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THE CONCENTRATIONS OF HEAVY METALS IN DIFFERENT TISSUES OF HORSESHOE CRABS COLLECTED FROM INTERTIDAL AREAS OF JOHOR, PENINSULAR MALAYSIA

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ABSTRACT

Horseshoe crabs (*Tachypleus gigas*) were collected from the intertidal areas of Tampok, Pontian Besar and Kg. Pasir Puteh in Johore, in May 2007. They were dissected into five parts namely carapace, muscle (from foot), operculum, gills and fillet. For each site, the different tissues from the animals were pooled and triplicates of each category were determined for the concentrations of Cd, Cu, Fe, Pb and Zn. Based on the metal distribution patterns in the *T. gigas* from all the sites, four distinctive patterns were found. It showed that different tissues of *T. gigas* accumulated different levels of metals. The gills accumulated the highest concentrations of Fe, Cu, Pb and Cd while the carapace accumulated the lowest concentrations of Cd, Cu, Fe, Pb and Zn. Except for Cd, all of the metal levels in the fillet were similar. Finally, the elevated concentrations of Cd and Zn in the fillet indicated anthropogenic-induced bioavailabilities of Cd and Zn. These results are useful baseline data for future comparisons.

Key words: Horseshoe crab (*Tachypleus gigas*), heavy metals

INTRODUCTION

The living fossils, horseshoe crabs (Order: Xiphosura) belong to three genera (*Limulus, Tachypleus* and *Carcinoscorpius*) of the family Limulidae (Sekiguchi et al., 1976). According to Chatterji et al. (1992), their ecological habitats include intertidal waters, generally on the sandy bottoms where they move about or burrow just beneath the surface, preying on other animals. The three living genera of limulids exhibit distinct geographical ranges, for example, *Limulus (L. polyphemus)* is restricted to eastern North America, ranging from Nova Scotia to the Yucatan region of Mexico; while *Tachypleus* (*T. tridentatus)* and *Carcinoscorpius* occur in Southeast Asia (Mohan et al., 1984; Shuster 1985). Two species of horseshoe crabs, *Carcinoscorpius rotundicauda* and *Tachypleus gigas* inhabit the Gulf of Thailand, where they are consumed by the local people (Kungsuwan et al., 1987). *Tachypleus gigas* was reported to be abundant in the shallow nearshore waters off the Balramgari coast of north eastern India. The ecological distribution of horseshoe crabs has been reported in Sarawak and Kudat, Malaysia; Singapore; the Gulf of Thailand; and the Torres Straits based on a publication by Sekiguchi et al. (1976).

Review of the literature showed that there was relatively high concentrations of heavy metals in both shorebird feathers and horseshoe crab eggs suggesting that the shorebirds were acquiring these metals from the horseshoe crabs' eggs (Burger, 1993, 1997; Burger et al., 2003). These ingested metals from the contaminated eggs may be stored in their bodies for later mobilization and sequestration in the feathers of the shorebirds (Burger, 1993). From the ecosystem standpoint, the horseshoe crab–shorebird relationship could be a factor in causing severe population declines and community disruption. Therefore, studies of heavy metal levels in horseshoe crabs are important in order to ascertain whether these

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heavy metals are posing toxicological risks to secondary consumers, such as the migratory shorebirds.

Although the presence of *T. gigas* has been reported in Malaysia, the metal distribution in the different tissues of horseshoe crabs, have not yet been reported in the literature. Therefore, the objective of this study was to determine the heavy metal concentrations (Cd, Cu, Fe, Pb and Zn) in the different tissue of *Tachypleus gigas* collected from the intertidal areas of Johore.

