping them in -20°C for 2 h and washed thoroughly with running tap water prior to dissection. Clean and sterilized scissors were used to dissect open the animal from ventral region and body parts such as Gill tissue, legs, mouth parts, gut and internal flesh tissues (Apodeme) were exercised and washed with distilled water and kept separately in labeled Petri dishes and left in hot air oven at 60°C for 8 days. After the samples were completely dried, they were ground using mortar and pestle and kept in labeled falcon tubes prior to digestion.

Acid digestion method: An accurate sample dry weight, 0.5 g of different tissues was gently digested for 3 h in 10 mL concentrated HNO_3 followed by 6 mL mix acid ($3HCl:1H_2O_2:2H_2SO_4$) and heated at 60°C until the digestion was completed. Then, 3 mL mixed acid ($2HNO_3:1H_2O_2$) was added to the solution and then heated to dryness. After cooling, the solution was then transferred to a 50 mL polypropylene vial and completed with 5% HNO_3 digestion. For each series of samples, three analytical blanks were prepared in a similar manner without samples to check the possible contamination. Finally, the samples were analyzed for Fe, Zn, Cu and Cd concentration by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Kamaruzzaman *et al.*, 2010b). Analytical quality was assessed using standard reference materials, DORM (dogfish muscle: National Research Council Canada). Recoveries of all the elements ranged from 96 to 105% of the certified value.

Statistical prediction: Data were analyzed with non-parametric procedures to compare concentrations among metals and tissues. Kruskal Wallis correlation coefficients were used to

Fig. 1: Map showing location where horseshoe crab samples were collected (Source: Kamaruzzaman *et al.*, 2010a)

compare the various metal concentrations (Corder and Foreman, 2009). Both arithmetic and geometric means are given to facilitate comparisons with other studies. Two way Anova test were used to check the influence of weight, sex, total length and carapace width over the bioaccumulation of different metals in different parts.

RESULTS

Overall there is no significant influence of parameters such as weight of the animal, total length, carapace width over the heavy metal accumulation in different body parts were observed ($p>0.05$) except animal sex which had meager influence. Female crabs accumulate considerabely higher amount of metals such as Fe, Zn and Cu in their body parts (except gill tissue). Two fold increase in bioaccumulation of Fe in male crab gill tissues were detected besides Zn and Cu which were also higher accumulated in comparison to female crabs (Fig. 2). Cadmium level were lower in male crab parts compared to female crabs (except in apodeme). It was also observed that both male and female crabs accumualates same level of cadmium content in their gill tissues (Fig. 3). Average concentration of heavy metals in different parts of *T. gigas* (regardless of sex) indicated that gill tissue accumulate more amount of Fe (1336.73 ppm) followed by mouth (965.6 ppm), Apodeme (942.83 ppm), legs (814.45 ppm) and gut (566.61 ppm). Zinc accumulation in different body parts flowed in Apetome (921.11 ppm) > Gill (912.68 ppm) > Leg (615.01 ppm) > Gut (606.44 ppm) > Mouth (605.36 ppm) order. It was evident from the present study that the accumulation Fe and Zn in comparison to Cu level was 3-13, 3-10 times higher in different body parts respectively. The flow of Cu concentration in horseshoe crabs showed Gut (183.42 ppm) > Apodeme (129.95 ppm) > Gill