MATERIALS AND METHODS

Samplings were conducted in the southwestern intertidal areas of western Peninsular Malaysia in Johore State in May 2007. Tampok is a mangrove area with some agricultural activities, Pontian Besar is a fishing village with some construction in the vicinity while Kg. Pasir Puteh is a shipping area and potentially receiving effluents from petro-chemical plants besides domestic wastes. Kg. Pasir Puteh has been previously reported as a metal-contaminated coastal area in the southern part of Johore (Yap et al., 2004a, 2004b, 2006)

During the samplings, *T. gigas* were collected from Tampok, Pontian Besar and Kg. Pasir Puteh, only one male individual was collected from each site and a female individual was also collected from Kg. Pasir Puteh (Table 1 and Fig. 1). The samples were then stored in an ice container at 10ºC until transportation to the laboratory. In the laboratory, the horseshoe crabs were measured for the allomatric parameters shown in Table 2. Then, they were carefully dissected into different parts namely carapace (prosoma and opithosoma), muscle (from foot), operculum, gills, and fillet. Eggs of the female individuals from Kg. Pasir Puteh were also dissected for analysis. For each site, the different type of the dissected samples from four animals were placed in aluminum foils and then were dried in the oven for 72 hours at 105ºC to constant dry weights.

The dried tissue type were digested in concentrated nitric acid (AnalaR grade, BDH 69%) by placing them in a hot-block digestor, first at low temperature for one hour and then they were fully digested at high temperature (140°C) for at least three hours. The digested samples were then diluted to 40 mL with double distilled water. After filtration, the samples were determined for Cd, Cu, Fe, Pb and Zn concentrations by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in μg/g dry weight basis. Multiple level calibration standards were analyzed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/L stock solution of each metal (Merck Titrisol).

To avoid possible contamination, all the glassware and equipment used were acid-washed and the accuracy of the analysis was checked with the blanks and quality control samples made up of standard solutions. The percentages of recoveries for the heavy metal analyses were 90-110%.

RESULTS AND DISCUSSION

The concentrations of heavy metals in the different tissues of *T. gigas* are given in Table 3 while the overall heavy metal concentrations of the different tissues are given in Table 4. Based on Table 3, the highest levels of Pb were found in carapace, muscle, operculum and fillet of samples from Tampok. Similarly for Fe, the highest levels were found in the carapace, muscle and operculum of samples from Tampok. The highest levels of Cu and Zn were found in the carapace, operculum and gills of horseshoe crabs from Pontian Kecil. The horseshoe crabs of Pontian Kecil also recorded the highest levels of Zn in the carapace, muscle, operculum and gills. There was no significant difference ($P > 0.05$) among the different sites for Zn in the fillet. For Cd, the levels

Table 1. Description of the sampling sites of the horseshoe crabs, *Tachypleus gigas*

No.	Location	Sampling date	Longitude	Latitude	Description of sampling site
1.	Tampok, Johor	09 May 2007	01º37.539' N	103º11.977' E	Mangrove area with some agricultural activities. A dam in the vicinity.
2.	Pontian Besar, Johor	09 May 2007	01º30.469' N	103º11.977' E	Fishing village with some construction activities.
3.	Kampung (Kg.) Pasir Puteh, Johor	10 May 2007	01º29.108' N	103º49.003' E	Aquacultural site, shipping area and potentially receiving effluents of petro-chemical plants and domestic wastes.

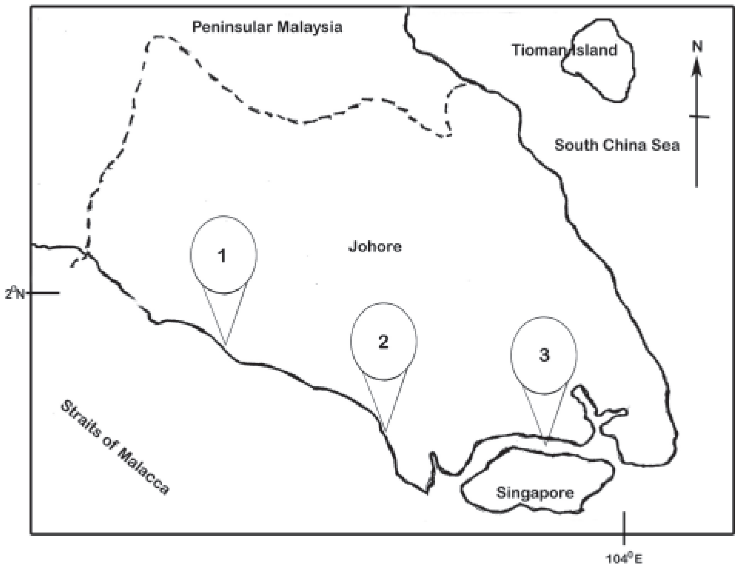

Fig. 1: Map showing the sampling sites of the horseshoe crabs, *Tachypleus gigas*; 1. Tampok; 2. Pontian Besar 3. Kg. Pasir Puteh

Table 2. Allometric data of the *Tachypleus gigas*

No	Site	Total wet weight (g)	Length (cm)	Width (cm)	Height (cm)
1.	Tampok	323.48	35.48	16.60	4.40
2.	Pontian Kecil	256.11	30.24	14.85	3.99
3.	Kg. Pasir Puteh (male)	262.87	32.50	15.51	4.28
	Kg. Pasir Puteh (female)	587.72	44.48	19.46	4.32

Note: the length of the horseshoe crab excluded the telson.

Table 3. The mean concentrations (µg/g dry weight) of heavy metals in the different tissues; carapace, foot muscle (muscle), operculum, gills and fillet of *Tachypleus gigas* from Tampok, Kg. Pasir Puteh (PP) and Pontian Kecil. N = 1 individual of horseshoe crab

No.		Part	Fe	SE	Cu	SE	Zn	SE	Cd	SE	Pb	SE
1.	Tampok	Carapace	120.98	2.21	6.44	0.28	23.94	1.11	0.25	0.11	1.30	0.47
		Muscle	245.77	5.64	64.77	4.78	223.45	13.3	0.73	0.09	2.78	0.18
		Operculum	817.56	13.08	80.20	1.45	87.36	1.96	0.76	0.08	2.78	0.74
		Gills	538.44	9.71	182.52	5.00	81.39	1.66	0.85	0.05	3.63	0.50
		Fillet	244.63	8.03	49.05	1.40	236.97	0.91	1.04	0.05	2.38	0.40
2.	Pontian Kecil	Carapace	100.2	25.61	8.84	2.54	20.98	3.12	1.21	0.12	0.93	0.12
		Muscle	223.53	2.62	63.60	3.70	216.17	1.62	1.41	0.03	2.02	0.34
		Operculum	667.83	93.43	63.36	0.27	84.51	1.25	1.44	0.04	2.01	1.14
		Gills	841.18	63.60	185.95	9.13	84.87	2.37	1.42	0.01	3.15	0.50
		Fillet	252.19	24.11	45.23	2.49	245.80	4.05	1.45	0.05	1.82	0.18
3.	PP- Male	Carapace	71.65	6.28	36.43	5.34	36.43	5.34	0.92	0.06	0.76	0.40
		Muscle	96.20	0.71	45.89	1.13	239.53	2.34	1.26	0.04	0.89	0.39
		Operculum	152.90	8.06	82.11	3.72	101.10	3.53	1.24	0.04	1.72	0.28
		Gills	546.58	3.29	208.66	2.81	99.51	2.47	1.25	0.09	3.86	0.34
		Fillet	255.17	12.4	69.01	1.02	249.27	13.25	1.42	0.08	1.05	0.50
	PP-Female	Carapace	88.22	1.83	6.28	0.57	10.38	0.46	0.97	0.01	0.94	0.09
		Muscle	98.75	4.99	20.19	1.07	238.68	2.30	1.37	0.01	1.33	0.34
		Operculum	217.63	14.59	62.32	0.52	98.23	2.01	1.38	0.07	1.54	0.36
		Gills	966.72	41.49	67.52	1.82	82.67	2.48	1.38	0.03	7.17	0.24
		Fillet	130.29	6.19	85.73	0.91	263.67	0.77	1.36	0.02	1.33	0.20
		Egg	244.23	0.79	70.13	4.70	135.78	1.95	1.3	0.06	0.97	0.29

Note: All the values are based on one individual horseshoe crab in which the different tissues were dissected and pooled for 3 replicates. SE= standard error.