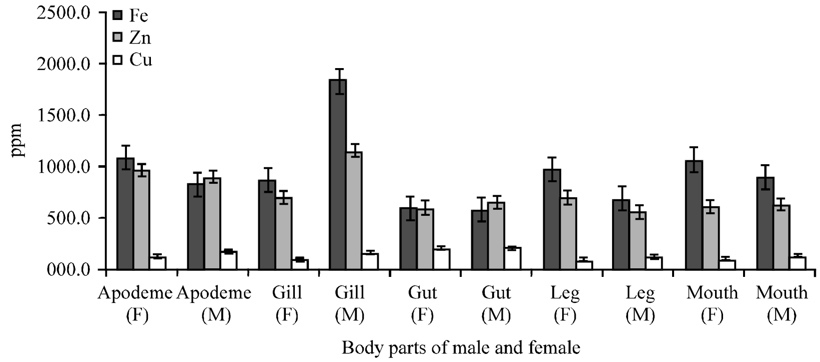

Fig. 2: Comparison of mean bioaccumulation level of Iron (Fe), Zinc (Zn) and Copper (Cu) in different parts of male and female *T. gigas*. X-axis represents different body parts of Male (M) and Female (F) horseshoe crabs and Y-axis represents the metal bioaccumulation level (ppm). Overall data represented as Mean±SE

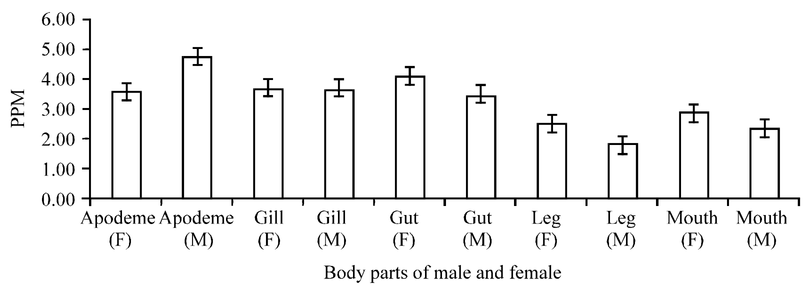

Fig. 3: Comparison of mean bioaccumulation level of Cadmium (Cd) in different parts of male and female *T. gigas*. X-axis represents different body parts of Male (M) and Female (F) horseshoe crabs and Y-axis represents the metal bioaccumulation level (ppm). Overall data represented as Mean±SE

Table 1: Overall means of metals in different tissues of horseshoe crab (*Tachypleus gigas*) collected from Pekan, Pahang in 2010

		Different body parts			
Metals (ppm)	Apodeme (flesh)	Gill	Gut	Leg	Mouth
Fe	942.83	1336.73	566.61	814.45	965.60
Zn	921.11	912.68	606.44	615.01	605.36
Cu	129.95	102.22	183.42	60.85	97.10
Cd	4.16	3.70	3.80	2.12	2.59

(102.22 ppm) > Mouth (97.10 ppm) > Leg (60.85 ppm) order. Cadmium accumulated in least concentration compared to other metals studied and bioaccumulative flow was observed in apotome (4.16 ppm) > Gut (3.80 ppm) > Gill (3.70 ppm) > Mouth (2.59 ppm) > Leg (2.12 ppm) order (Table 1). Overall metal bioaccumulation in different tissues of *T. gigas* collected from Pekan coastal area, Pahang was in Fe > Zn > Cu > Cd order.

Comparative study of detected bioaccumulation in present study with earlier studies (Burger *et al.*, 2003) and national and international Maximum Permissible standard Limits (MPL) clearly showed the exceeded level of metals in *T. gigas* from Pekan coast (Fe = 817.59±279.35 ppm;

Table 2: Guidelines on heavy metals concentration (μg g^{-1} dry weight) for food safety set by local and international body and comparison of results with previous studies on Atlantic horseshoe crab (*L. polyphemus*)

Metals	Certified reference material	MPL (WHO, 1982) (ppm)	MPL (FDA, 2001) (ppm)	MPL (MFR, 1985) (ppm)	Concentration (ppm) In *L. polyphemus* New jersey	Delaware bay (year 2003)	Delaware bay (year 1997)	Concentration (ppm) in *T. gigas* (present study)
Fe	142±10	-	-	-	-	-	-	817.59±279.35
Zn	25.6±2.3	100	150	100	-	-	-	638.22±168.74
Cu	2.34±0.16	10	100	30	-	-	-	103.19±45.60
Cd	0.043±0.008	2	0.2	1	23±10	19.2±2	13±2	2.87±0.86

MPL: Maximum permissible limit, WHO: World health organization, FDA: Food and drug administration and MRF: Malaysian regulation on Food. Data represented in Mean±SD

Zn = 638.22±168.74 ppm; Cu=103.19±45.6 ppm; Cd = 2.87±0.86 ppm) with reference to MPL while the conspecifics of *T. gigas* in Atlantic coast (*L. polyphemus*) accumulate 5-8 fold increase (13-23 ppm) in cadmium concentration (Table 2).