Table 4. Overall concentrations (µg/g dry weight) of the heavy metal distribution in the different tissues of the *Tachypleus gigas*. N= 4 individuals of horseshoe crab

		Fe	Cu	Zn	Cd	Pb
Carapace	Minimum	71.65	6.28	10.38	0.25	0.76
	Maximum	120.98	36.43	36.43	1.21	1.30
	Mean	95.26	14.50	22.93	0.84	0.98
	SE	10.38	7.33	5.36	0.21	0.11
Fillet	Minimum	130.29	45.23	236.97	1.04	1.05
	Maximum	255.17	85.73	263.67	1.45	2.38
	Mean	220.57	62.26	248.93	1.32	1.65
	SE	30.17	9.40	5.55	0.09	0.29
Gills	Minimum	538.44	67.52	81.39	0.85	3.15
	Maximum	966.72	208.66	99.51	1.42	7.17
	Mean	723.23	161.16	87.11	1.23	4.45
	SE	107.45	31.75	4.20	0.13	0.92
Muscle	Minimum	96.20	20.19	216.17	0.73	0.89
	Maximum	245.77	64.77	239.53	1.41	2.78
	Mean	166.06	48.61	229.46	1.19	1.76
	SE	39.86	10.41	5.77	0.16	0.41
Operculum	Minimum	152.90	62.32	84.51	0.76	1.54
	Maximum	817.56	82.11	101.10	1.44	2.78
	Mean	463.98	72.00	92.80	1.21	2.01
	SE	164.33	5.31	4.05	0.15	0.27
Eggs of PP Female	Minimum	243.30	63.53	133.11	1.20	0.44
	Maximum	245.79	79.22	139.58	1.40	1.44
	Mean	244.23	70.13	135.78	1.3	0.97
	SE	0.79	4.7	1.95	0.06	0.29

Note: PP= Kg. Pasir Puteh; SE= standard error.

Table 5. The concentration patterns of Cd, Cu, Fe, Pb and Zn in the different tissues of *Tachypleus gigas* collected from Tampok, Pontian Besar and Kg. Pasir Puteh

Location	concentration pattern
Fe	Gills > Operculum> Fillet> Eggs> Muscle> Carapace
Cu	Gills > Operculum> Eggs> Fillet> Muscle> Carapace
Zn	Fillet> Muscle> Eggs> Operculum> Gills> Carapace
Cd	Fillet> Eggs> Gill> Operculum> Muscle> Carapace
Pb	Gills > Operculum> Muscle> Fillet> Carapace> Eggs

were found to be consistently the highest in all the different tissues of the female individuals of Kg. Pasir Puteh while the samples from Tampok, it consistently recorded the lowest levels of Cd in all the different tissues. Based on the above comparison, the metal-polluted site at Kg. Pasir Puteh, potentially receiving effluents from petrol-chemical plants, domestic wastes and shipping and port activities as previously reported by Yap et al. (2004a, 2004b, 2006), did not show the highest levels of metals in the different tissues except for Cd. This could be attributed to limited number of sample size and regulative mechanisms for Cu, Zn, Fe and Pb by *T. gigas* collected from Kg. Pasir Puteh.

The metal concentrations patterns in the different tissues of *T. gigas* are shown in Table 5. Four distinctive patterns are found: First, different tissues of *T. gigas* accumulated different levels of metals. Second, the gills had the highest concentrations of Fe, Cu, Pb and Cd while the carapace possessed the lowest concentrations of Cd, Cu, Fe, Pb and Zn. Third, except for Cd, most of the metal levels in the fillet are similar. Fourth, the elevated concentrations of Cd and Zn in the fillet indicated the anthropogenic-induced bioavailabilities of Cd and Zn. The Elevated levels of Fe, Cu, Pb and Cd in the gills can be explained by the fact that this organ has a large surface contact with their surrounding environment (Yap et al., 2003a).The observation that fillet and muscle accumulated lower levels of Fe, Cd and Pb could be due to their having less surface contact with the surrounding environment. Our finding of the low levels of metals (essential and nonessential ones) in the carapace is very interesting. This finding is different from what had been found in the hard tissue or shells of molluscs which accumulated higher levels of nonessential metals Cd and Pb than the soft tissues (Yap et al., 2003b). The metals found in the carapace could be those that were sequestered in this chitinous shield covering the horseshoe crab as a method of metal excretion (Burger, 1992, 1994).

Interpreting the levels of metals in invertebrates is difficult because toxicity occurs when the rate of uptake exceeds the rates of detoxification and/or excretion, rather than the absolute body concentrations (Rainbow, 1996). Therefore, it is important to understand the metal levels in horseshoe crabs because there is considerable concern of the population numbers due to overharvesting and the animal also being as food source for migratory shorebirds during their important spring stopover in Malaysia (Castro et al., 1989; Castro and Myers, 1993; Tsipoura and Burger, 1999).

The metal levels found in the eggs of the female horseshoe crabs indicated that the eggs were accumulating high levels of metals (Table 3). The metal concentrations found in the eggs of *T. gigas* are very important from the food chain transfer point of view as was discussed previously. According to Burger (1993) and Burger et al. (2003), the levels of Pb, Cd, Se, Cr, and Mn in feathers were higher in shorebirds than in the feathers of other birds. This was due to the food consumed by these birds which consisted of metal-contaminated eggs of horseshoe crabs. According to Botton et al. (1998), the relatively high tolerance of horseshoe crab eggs to heavy metals suggested that the eggs might pose toxicity problems to the consumers. Later, Botton et al. (2000) also found elevated levels of Cd and Pb in the eggs of *Limulus polyphemus*, indicating potential toxicological risks to the consumers. Therefore, more detailed studies on heavy metal in the eggs and also the different parts of horseshoe crabs in Malaysia should be done in near future especially as part of a migratory bird conservation program.

CONCLUSIONS

The metal distributions in the different tissues of *T. gigas* indicated the different affinities of metals in these tissues. Most note worthy is the higher concentrations of Cd and Zn in the fillet compared with the other tissues. This indicated anthropogenic sources (possibly due to domestic wastes and agricultural activities in Tampok and Pontian Besar, and effluents of petrol-chemical plants and domestic wastes besides shipping activities at Kg. Pasir Puteh) of Cd and Zn since fillet is usually an organ with low binding affinity. The redistribution pattern of Cd and Zn in the fillet due to a detoxification mechanism could cause the elevated levels of Cd and Zn in this particular tissue. This study should prompt further studies on horseshoe crabs from the heavy metal ecotoxicological point of view. A study should be done to establish this living fossil as a biomonitor of heavy metal pollution in the intertidal area of Malaysia.

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REFERENCES

Botton, M.L. 2000. Toxicity of cadmium and mercury to horseshoe crab (*Limulus Polyphemus*), embryos and larvae. *Bulletin of Environmental Contamination and Toxicology*, **64:** 137–143.

Botton, M.L., Johnson, K. & Helleby, L. 1998. Effects of copper and zinc on embryos and larvae of the horseshoe crab, *Limulus Polyphemus*. *Archives of Environmental Contamination and Toxicology*, **64:** 25–32.

Burger, J. 1992. Trace element levels in Pine Snake hatchlings: tissue and temporal differences. *Archives of Environmental Contamination and Toxicology*, **22:** 209–213.

Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. *Review of Environmental Toxicology*, **5:** 203–311.

Burger, J. 1994. Heavy metals in avian eggshells: another excretion method. *Journal of Toxicology and Environmental Health,* **41:** 207–220.

Burger, J. 1997. Heavy metals in the eggs and muscle of horseshoe crabs (*Limulus polyphemus*) from Delaware Bay. *Environmental Monitoring and Assessment*, **46:** 279–287.

Burger, J., Dixon, C., Shukla, T., Tsipoura, N., Jensen, H., Fitzgerald, M., Ramos, R. & Gochfeld, M. 2003. Metals in Horseshoe

Crabs from Delaware Bay. *Archives of Environmental Contamination and Toxicology*, **44:** 36–42.