DISCUSSION

The knowledge on metal bioaccumulation in native species is very important for their management, utilization of these species for human consumption and to determine the useful bioindicator species through various biomonitoring programs. It was suggested that interpreting the levels of metals in invertebrates is difficult because both toxicity and susceptibility differ due to their detoxification ability (Burger *et al*., 2002). Considerable amount of studies were carried out on Atlantic horseshoe crab (*L. polyphemus*) due to their higher population density, their utilization in bite fishery and biomedical industries besides their importance in balancing migratory shorebirds food chain along the Mexican coasts (Botton *et al*., 1994). It was also found out that heavy metal pollution is a major factor responsible for the declining horseshoe crab population in Delaware bay (Botton, 2000). The environmental and toxicological studies on other three conspecific species are still scanty. It is evident from present study that the essential metals bioaccumulate in higher quantity than the nonessential metals. The elevated concentration of Fe, Zn and Cu in horseshoe crab might be due to the major role played by these elements in maintaining the proper physiological functions of the organism through various enzymatic activities. This observation was well corresponded with the previous study by Kanakaraju *et al*. (2008) who postulated that these metals play an important role as an essential element in all living systems from invertebrates to human, hence the organisms tend to accumulate high concentration of Fe from the surrounding environment. This observation might also be due to the organism's capacity to regulate and accumulate elevated concentration of these metals (Kamaruzzaman *et al*., 2010c). The observed high concentration of metals in female crabs might be due to (1) their long term exposure to the bottom sediments compared to male (2) their feeding behavioral pattern, physiologic condition and metabolic activity of the animal.

Comparison with earlier studies on conspecific species from Mexican coast (*L. polyphemus*), *T. gigas* accumulate 5-8 times less amount of cadmium. This may relate partly to the lack of industry near the nesting beaches along the Malaysian coasts and to the relative distance from ocean which is a source of some naturally occurring metals (mercury, cadmium) (Burger *et al*., 2002). Comparison with the recent report suggested that the source of Cd in Pekan coast was primarily through the natural sources and virtually no anthropogenic activities through various industrial processes were observed along this coast. Hence the minimal enrichment concentration of Cd in nesting ground of *T. gigas* would have been reflected in low accumulated concentration of Cd in different body parts of horseshoe crab (Zaleha *et al*., 2010) whereas industrialization and various anthropogenic activities would influenced the higher accumulation of Cd in horseshoe crab samples from New Jersey and Delaware bay (Burger *et al*., 2002).

The detected concentration of heavy metals in different parts of horseshoe crabs was more than Maximum Permissible Limit (MPL) and hence *T. gigas* from Pekan site should not be used for human consumption. These heavy metal contaminated horseshoe crabs might result in bioaccumulation of toxic metals in the human system via food chain and may lead to adverse health effects (Bergback *et al*., 1992; Koller *et al*., 2004).

CONCLUSION

Unlike in the Mexican coast, horseshoe crab in Malaysian coast neither being used for bait fishery nor their eggs are being prayed upon by the shore birds due to low population density of horseshoe crabs and less number of landing during mating season due to site preference. Hence, only limiting source of their population would be the predatory activity or environmental pollution. Thus the knowledge on various contaminants in the horseshoe crabs and their surrounding environment would pave the way in understanding the healthiness of the environment and enacting various managemental laws to protect the horseshoe crabs in Malaysian coasts.

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