Castro, G. & Myers, J.P. 1993. Shorebird predation on eggs of horseshoe crabs during spring stopover on Delaware Bay. *Auk*, **110:** 927–930.

Castro, G., Myers, J.P. & Place, A.R. 1989. Assimilation efficiency of sanderlings (*Calidris alba*) feeding on horseshoe crab eggs. *Physiological Zoology,* **62:** 716–731.

Chatterji, A., Mishra, J.K. & Parulekar, A.H. 1992. Feeding behaviour and food selection in the horseshoe crab, *Tachypleus gigas* (Muller). *Hydrobiologia,* **246:** 41-18.

Kungsuwan, A., Nagashima, Y., Noguchi, T., Shida, Y., Suvapeepan, S., Suwansakornkul, P. & Hashimoto, K. 1987. Tetrodotoxin in the horseshoe crab, *Carcinoscorpius rotundicauda* inhabiting Thailand. *Nippon Suisan Gakkaishi,* **53:** 261-266.

Mohan, S., Doral, D.T., Srimal, S., Bachhawat, B.K. & Das, M.K. 1984. Circular dichroism studies on carcinoscorpin, the sialic acid binding lectin of horseshoe crab, *Carcinoscorpius rotundacauda. Indian Journal of Biochemical Biophysics*, **21:** 151-154.

Rainbow, P.S. 1996. Heavy metals in aquatic in vertebrates. In: *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations.* W.N. Beyer, G.H. Heinz and A.W. Redmon-Norwood (Eds.). Lewis Pubblicaton, Boca Raton, FL, pp. 405–425.

Sekiguchi, K., Nakamura, K., Sen, T.K. & Sugita, H. 1976. Morphological variation and distribution of a horseshoe crab, *Tachypleus gigas,* from the Bay of Bengal and Gulf of Siam. *Proceedings of the Japanese Society of Systematic Zoology,* **12:** 13-20.

Shuster, C.N. Jr. 1985. Introductory remarks on the distribution and abundance of the American horseshoe crab, *Limulus polyphemus,* spawning in the Chesapeake Bay area. In: *Proceedings from the Chesapeake Bay Symposium*. V. Chase (Ed.). Baltimore, MD, pp. 34-38.

Tsipoura, N. & Burger, J. 1999. Shorebird diet during spring migration stop-over on Delaware Bay. *Condor*, **101:** 35–644.

Yap, C.K., Ismail, A. & Tan, S.G. 2003a. Cd and Zn in the straits of Malacca and intertidal sediments of the west coast of Peninsular Malaysia. *Marine Pollution Bulletin,* **46:** 1348-1353.

Yap C.K., Ismail, A., Tan, S.G. & Abdul Rahim, I. 2003b. Can the shell of the green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn? *Estuarine and Coastal Shelf Sci*ence, **57:** 623-630.

Yap, C.K., Tan, S.G., Ismail, A. & Omar, H. 2004a. Allozyme polymorphisms and heavy metal levels in the green-lipped mussel *Perna viridis* (Linnaeus) collected from contaminated and uncontaminated sites in Malaysia. *Environment International*, **30:** 39-46.

Yap, C.K., Ismail, A, Tan, S.G. & Rahim Ismail, A. 2004b. The impact of anthropogenic activities on heavy metal (Cd, Cu, Pb and Zn) pollution: Comparison of the metal levels in green-lipped mussel *Perna viridis* (Linnaeus) and in the sediment from a high activity site at Kg. Pasir Puteh and a relatively low activity site at Pasir Panjang. *Pertanika Journal of Tropical Agricultural Science,* **27(1):** 73-78.

Yap, C.K., Ismail, A., Edward Berandah, F., Tan, S.G. & Siraj, S.S. 2006. Use of different soft tissues of *Perna viridis* as biomonitors of bioavailability and contamination by heavy metals (Cd, Cu, Fe, Pb, Ni and Zn) in a semi-enclosed intertidal water, the Johore Straits. *Toxicological and Environmental Chemistry*, **88(4):** 683 - 695.